MOTOR CORTEX PLASTICITY AND SKILL ACQUISITION IN ENDURANCE-TRAINED ATHLETES

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List of Publications

This thesis consists of a combination of published journal articles and text written in manuscript form, in accordance with the Academic Program Rules of The University of Adelaide.

The experimental chapters of the thesis are based on the following published journal articles and text written in manuscript form:

> **Hand, B. J.**, Opie, G. M., Sidhu S. K. and Semmler J. G. (2020). "TMS coil orientation and muscle activation influence lower limb intracortical excitability." *Brain Research,* **1746**: 147027.
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> **Hand, B. J.**, Opie, G. M., Sidhu S. K. and Semmler J. G. (2021). "Motor cortex plasticity and visuomotor skill learning in upper and lower limbs of endurance-trained cyclists." *European Journal of Applied Physiology*, doi: 10.1007/s00421-021-04825-y.
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> **Hand, B. J.**, Opie, G. M., Sidhu S. K. and Semmler J. G. “Motor cortex plasticity is greater in endurance-trained cyclists following acute exercise.” *Text in manuscript form.*

The following journal articles are of close relevance to the main research topic but are not included.

> Opie, G. M., **Hand, B. J.**, Coxon, J. P., Ridding, M. C., Ziemann, U. and Semmler, J. G. (2019). "Visuomotor task acquisition is reduced by priming paired associative stimulation in older adults." *Neurobiology of Aging* **81**: 67-76.
>
> Opie, G. M., **Hand, B. J.** and Semmler, J. G. (2020). "Age-related changes in late synaptic inputs to corticospinal neurons and their functional significance: A paired-pulse TMS study." *Brain Stimulation* **13**(1): 239-246.
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> Opie, G. M., Sasaki, R., **Hand, B. J.** and Semmler, J. G. (2021). "Modulation of Motor Cortex Plasticity by Repetitive Paired-Pulse TMS at Late I-Wave Intervals Is Influenced by Intracortical Excitability." *Brain Sciences* **11**(1): 121.
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> Semmler, J. G., **Hand, B. J.**, Sasaki, R., Merkin, A. and Opie, G. M. (2021). "Age-related changes in motor cortex plasticity assessed with non-invasive brain stimulation: an update and new perspectives." *Experimental Brain Research.* **239**(9): 2661-2678.

Abstract

Numerous research studies utilising transcranial magnetic stimulation (TMS) have demonstrated that physical exercise has the potential to modulate motor cortex excitability and plasticity in humans. However, it is unknown how plasticity is modified in endurance-trained athletes. Therefore, the overarching aim of this thesis was to investigate how motor cortex plasticity is modified for both exercised (lower limb) and non-exercised (upper limb) muscle groups of endurance-trained individuals.

To do so, we first had to identify TMS parameters that are appropriate for measurements of lower limb muscles (study 1, Chapter 2). This study optimised muscle-specific TMS parameters (coil type and orientation) for single-pulse measures of corticospinal excitability and paired-pulse measures of intracortical circuits, the activity of which can contribute to neuroplasticity. We found that with a figure-of-eight TMS coil, a medio-lateral coil orientation was the most effective for TMS measures of lower limb muscles, requiring lower TMS intensities than alternative coil orientations. These findings were necessary for the subsequent investigation of how regular physical activity (endurance cycling) influences neuroplasticity induced by skill training involving the upper (non-exercised) and lower (exercised) limb muscles (study 2, Chapter 3). The primary outcome was that endurance-trained participants showed greater motor cortex plasticity following visuomotor skill training. This was found in both upper and lower limb muscles, indicating that the heightened plasticity in endurance-trained individuals is not exclusive to muscle groups directly involved in the exercise. Despite this, visuomotor skill acquisition was not differentially modulated between endurance-trained cyclists and sedentary individuals.

Study 3 (Chapter 4) aimed to determine if regular endurance training increased TMS-induced plasticity following acute aerobic exercise involving high-intensity interval training. In a novel finding, the endurance-trained group demonstrated greater TMS-induced motor cortex plasticity following acute exercise than sedentary participants. Furthermore, TMS-induced plasticity was significantly greater when preceded by acute exercise, compared with a no-exercise control. Together, these studies provide novel evidence showing that regular endurance training promotes both use-dependent and experimentally induced motor cortex plasticity.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, 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. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Signed: *Date: 28/03/2022*

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1. Literature review

It is now well known that exercise provides widespread benefits for the human body, promoting several aspects of health and wellbeing while improving disease outcomes. Evidence from the ever-expanding body of literature demonstrates that exercise aids attention and memory, cognition, executive function, and motor control. Many of these outcomes are likely due to the modulation of cortical plasticity – the brain's capacity to change the nature of its neural circuitry. With regards to the motor system, plasticity is known to be a critical component of motor skill acquisition and retention. While exercise has been shown to favourably influence cortical plasticity and motor function, the interactions between acute and regular exercise, use-dependent plasticity and motor skill acquisition are not well understood.

This introduction aims to provide an overview of the literature surrounding motor cortex plasticity with aerobic exercise and skill acquisition. Firstly, I explore the relevant anatomy of the brain (specifically the motor cortex) and central nervous system, before a discussion of some important physiological mechanisms involved with neural control of movement. This includes an overview of the primary motor cortex (M1) and the descending pathways required for the control of skeletal muscles. I then explore non-invasive brain stimulation as a research tool, followed by a discussion of how corticospinal and intracortical networks are both assessed and functionally modulated using transcranial magnetic stimulation (TMS). Subsequently, this introduction includes an overview of the various mechanisms that contribute to cortical plasticity. Finally, I discuss the relevant physiological and functional associations between exercise, plasticity and motor skill acquisition.

1.1 Neural control of movement

1.1.1 Human motor cortex

The human motor cortex encapsulates the principal cortical areas associated with motor control. The motor cortex is estimated to include more than 1 billion of the 19 – 23 billion cortical neurons that make up the cerebral cortex (for review, see Pakkenberg and Gundersen 1997, Ziemann 2017). It is now understood that multiple distinct regions within the cortex are associated with motor control. These regions are known to be functionally interconnected, however many of the precise details are difficult to determine (Donoghue and Sanes 1994). The motor cortex is located at the posterior of the frontal lobe and can be divided into independent cortical regions. Firstly, primary motor cortex (M1 or Brodmann's area 4), located on the precentral gyrus, is the primary source of motor output from the cortex and is known to be particularly dynamic in nature, with significant potential for structural and functional reorganisation. For these reasons, M1 is therefore a frequent target for cortical stimulation in motor studies (Sanes and Donoghue 2000, Ziemann 2017) and is a central focus of this thesis. Anteriorly to M1, the supplementary motor area (SMA) and pre-motor area (PMA) are located on the medial and lateral aspect of Brodmann's area 6, respectively (Donoghue and Sanes 1994). Together, the SMA and PMA are heavily involved with planning and guiding of movement, however, these regions are beyond the scope of the current thesis and will not be addressed further.

1.1.2 Primary motor cortex (M1)

M1 has been thought to include discreet representations of individual muscles since the late 19th century, when it was observed that convulsions in an epileptic seizure often travel between nearby muscle groups, following an orderly representation (Jackson 1873). In the early 20th century, the idea of functionally independent areas within the cortex was expanded based on the unique cellular content between regions. This provided an anatomical map of the human cortex (Brodmann 1909). Subsequent electrical stimulation research from the early/mid-20th century allowed for refinement of this concept and was pivotal in the understanding of M1 organisation and function. Namely, the development of a representative map (homunculus) for the human sensory cortex (Penfield and Boldrey 1937) and motor cortex (Penfield and Rasmussen 1950). These developments spawned from direct stimulation of the human cortex in epileptic patients during surgery. The motor homunculus follows a general organisation of lower limbs being represented medially, while upper limb, head and face representations are progressively more lateral (Sanes and Donoghue 2000). Subsequent animal studies using intracortical microsimulation have indicated that this 'homunculus' is a continuous layout of all muscular regions in a non-overlapping fashion, however with distorted proportions. It was suggested that the organisation of the motor homunculus involved high specificity of neural elements, allowing accurate, individualised control of muscles (Asanuma and Ward 1971). However, recent studies have demonstrated that there is a complex intermingling of cortico-motoneuronal cells involved with movement. So widespread, that 15-16% of these cells originate from outside M1. Specifically, in region 3a – primary somatosensory cortex (Rathelot and Strick 2006, Rathelot and Strick 2009). Further research has demonstrated that motor representations within M1 are likely to be widely distributed, overlapping and not clearly distinguishable (Indovina and Sanes 2001, Schieber and Santello 2004). To further support this notion, research suggests that some specific neuronal populations within M1 are involved with the kinematics of movement and are not confined to specific motor cortical regions (Georgopoulos et al. 1986, Wessberg et al. 2000).

1.1.3 Cortical neurons

Cells of the human cortex can be distinguished into two broad categories, which differ in both structure and function. Non-pyramidal cells, otherwise known as stellate cells, project locally within the cortex and have a star-shaped soma (cell body). Stellate cells demonstrate considerable variation in morphology and function and can act as either excitatory or inhibitory neurons. Excitatory non-pyramidal cells, known as spiny stellate cells use glutamate as their neurotransmitter while inhibitory, non-spiny stellate cells rely on γ-aminobutyric acid (GABA) (DeFelipe 1997). The other broadly categorised division of cortical neurons is pyramidal cells, which demonstrate a triangular soma. Like spiny stellate cells, pyramidal cells use glutamate as their neurotransmitter and are always excitatory in nature. Unlike stellate cells, these neurons have projections both within and between cortices and subcortical areas, often having distant targets (Molnar and Cheung 2006).

The distribution of these different cortical neurons has specific anatomical significance as their distribution within the brain delineates 6 cortical layers (Brodmann 1909). Labelled I – VI, these layers span from the outer (pial) surface of the brain (layer I) to the white matter (layer VI). The anatomical makeup of these layers varies. For example, pyramidal cells are present primarily in layers III, V and VI, whereas stellate cells are found to some extent in all layers. These variations in cell populations result in structural and functional differences of cortical layers, which can vary between brain regions. For example, M1 tissue demonstrates thick layers III and V while IV is hardly present, whereas the visual cortices demonstrate a broad layer IV and compact layers III and V (Kandel et al. 2000).

1.1.4 Descending pathways

To facilitate the communication of motor cortical commands to skeletal muscles, there are multiple anatomically and functionally distinct descending pathways. Originating in either the brainstem or cortex, descending corticospinal neurons project to pools of motor neurons within the spinal cord, known as motor nuclei. Arranged to form longitudinal columns, these motor nuclei span multiple vertebral segments and are somatotopically organised: motor nuclei responsible for innervation of proximal muscle tissue lie medially, while those innervating distal muscle are represented laterally (Romanes 1964). The corticospinal system involves descending pathways, which originate in the cortex. Pyramidal cells of cortical layer V form the most substantial portion, representing over 80% of the corticospinal system. However, descending neurons also originate in other cortical regions including the premotor and supplementary motor areas, sensory and posterior parietal cortices (Murray and Coulter 1981, Lemon 1997). These cells project to the brainstem and form medullary pyramids, where more than 70% of descending fibres cross to the contralateral side of the body, forming the pyramidal decussation (Chouinard and Paus 2006). These descending fibres innervate dorsolateral motor nuclei and form the lateral corticospinal tract, which is primarily concerned with the control of distal limb musculature. The remaining fibres descend without crossing the midline, follow the medial column and innervate ventromedial motor nuclei bilaterally, forming the ventral corticospinal tract. This pathway is concerned with the control of axial and proximal limb musculature (Kuypers 1964).

1.1.5 The functional significance of glutamate and gamma-aminobutyric acid (GABA)

1.1.5.1 Glutamate

Glutamate is one of the most abundant amino acids in mammalian species, with roles in nutrition, metabolism, and neural signalling (Brosnan and Brosnan 2013). This protein acts as the primary excitatory neurotransmitter in the mammalian central nervous system and acts through two receptor types; ionotropic glutamate receptors (iGluRs) and metabotropic glutamate receptors (mGluRs) (Schubert et al. 2004, Kew and Kemp 2005). Binding of glutamate to iGluRs results in the direct opening of the receptor’s ion channel, resulting in the influx of calcium and sodium into the cell. Movement of these positively charged ions into the cell causes excitatory post-synaptic potentials (EPSPs), raising the membrane potential of the cell toward the threshold for activation, at which point an action potential occurs (Tremblay 2010). This process is known to occur particularly within the motor system and has important roles in regulating global cortical excitability (Stagg et al. 2011) as well as synaptic plasticity (Gécz 2010) (see section 1.3.1). Alternatively, glutamate binding with mGluRs activates ion channels either directly by $\beta\gamma$ subunits of the G-protein, or indirectly through second messenger pathways (Kew and Kemp 2005). In addition to its role directly in the CNS, glutamate is an important metabolic precursor for GABA via glutamate decarboxylase (Brosnan and Brosnan 2013).

1.1.5.2 Gamma-aminobutyric acid (GABA)

GABA is considered the brain's primary inhibitory neurotransmitter and has been shown to be present in > 40% of neural synapses (DeFelipe 1993). Having such a prevalence within the CNS, GABA is a critical component of cortical activity. GABAergic inhibition is involved with regulating the oscillatory activity of the brain, synchronisation of neural networks through pyramidal cell timing, and aids in motor control (Rudy et al. 2011, Hermans et al. 2018). For example, GABAergic inhibition is reduced when a specific muscle is activated (Ridding et al. 1995). Accordingly, GABAergic inhibition is thought to be critical for precise movements. GABA-mediated inhibitory processes within the cortex are regulated by several neuronal subtypes, which vary in morphology, physiology and function (Druga 2009). The specific function of these neurons is partly dependent on the nature and location of their synaptic connections with pyramidal cells (Markram et al. 2004).

There are several different GABA receptor classes with varying synaptic profiles: $GABA_A$, $GABA_B$ and $GABA_C$. Both $GABA_A$ and $GABA_B$ receptors are highly prevalent in the human brain, while $GABA_C$ is prevalent in the retina of many species (Matsumura et al. 1992, Zhang et al. 2001). $GABA_A$ receptors are ionotropic with 5 transmembrane domains and a central ion pore. Located on the postsynaptic cells, binding of $GABA_A$ receptors causes chloride influx and hyperpolarisation, resulting in an inhibitory postsynaptic potential (IPSP). In contrast, $GABA_B$ receptors are G-protein coupled with a heterodimeric structure. Unlike $GABA_A$, $GABA_B$ is located on both pre and post-synaptic membranes (Benarroch 2012). Stimulation of $GABA_B$ receptors at the post-synaptic cell activates rectifying K^+ channels which cause a slow inhibitory postsynaptic current (IPSC) that occurs much later than $GABA_A$ mediated IPSPs, peaking at 50-250 ms (Bettler et al. 2004). One key difference between these two GABA subtypes is the level of observed conservation in the receptor type; there appear to be many types of $GABA_A$ receptors, which vary depending on their specific sub-unit expression/structure. Divergently, $GABA_B$ shows high-level structural conservation, with only 2 known sub-types (Benarroch 2012, Sigel and Steinmann 2012). Glutamate and GABA are known to be critical for regulating corticospinal excitability within the motor cortex and the activity of both glutamatergic excitatory and GABAergic inhibitory circuits can be indirectly assessed with non-invasive brain stimulation.

1.2 Non-invasive brain stimulation

Early research that utilised electrical stimulation directly to the exposed cortex (Penfield and Boldrey 1937, Penfield and Rasmussen 1950) provided significant advancements in the understanding of the somatosensory and motor homunculus in humans. However, there were clear limitations for the widespread use of direct stimulation to the cortex for research. Gualtierotti and Paterson (1954) demonstrated that cortical tissue could be activated non-invasively with continuous electric currents. However, this method was not put to widespread use given the high stimulation amplitude required, and resultant participant discomfort (Suihko 2002). This concern was identified by Merton and Morton (1980), who subsequently demonstrated that the human cerebral cortex could be stimulated non-invasively by applying brief, high-voltage pulses to the scalp, a technique known as transcranial electric stimulation (TES). When applied over M1, this technique activated skeletal muscles on the contralateral side. Early TES research provided important information about the nature of neural pathologies such as Parkinson’s disease (Cowan et al. 1984) and multiple sclerosis (Dick et al. 1984). Unfortunately, as is the case with continuous electric current stimulation, very little of the current from TES penetrates deep enough to activate cortical tissue. This current instead stimulates more superficial tissue, causing activation of scalp muscles and nociceptors (Suihko 2002), resulting in significant discomfort (Amassian and Maccabee 2006).

As a revolutionary alternative, Barker et al. (1985) established that strong magnetic pulses applied over the scalp could activate underlying cortical tissue as, unlike electrical current, magnetic fields are not impeded by the high resistance of the skull and scalp tissue. This technique, known as transcranial magnetic stimulation (TMS), involves discharging a brief electrical current from high capacitance devices into a copper wire coil, which subsequently emits an electromagnetic pulse. The electromagnetic discharges from a TMS coil activate underlying conductive (neuronal) tissue. When applied over the motor cortex, depolarisation of M1 tissue results in the activation of descending corticospinal neurons, which project to lower motor neurons and subsequently generate a discernible response in peripheral muscles on the contralateral side of the body (Hallett 2000). The magnetic field produced by the coil is aligned perpendicular to the plane of the coil, and the characteristic of this field varies depending on the type of coil used. For example, figure-of-eight shaped coils are known for high focality due to the high current at the intersection of each 'wing', as will be discussed further in section 1.2.4.1. TMS is now commonplace in many neurophysiological research settings, providing an avenue for the investigation of both excitatory and inhibitory intracortical circuits, with good spatial and excellent temporal resolution (Bolognini and Ro 2010, Ziemann 2017, Chail et al. 2018). TMS remains a favourable choice for studies of motor control, as it is painless, very low risk and easy to administer (Ziemann 2017). Consequently, TMS has been critical in building our understanding of motor control, including investigations of motor map representations, mechanisms of motor output (both corticospinal and intracortical networks), as well as functional neuroplasticity and pathology of M1 (for reviews, see Ziemann 2017, Chail et al. 2018).

1.2.1 TMS physiology and the descending volley

When applied over the brain's motor areas, TMS can generate action potentials within corticospinal neurons, which results in the activation of lower motor neurons that innervate skeletal muscles. The electrophysiological response in the muscle can be recorded using electromyography (EMG) and is referred to as a motor-evoked potential (MEP) (Dimitrijević et al. 1992). Adjustment of the coil position over different parts of the motor strip allows for the targeted activation of skeletal muscle groups (Hallett 2000). Motor cortex TMS generally targets a muscle in the hand or upper limb. This is because these muscles have large cortical representations that are located superficially, giving them a low TMS threshold (Roux et al. 2020). In contrast, the cortical representation of lower limb muscles is located within the deeper cortical tissue of the interhemispheric fissure. Accordingly, muscles of the lower limb are typically more difficult to activate with TMS, as is discussed further in section 1.2.4.2 (Penfield and Boldrey 1937, Terao et al. 1993, Cacchio et al. 2009, Hassanlouei et al. 2017).

Application of TMS over M1 results in a complex volley of activity within corticospinal neurons. A descending volley includes several discernible waves that result from trans-synaptic activation of intracortical neurons (Hallett 2000, Di Lazzaro et al. 2012). This concept builds on early animal studies from the mid-twentieth century which demonstrated that electrical stimulation to the cortex elicits multiple descending 'waves'. The term volley is given to the group of descending waves, and it is understood that each wave demonstrates different characteristics from one another (Patton and Amassian 1954). In such research, electrical stimuli were applied directly to the cortex of anesthetised mammals (cat or monkey models). Insulated wire electrode recordings were used to assess activity (voltage changes) from the pyramidal decussation. This research demonstrated that the first wave in the descending volley displays unique characteristics from subsequent waves, and it was postulated that this initial direct (D-wave) is likely generated from direct activation of corticospinal neurons (Patton and Amassian 1954). Subsequent waves in the volley occurred at an interval of ~1.5 ms and were instead suggested to reflect trans-synaptic activation of corticospinal neurons from interneuronal circuits; these were therefore referred to as Indirect (I) waves (Phillips 1956, Kernell and Chien-ping 1967). More specifically, early I waves (I1) are thought to occur through local activation of monosynaptic cortico-cortical connections that project onto descending corticospinal neurons (Di Lazzaro et al. 2008), whereas later I waves (I2/I3) are thought to involve complex neural circuitry that result in the repeated discharge of pyramidal tract neurons (Di Lazzaro et al. 2008).

Recent human studies have demonstrated this concept with TES and TMS, however with slight variances in the composition of the descending volley produced by each technique. In line with earlier animal studies, TES has been shown to activate D-waves first, while TMS appears to elicit descending volleys with a comparatively delayed onset latency. Specifically, TMS is thought to produce a descending volley that begins with an I1 wave, occurring approximately 1.5 ms after the onset of a TES response (Di Lazzaro et al. 2012). As stimulus intensity increases, there is progressive recruitment of later I-waves, followed at high intensity by a D-wave (Di Lazzaro et al. 2008). However, the order in which I-waves are recruited has also been shown to depend on the orientation with which the TMS coil is held on the scalp (see section 1.2.4.2). Together, this research demonstrated that various TMS stimulation parameters (especially stimulation intensity and coil orientation) influence the specific composition of the descending volleys. Despite this, it is commonly accepted that most TMS parameters that are used to stimulate M1 activate corticospinal neurons trans-synaptically (preferential I-wave recruitment). Fortuitously, this allows TMS to be used for measurement of both corticospinal and intracortical networks (see sections 1.2.2-3) as well as for plasticity induction paradigms that are known to influence I-wave generating circuits (see section 1.3.2.1).

1.2.2 Single-pulse TMS

Single-pulse TMS is a standard method of measuring corticospinal excitability. Specifically, the peak-to-peak amplitude of MEPs generated by TMS is expressed relative to stimulus intensity (generally stated as the percentage of maximum stimulator output - %MSO). The amplitude of the MEP reflects the excitability of the neural elements activated by TMS, including intracortical circuits, corticospinal cells and spinal motor neurons. These responses are influenced by many physiological characteristics, including motor neuron recruitment, synchronisation and background muscle activity (Kiers et al. 1993).

There are numerous single-pulse measures that can be utilised to assess corticospinal excitability. For example, the motor threshold is defined as the lowest TMS intensity required to produce an MEP and is thought to reflect the membrane excitability of cortico-cortical axons within the motor cortex, as well as the excitability of spinal motor neurons (Amassian et al. 1987, Kobayashi and Pascual-Leone 2003, Shimazu et al. 2004). A motor threshold can be recorded for skeletal muscles in either a resting state (resting motor threshold, RMT) – or in an active muscle (active motor threshold, AMT). RMT is typically defined as the minimum stimulator intensity required to produce an MEP of $\geq$ 50 µV in 5 out of 10 responses. Activation of a skeletal muscle lowers the required stimulus intensity to elicit a response. Accordingly, AMT is often defined as the minimum intensity required to produce an MEP of $\geq$ 200 µV in 5 out of 10 trials in an active muscle (5-10% maximum voluntary contraction) (Rossini et al. 2015). As a more comprehensive characterisation of motor cortical excitability, input/output (I/O) curves can be produced by applying a number of stimuli (i.e., 20 stimuli) at several TMS intensities. As greater stimulus intensities are applied, MEP amplitude increases according to a sigmoidal function. These data are often quantified by deriving the slope of the IO curve, which is used to characterise the gain in the corticospinal system resulting from the strength of corticospinal projections (Chen et al. 1998, Kukke et al. 2014). Alternatively, an *area under the curve* analysis can be utilised as a more robust measure of corticospinal projections that does not rely on appropriate slope fitting (Iyer and Madhavan 2019).

Single-pulse TMS measures including I/O curves are commonly utilised to assess and compare corticospinal excitability between participant cohorts (e.g., age or gender, Pitcher et al. 2003), or as an experimental outcome in interventional studies in which a change in corticospinal excitability is measured. As a simple alternative to I/O curves, a *test TMS* measure can be utilised. This involves identifying the stimulation intensity required to produce a specific MEP amplitude (usually ~ 1 mV) or applying a fixed intensity of stimulation (usually 110-140% of RMT) (Sondergaard et al. 2021). These intensities are used as they elicit a mean MEP amplitude that sits approximately in the middle of the linear portion of the input/output curve (Fisher et al. 2002). This ensures that changes in excitability are measurable without floor or ceiling effects and avoid non-linear changes in MEP responses following an intervention. These single-pulse protocols of corticospinal excitability (particularly I/O curves and test TMS) have been utilised in a wide range of interventional studies where measures are obtained prior to and following an intervention, with a change in mean MEP amplitude considered a marker for the induction of plasticity. For example, this approach has been used before and after a bout of exercise (Singh et al. 2014, Mooney et al. 2016), motor learning (Perez et al. 2004, Paparella et al. 2020) or a plasticity-inducing brain stimulation paradigm (Cash et al. 2009, Jung and Ziemann 2009) (discussed further in section 1.3.3-4). Due to the highly variable nature of TMS responses, numerous individual responses are generally collected to improve the reliability of study outcomes (Rossini et al. 1994).

1.2.3 Paired-pulse TMS

While measures of single-pulse TMS provide inferences that are mostly limited to the excitability of corticospinal projections, paired-pulse TMS instead expands this to specifically test excitability within intracortical circuits. Paired-pulse TMS protocols involve two stimuli at highly specific inter-stimulus intervals (ISIs) in a conditioning-test like paradigm. This results in changes in the amplitude of the test stimulus when compared to the amplitude of the test stimulus given in isolation (see Fig. 1.1). The paired-pulse (conditioned) responses are analysed as a proportion of the mean unconditioned MEP size (Ilić et al. 2002). Modulation of the MEP with paired-pulse TMS is thought to indicate the activity of intracortical inhibitory or facilitatory circuits by the conditioning stimulus that then influences the excitability of corticospinal neurons upon which they synapse. The specific effect on motor output (inhibitory or excitatory) is dependent on the intensity of the conditioning stimulus and the inter-stimulus interval (ISI) between the conditioning and test stimulus. The following sections will focus on four paired-pulse paradigms commonly applied to assess the activity of interneuron networks known to be important for motor function.

1.2.3.1 Intracortical inhibition

One of the most established paired-pulse TMS protocols is referred to as short-interval intracortical inhibition (SICI, Kujirai et al. 1993). This paired-pulse TMS protocol involves a subthreshold conditioning pulse (S1) applied 1-5 ms prior to a suprathreshold ‘test TMS’ pulse (S2) (Kujirai et al. 1993, Di Lazzaro et al. 1998). The conditioning S1 pulse inhibits the MEP generated by S2 through pre-synaptic inhibition, with evidence suggesting that SICI is regulated by GABAergic transmission. Pharmacological studies using paired-pulse

TMS have shown that the magnitude of inhibition recorded during SICI is increased following a dose of lorazepam, a $GABA_A$ receptor agonist (Ziemann et al. 1996, Di Lazzaro et al. 2000). These findings are specific to paired-pulse SICI with an ISI of 2 ms or greater (largest effect at 4 and 5 ms). Suppression of the MEP with an ISI of 1 ms is likely due to factors other than $GABA_A$ activation, such as axonal refractoriness or extrasynaptic inhibition (Fisher et al. 2002, Roshan et al. 2003). Epidural recordings of the descending volley show that SICI reduces late (I3) waves, without influencing D-waves or early I-waves (Di Lazzaro et al. 1998). Evidence suggests that this paired-pulse protocol does not modify H-reflex amplitude (a neuromuscular twitch response to electrical stimulation of 1a sensory fibres, used as a measure of spinal/peripheral excitability) (Kujirai et al. 1993, Ziemann et al. 1996, Palmieri et al. 2004). Furthermore, (Chiou et al. 2020) demonstrated modulation of SICI after voluntary activity without a change in response to cervicomedullary stimulation (which activates the corticospinal tract directly). Together, these outcomes suggest that it is unlikely that there are spinal contributions to suppression of the test MEP with this protocol.

SICI has received considerable interest in research in the past two decades, as this measure is known to reflect $GABA_A$ activity (Di Lazzaro et al. 1998) and is modulated by several interventions such as exercise and motor skill learning (Coxon et al. 2014, Berghuis et al. 2017, Stavrinos and Coxon 2017). Ridding et al. (1995) initially demonstrated that SICI is reduced during a voluntary contraction. Subsequently, it was demonstrated that SICI was reduced 95 msec prior to the onset of EMG activity of a voluntary contraction (Reynolds and Ashby 1999). Together, these outcomes suggest that SICI has a critical role in modulating motor output to skeletal muscles. In addition, SICI measured in an active muscle has been shown to increase 30 ms before the onset of relaxation, indicating that SICI is critical in suppressing resting-state corticospinal output (Buccolieri et al. 2004). There appears to be high specificity with regards to SICI modulation during motor tasks.

For example, during a precise FDI movement task (in which the rest of the hand was intentionally relaxed), there is a reduction in SICI that is isolated to the cortical representation for the FDI and an increase in SICI for neighbouring muscles (Stinear and Byblow 2003). Further evidence for the importance of SICI for motor control is the considerable decrease of SICI in movement disorders such as Tourette syndrome (Ziemann et al. 1997) or Parkinson's disease (Ridding et al. 1995). Finally, numerous recent studies have demonstrated acute changes in SICI following a motor skill training task, albeit with somewhat mixed findings (Coxon et al. 2014, Mooney et al. 2019, Cirillo et al. 2020).

Paired-pulse TMS can also be applied with a suprathreshold conditioning stimulus at longer intervals of 50 – 200 ms (Valls-Solé et al. 1992, Nakamura et al. 1997) to assess a separate form of intracortical inhibition, known as long-interval intracortical inhibition (LICI). Epidural recordings have been utilised to determine how LICI influences I-waves of the descending volley. Such research indicated a reduction in late I-waves (I2 and I3), while D- and I1-waves remain unaffected (Di Lazzaro et al. 2002). It is currently unclear if changes in spinal excitability from the suprathreshold conditioning pulse are involved with suppression of the MEP with LICI. Some evidence suggests that ISIs of 100-200 ms are sufficiently long enough to allow spinal excitability to recover from the conditioning pulse (Nakamura et al. 1997, Di Lazzaro et al. 2002). In contrast, more recent work by McNeil and colleagues utilised cervicomedullary stimulation to investigate the cortical and non-cortical contributions to LICI (McNeil et al. 2009, McNeil et al. 2011). However, TMS research involving electroencephalography (EEG) has shown significant correlations between a TMS-evoked cortical potential (TEP) and the amplitude of a peripheral MEP with LICI (Rogasch et al. 2013), suggesting that this mechanism may be related to cortical activity. Similarly to SICI, evidence suggests that LICI is modulated by the neurotransmitter GABA. However, pharmacological research indicates that the GABA_B

receptor subtype is responsible for this inhibition, with increased LICI resulting from the application of a $GABA_B$ receptor agonist (McDonnell et al. 2006).

1.2.3.2 Intracortical facilitation

Paired-pulse TMS is also used routinely for the assessment of facilitatory intracortical networks. Short interval intracortical facilitation (SICF) was developed as a method of quantifying the activity of I-wave circuitry (Tokimura et al. 1996, Ziemann et al. 1998). The TMS protocols that are used to assess SICF involve pairs of near-threshold TMS stimuli, which results in significant facilitation of the MEP at the approximate I-wave intervals (1-1.5, 2.5-3.0 and 4.5 ms)(Tokimura et al. 1996, Ziemann et al. 1998). SICF typically involves an initial suprathreshold S1 and a perithreshold S2 (Ziemann et al. 1998, Hanajima et al. 2002). The specific mechanisms that regulate SICF remain unknown; however, it has been suggested that S2 directly activates cortical elements (e.g., cell bodies or initial segments of neurons) that were made hyperexcitable by S1, although below the activation threshold. Facilitation of the MEP is therefore thought to result from the summation of corticomotoneuronal EPSPs induced by S1 and direct depolarization of interneurons by S2 (Amassian et al. 1990, Di Lazzaro et al. 1999, Hanajima et al. 2002). Accordingly, the degree of facilitation with SICF is thought to indicate the excitability of I-wave circuity, and the timing of SICF peaks reflects the temporal characteristics of such circuits (see Opie and Semmler 2020, for review). Despite this, we cannot determine exactly which components of the descending volleys are contributing to the observed response, even with paired-pulse ISIs that align with the known I-wave intervals. Furthermore, while it is accepted that SICF most likely has cortical origins, evidence suggests that there may be spinal contributions (Cirillo et al. 2015). Therefore, interpretations regarding the mechanisms and modulation of SICF must be considered carefully. As with SICI, evidence suggests that SICF may be correlated with motor function; however, the specific nature of these interactions is difficult to determine. For example, decreased SICF in older adults has also been associated with increased unilateral pegboard performance (Clark et al. 2011; Opie et al. 2020a), however increased SICF has been associated with improved bimanual dexterity in older adults (Clark et al. 2011). Furthermore, an upregulation of SICF has been demonstrated with action observation and movement planning (Cretu et al. 2020; Cattaneo et al. 2005).

In addition to SICF, paired-pulse TMS at longer ISIs of 8-20 ms elicits a separate form of MEP facilitation known as intracortical facilitation (ICF). The TMS protocol for ICF utilises a subthreshold S1 and suprathreshold S2, as is the case with SICI (Kujirai et al. 1993, Ziemann et al. 1996). Evidence suggests that ICF is mediated by glutamatergic neurotransmission, as the administration of Riluzole (a glutamate antagonist that has both pre- and post-synaptic effects) suppresses ICF (Liepert et al. 1997). The exact neural mechanisms of ICF remain unclear; however, it has been surmised that this mechanism most likely results from activation of cortico-cortically projecting pyramidal cells located in superficial cortical layers that have poly-synaptic connections with the corticospinal neurons of layer V (Asanuma and Rosén 1973, Ziemann et al. 1996). While the functional importance is ICF remains unclear, it is thought to be an important factor in regulating corticospinal output. Interestingly, Abbruzzese et al. (1999) demonstrated that ICF is abolished with voluntary activation. In addition, evidence shows that ICF is modulated by regular exercise, however, the findings are inconsistent. For example, Singh et al. (2014) demonstrated an increase in ICF after exercise in a population of moderately active individuals, whereas Lulic et al. (2017) indicated a decrease in ICF with exercise in groups of both high and low levels of physical activity levels.

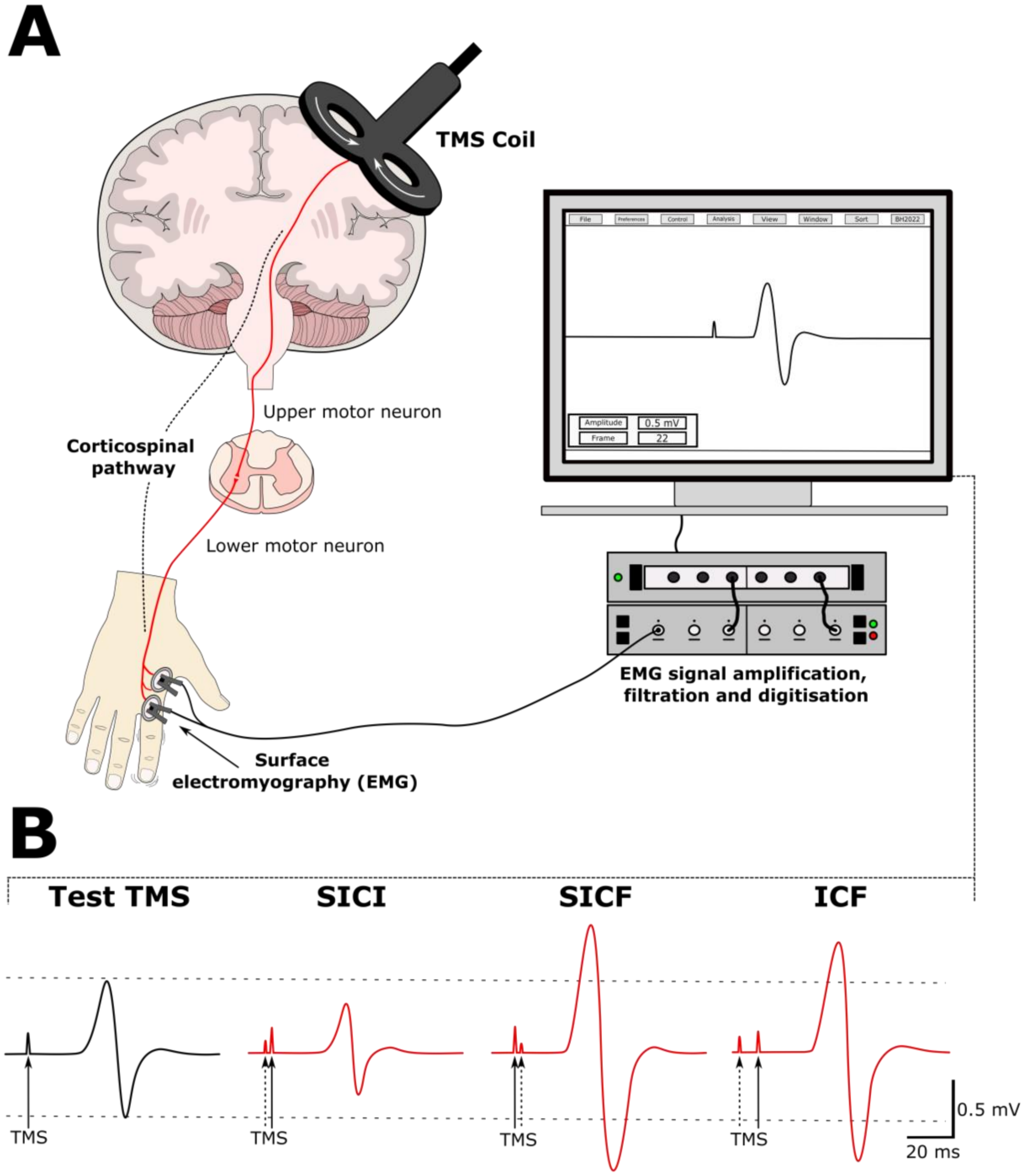

Figure 1.1: *TMS Schematic diagram. Plot A depicts TMS coil application over primary motor cortex (M1) thereby activating the corticospinal tract, including upper and lower motor neurons. A resultant motor evoked potential (MEP) is recorded via surface electromyography (EMG). The EMG signal is amplified, filtered and digitised for computer analysis. Plot B depicts example data of MEPs recorded from single-pulse test TMS, as well as paired-pulse TMS measures of short-interval intracortical inhibition and facilitation (SICI and SICF) as well as intracortical facilitation (ICF). Horizontal dashed lines represent the amplitude of the test MEP (unconditioned). Arrows illustrate the TMS stimuli; solid arrows depict test TMS stimulus and dashed arrows represent conditioning stimulus.*

1.2.4 Factors that influence TMS

1.2.4.1 TMS coil type

Early TMS research identified that one of the most influential factors for TMS measurements is the type of coil used (i.e., circular, figure-of-eight, double-cone). The effect of different coil types on the induced current has now been studied thoroughly, through theoretical calculations and stimulation modelling (Tofts 1990, Saypol et al. 1991, Esselle and Stuchly 1992, Ravazzani et al. 1996, Thielscher and Kammer 2004, Lontis et al. 2006, Salinas et al. 2007, Deng et al. 2013, Gomez-Tames et al. 2018) and in vivo models (Tay et al. 1991, Wagner et al. 2004). Variation between TMS coil types and the associated induced current has become the focus of a large literature in human participants (Rosler et al. 1989, Terao et al. 2000, Di Lazzaro et al. 2002, Hardwick et al. 2014, Fernandez et al. 2018). While the first TMS study used a circular coil for its simple geometry and construction (Barker et al. 1985), this type of coil was subsequently found to produce a non-focal electromagnetic field that activated a wide region of underlying cortical tissue. Although there have been developments to the circular coil (mainly modification of the coil windings) to increase its focality (Ruohonen et al. 1997, Hsiao and Weh-Hau Lin 2001), these have only resulted in marginal improvement (Deng et al. 2013). In contrast, the development of the figure-of-eight coil was a considerable step forward in coil design, extending the capability of TMS. A figure-of-eight coil design utilises two adjacent coil loops in which the current flow is in opposite directions, producing an electromagnetic field that is maximal underneath the intersection of the two loops, and more focal than can be achieved with previous circular designs (Ueno et al. 1988). It is understood that there is a depth-focality trade-off with regards to coil design, wherein coils with high focality have reduced stimulating depth (Deng et al. 2013). This phenomenon is exemplified by comparing the electromagnetic field generated by a figure-of-eight coil (which have high focality but low stimulating depth) to that generated by a double-cone coil (which has high stimulating depth, but low focality) (Lontis et al. 2006, Deng et al. 2013). There has now been a plethora of technical modifications in an attempt to produce coils with improved focality, however figure-of-eight coils still remain the most popular choice for TMS research As they provide a balance between depth and focality that is optimal for most TMS-based research (Deng et al. 2013).

1.2.4.2 TMS coil orientation and target muscle

It is now well understood that coil orientation, i.e., the direction of the TMS coil relative to the head position, is an important consideration for TMS application. Numerous studies have demonstrated that the specific orientation of the coil has a major influence on the composition of the descending volley, and therefore the characteristics (e.g., amplitude and latency) of the resultant MEP (Brasil-Neto et al. 1992, Mills et al. 1992, Werhahn et al. 1994, Terao et al. 2000, Richter et al. 2013, Laakso et al. 2014, Janssen et al. 2015, Gomez-Tames et al. 2018). It was initially suggested that the TMS coil orientation could be individualised for each participant, adjusting the coil position incrementally to determine which orientation produces the greatest average MEP amplitude (Richter et al. 2013). However, doing so is experimentally arduous and typically adds many total stimuli to experimental sessions. For these reasons, this process is regularly omitted from session protocols in favour of using standard orientations that have been determined as the most effective for a muscle of interest (Groppa et al. 2012, Richter et al. 2013). For example, it is now accepted that optimal coil orientation (producing the greatest MEP amplitudes at a given stimulator intensity) differs between the upper and lower limb muscles (Terao et al. 2000, Richter et al. 2013). Typically, it is much more difficult to activate lower limb muscles using TMS as the cortical representation for leg muscles is smaller than that of upper limb muscles and is located deep within the interhemispheric fissure (Allison et al. 1996, Terao et al. 2000). With a monophasic TMS pulse, a current that flows in an antero-medial direction within the cortex (commonly referred to as posterior-anterior – PA stimulation), is known to be most effective for upper limb measurements (Mills et al. 1992, Kammer et al. 2001), whereas for lower limb TMS, a medio-lateral induced current within the cortex (ML) is most effective (Terao et al. 2000, Smith et al. 2017). Fox et al. (2004) initially proposed that the induced field from a TMS stimulus is greatest when the induced currents flow perpendicularly to the cortical surface and that neurons are stimulated most effectively when their axons curve away from the direction of the induced current. However, recent evidence suggests that cortical activation may originate from sites other than deep within the sulci where the induced current is perpendicular to the cortical surface. Rather, neuronal activation appears to be related to the magnitude of the electric field, and originate close to the lip or crown of the gyrus. Furthermore, contrary to influential research which suggested that TMS activates cortical neurons at the soma or initial segment (Pashut et al. 2011, Seo et al. 2016), it was recently demonstrated that TMS preferentially activates axonal terminals in all cell types, with pyramidal cells from layers II, III and V activated at most TMS intensities (Aberra et al. 2020, Gomez-Tames et al. 2020).

Another important aspect of coil orientation is the influence of current direction on the composition of the elicited descending volley and the latency of the subsequent MEP. For upper limb muscles, the shortest latency response has been demonstrated with lateral-medial (LM) induced current which is thought to activate pyramidal tract neurons at the axonal level, resulting in preferential recruitment of D-waves (Di Lazzaro et al. 2012). In contrast, PA coil orientation has been shown to preferentially activate early I-waves (Hanajima et al. 2002) resulting in an MEP that occurs approximately 1.5 ms after that of LM stimulation, while an anterior-posterior (AP) directed current preferentially induces late (I2/I3) I-waves, resulting in an MEP that is delayed by approximately 4.5 ms relative to LM stimulation (Zoghi et al. 2003, Di Lazzaro et al. 2008). While it was initially suggested that different coil orientations activate inputs to descending corticospinal neurons from a common neuronal population, growing evidence suggests that modifying coil orientation may result in the activation of unique I-wave inputs (for review, see Opie and Semmler 2020). These findings are an important consideration for TMS studies, especially those that utilise paired-pulse protocols to examine intracortical networks, which are known to modify the activity of specific I-waves of the descending volley. For example, an AP orientation has been suggested to provide a more sensitive measure for SICI and LICI as it preferentially activates late I-wave circuits. However, it may be that it reflects activity in separate late I-wave circuits that have different properties (Nakamura et al. 1997, Sakai et al. 1997, Di Lazzaro et al. 1998, Hanajima et al. 1998, Zoghi et al. 2003, Di Lazzaro et al. 2012), with several studies of upper limb muscles showing greater inhibition using an AP coil orientation for measures of both SICI (Sale et al. 2015, Cirillo and Byblow 2016, Mooney et al. 2018, Wessel et al. 2019) and LICI (Cirillo and Byblow 2016). Evidence surrounding the influence of coil orientation on intracortical facilitatory circuits is varied. For upper limb measurements, an AP coil orientation has been shown to result in both increased (Delvendahl et al. 2014) and decreased (Cash et al. 2015, Opie et al. 2020) measures of SICF, relative to PA orientation. For measures of ICF, Wessel et al. (2019) demonstrated no difference between coil orientations. The influence of coil orientation for measures of intracortical excitability is not yet known for TMS measurements of the lower limb. This provides a limitation for TMS research looking to compare the activity of intracortical networks between upper and lower limb muscles.

1.2.4.3 Voluntary activation and TMS

Activation of the target muscle is another factor that has been shown to influence TMS responses. Relative to resting muscle, voluntary contraction increases the excitability of corticospinal and spinal motor neurons, resulting in facilitation of all I-waves of the descending volley and greater MEP amplitudes for a given stimulation intensity (Di Lazzaro et al. 1998). For TMS measures in the upper limb, an active muscle state has been shown to influence measures of intracortical inhibition (Ridding et al., 1995; Abbruzzese et al., 1999; Fisher et al., 2002) and facilitation (Ilić et al., 2002; Ortu et al., 2008). Given the difficulty with stimulating lower limb muscle with TMS, volitional contraction is often utilised to lower the threshold for activation (Roy 2009, Brownstein et al. 2018, Krishnan 2019). However, there is currently no evidence indicating whether volitional muscle contraction influences measures of intracortical inhibition and facilitation for lower limb muscles. This factor, together with the unknown influence of coil orientation discussed in section 1.2.4.2, highlight distinct gaps in the current understanding of TMS for lower limb measurement. These points, therefore, provide the objective for Chapter 2, as discussed further in section 1.5.

1.3 Cortical plasticity

Neuroplasticity can be defined as the ability of the nervous system to adapt in response to learning, experience or injury (Bachtiar and Stagg 2014). There are numerous mechanisms that are understood to underpin cortical plasticity. These include synaptic related processes involved with rapidly induced plasticity, including activity-dependent changes in synaptic strength (Baranyi and Fehér 1978, Sanes and Donoghue 2000), change in excitability of post-synaptic neurons (Woody et al. 1991) and the unmasking of pre-existing connections via decreased GABA transmission (Jacobs and Donoghue 1991). Alternatively, plasticity can occur through long-term structural adaptations of neural networks, including synaptogenesis and neurogenesis that underpin motor map reorganisation (Gould et al. 1999, Kleim et al. 2004).

1.3.1 Mechanisms of cortical plasticity

Synaptic plasticity involves a change in synaptic strength resulting in long-term potentiation (LTP) or long-term depression (LTD) of synaptic communication (Schinder and Poo 2000, Wang and Van Praag 2012). There are several mechanisms that have been shown to contribute to these activity-dependent changes in synaptic strength, and these mechanisms are specific to the direction of synaptic modulation (i.e., up- or down-regulation of synaptic strength). Synaptic plasticity in the form of LTP is dependent on the summation of EPSPs generated by repeated (high-frequency) synaptic inputs, or via associative stimulation that occurs through the precise timing of inputs from multiple pre-synaptic sources converging at the post-synaptic cell. These processes are thought the be dependent on an initial influx of sodium through glutamate-activated alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) channels, or calcium through voltage-gated calcium channels (Westenbroek et al. 1990, Kew and Kemp 2005). This resultant shift to a more positively charged intracellular environment will remove a magnesium block from N-methyl-D-aspartate (NMDA) receptors, which act to further transport calcium and sodium molecules into the cell (Collingridge and Bliss 1995). Strong shifts in postsynaptic calcium levels trigger a protein kinase pathway, which results in the translocation of additional AMPA receptors to the postsynaptic membrane, as well as phosphorylation of AMPA receptors in the postsynaptic cell membrane. These processes upregulate the potential of the postsynaptic cell for glutamatergic activation and depolarization with weaker inputs (Weisskopf and Nicoll 1995, Rioult-Pedotti et al. 2000, Ziemann et al. 2004). NMDA-receptor dependent plasticity is governed by GABAergic inhibition. Specifically, $GABA_A$-mediated inhibition is released with high-frequency synaptic transmission via $GABA_B$ autoreceptors, allowing for the necessary activation of NMDA receptors to facilitate LTP. In contrast to LTP, low-frequency activation of a synapse results in reduced calcium entry in the post-synaptic cell (compared with high-frequency stimulation). The low post-synaptic calcium entry actives a protein phosphatase cascade that is responsible for an LTD response (Bear and Malenka 1994). The intracellular cascade associated with LTD acts to reduce the opening potential of AMPA receptors through dephosphorylation (Isaac 2001), as well as the removal of these receptors from the membrane altogether (Malinow and Malenka 2002). This form of LTD is input-specific, meaning that only the directly stimulated synapses demonstrate the effect, termed homosynaptic LTD. Interestingly, when NMDA receptors are pharmacologically blocked, homosynaptic LTD is inhibited, indicating that calcium entry through NMDA receptors is critical for this form of LTD induction (Dudek and Bear 1992). While the majority of research has indicated that NMDA receptors have an important role in the induction of synaptic LTD and LTP, it is worth noting that there is also evidence for NMDA receptor-independent LTP (Johnston et al. 1992) and LTD (Bear and Malenka 1994).

With regards to the motor system, Hess and Donoghue (1994) demonstrated that non-invasive brain stimulation has a greater capacity to induce LTP-like changes in a rat motor cortex (M1) when GABA receptors were blocked with bicuculline. In humans, an increase in $GABA_A$-mediated inhibition through the receptor agonist lorazepam has been shown to depress plasticity in the motor cortex (Ziemann et al. 2001, Teo et al. 2009). Furthermore, a decrease in GABA-mediated inhibition through ischaemic nerve block enhances the LTP like modulation with rTMS (Ziemann et al. 1998). In addition to GABA-mediated inhibition, there are many endogenous factors known to influence the induction of synaptic plasticity. This includes the dopaminergic, cholinergic, serotonergic and adrenergic systems. A review by Ziemann et al. (2015) indicates that there is no clear dose-response relationship in many cases, and the overall modulation of synaptic plasticity involving these systems is dependent on many specific factors, such as receptors subtype. For example, with relevance to the adrenergic system, evidence suggests that activation of α1-receptors promote LTD (Marzo et al. 2010) whereas α2-receptor activation reduces LTD (DeBock et al. 2003).

Functional plasticity of pre-existing synapses through LTP-like mechanisms are thought to be an important component of early motor skill acquisition in humans (Butefisch et al. 2000, Muellbacher et al. 2002). However, neuroplasticity is not confined to the modulation of synaptic activity, and there is considerable evidence that structural changes to neuronal circuity are also an important component of long-term plasticity. Structural plasticity is often characterised into two primary forms; neurogenesis and synaptogenesis, which refer to the generation of new neurons and synapses, respectively. While it was initially thought that these processes do not occur in the mammalian brain after early development, more recent evidence now suggests the contrary. Neurogenesis has been demonstrated in the hippocampus of rodents in response to associative learning tasks (Gould et al. 1999), while synaptogenesis within the cerebellum and motor cortex has been shown following motor skill learning paradigms (Black et al. 1990, Kleim et al. 1996). In the rat motor cortex, evidence suggests that synaptogenesis precedes motor map reorganisation during the late phases of motor learning (Kleim et al. 2004). These findings indicate that use-dependent plasticity within M1 (plasticity resulting from a history of repeated activations) may involve a co-occurrence of functional and structural plasticity. In humans, TMS research has demonstrated altered structural and functional plasticity in highly trained pianists, who display modified cortical representations (motor maps) of trained muscles, and differential interhemispheric inhibition compared with naïve participants (Chieffo et al. 2016). Furthermore, work utilising functional magnetic resonance imaging (fMRI) has revealed that trained pianists also display greater utilisation of prefrontal, sensorimotor and parietal regions during learning of a serial reaction time task (Landau and D'Esposito 2006). Use-dependent plasticity will be discussed further in section 1.3.3.

1.3.2 Assessing plasticity with TMS

Neuroplasticity can involve a change in excitability within the cortex when assessed with TMS. Accordingly, measures that are sensitive to corticospinal excitability (including single-pulse TMS) can therefore be used to index plasticity induction. Consequently, a change in MEP amplitude with TMS (typically measured as the pre-post difference in amplitude following an intervention) is a non-invasive way to indirectly assess the induction of corticospinal plasticity (Pascual-Leone et al. 1999). Paired-pulse TMS provides an avenue to assess the plasticity of intracortical inhibitory and facilitatory circuits, the activities of which are known to be functionally independent. For example, TMS has been used to monitor modulation of SICI and SICF during motor skill learning tasks (Cirillo et al. 2011, Clark et al. 2011) as well as following acute exercise (Singh et al. 2014, Mooney et al. 2016, Neva et al. 2017).

Despite the useful capability of TMS for the assessment of corticospinal and intracortical excitability, TMS has some limitations for the interpretation of cortical plasticity. For example, the neural elements activated by TMS are likely to differ from those activated by voluntary activation (Bestmann and Krakauer 2015). Furthermore, MEP changes may be due, at least in part, to adaptations at the spinal level (Bestmann and Krakauer 2015). However, measures of spinal excitability (cervicomedullary motor evoked potentials, CMEPs; Hoffman-, H-reflex) can be conducted alongside TMS measures of corticospinal excitability to more accurately identify the site of plasticity induction (i.e., spinal and/or cortical) (Kujirai et al. 1993, Chen et al. 1998, McNeil et al. 2009, Sidhu et al. 2012). Furthermore, spinal contributions to the MEP can be minimized by optimized TMS methodology, particularly with paired-pulse measures. For example, for assessment of LICI, utilising an ISI of greater than 100 ms is thought to remove any spinal contribution to the observed inhibition (Nakamura et al. 1997, Di Lazzaro et al. 2002). Finally, TMS-evoked MEPs generally exhibited considerable trial-to-trial variability (Kiers et al. 1993, Cuypers et al. 2014). MEP variability likely represents continual fluctuations in neurophysiological processes including ongoing oscillatory rhythms (Sauseng et al. 2009), attention and arousal (Mars et al. 2007), and pre-stimulus muscle activation (Darling et al.

2006). Work by Goldsworthy et al. (2016) and Cuypers et al. (2014) has seen to optimise TMS parameters in an attempt to mitigate the high variability associated with TMS-evoked MEPs. Findings indicate that 20-30 MEPs are required for a reliable measure of MEP amplitude, with more than 30 trials per block providing no additional benefit (Goldsworthy et al. 2016). Despite some limitations, assessment of MEP amplitude with TMS remains a popular method of non-invasively assessing neuroplastic changes in the motor cortex, and has provided vast information that has shaped our understanding of motor control today.

1.3.2.1 Experimentally inducing plasticity

Although single and paired-pulse TMS has been used to assess corticospinal and intracortical excitability, it has been shown that trains of stimuli can be used to induce a change in corticospinal excitability (for review, see Hallett 2007). These changes outlast the period of stimulation, have been shown to be reversible, are dependent on GABA-mediated inhibition and NMDA receptor activation (i.e. the characteristics of plasticity) (Huang et al. 2007, Stagg et al. 2009). Consequently, they have been suggested to reflect the induction of LTP/D like effects, indicating that TMS can be additionally used to induce short-term neuroplastic changes experimentally (for review, see Cárdenas-Morales et al. 2010).

Various plasticity-inducing repetitive TMS (rTMS) protocols have now been developed and can have either an up- or down-regulatory effect on corticospinal excitability. The direction of modulation seen with rTMS depends on the characteristics (frequency, duration, and intensity) of the protocol itself. Continuous trains of stimulation at low frequencies (1 Hz) have been shown to depress corticospinal excitability (Chen et al. 1997, Muellbacher et al. 2000), whereas high-frequency continuous stimulation ($\geq$ 5 Hz) facilitates corticospinal excitability (Berardelli et al. 1998, Pascual-Leone et al. 1998). While high-and low-frequency rTMS protocols have since been utilised and demonstrated in numerous studies, evidence suggests that the effect of these protocols also depends on the duration and intensity of stimulation. For example, high-frequency continuous stimulation can have a depressive effect on corticospinal excitability if is it applied for short durations (<13 TMS stimuli) (Modugno et al. 2001) or at low intensities (Todd et al. 2006). Pharmacological evidence suggests that synaptic LTP and LTD may underpin the effects seen with rTMS. Fitzgerald et al. (2005) demonstrated that the effect of low-frequency rTMS was abolished when participants were administered with either dextromethorphan, an NMDA receptor antagonist, or lorazepam, a GABA agonist. These findings suggest that plasticity induction with rTMS is dependent on both GABA and NMDA receptor systems. While rTMS can modulate corticospinal excitability through either LTP- or LTD-like mechanisms, there is considerable variability with regards to its influence on both inhibitory and excitatory intracortical circuits (Fitzgerald et al. 2006).

Since the introduction of rTMS, numerous other plasticity-inducing TMS protocols have been developed. Such techniques are based on naturally occurring physiological processes. For example, patterned stimulation protocols such as theta burst stimulation (TBS), which was intended to replicate theta (5 Hz)-gamma (50 Hz) coupling apparent in the animal hippocampus (Lisman and Buzsáki 2008). Specifically, TBS involves bursts of three magnetic pulses applied at 50 Hz, with intervals of 200 ms. TBS can either up-regulate (via continuous TBS, cTBS) or downregulate (via intermittent TBS, iTBS) corticospinal excitability (Huang et al. 2005). Despite the widespread use of TBS protocols, they do involve risks that need to be considered in the experimental design. For example, a small but important risk for high-frequency rTMS and TBS is the induction of seizures due to significant and widespread upregulation of corticospinal excitability (Thickbroom et al. 2006, Rossi et al. 2009). Fortuitously, the risk of seizure with TBS remains very low, approximately 0.02% (Oberman et al. 2011). Other side effects include syncope and vasovagal reactions. Despite these observed side-effects, the risks associated with rTMS protocols can be limited with suitable screening and appropriate methodology (Rossini et al. 1994, Grossheinrich et al. 2009, Rossi et al. 2009, Oberman et al. 2011).

Another theory of neural modulation from which plasticity-inducing TMS paradigms have been developed is that of associative, or *Hebbian* plasticity. Stefan and colleagues initially demonstrated modulation of motor cortical networks in an input specific, spike-timing-dependent manner with a TMS technique termed paired associative stimulation (PAS) (Stefan et al. 2000). This technique involves the repeated coupling of afferent signals generated by percutaneous electrical stimulation of peripheral nerves with corticospinal activation generated by TMS, with high temporal resolution (Stefan et al. 2000, Suppa et al. 2017). The resultant change in corticospinal excitability from PAS is thought to reflect changes at the cortical level and is highly dependent on the specific timing between the afferent (peripheral stimulation) and central (TMS) signals (Müller-Dahlhaus et al. 2010). PAS application with an ISI of 20 – 25 ms (PAS_{LTP}) results in facilitation of the MEP, whereas an ISI of 10 ms (PAS_{LTD}) reduces MEP amplitude (Wolters et al. 2003).

In addition to PAS, an alternative spike-timing-dependent TMS intervention known as I-wave repetitive TMS (iTMS) has been developed (Thickbroom et al. 2006). This intervention involves repeated paired stimulation at or above threshold intensity, applied with an ISI based on the known I-wave intervals (most commonly 1.5 ms) at a frequency of 0.2 Hz (Kidgell et al. 2016). These pairs of stimuli are indistinguishable from those utilised for paired-pulse measures of SICF, as discussed in section 1.2.3.2, however are administered repeatedly for an extended duration. The seminal study by Thickbroom and colleagues utilised an iTMS protocol that was 30 minutes in duration, which was effective in producing large increases (~500%) in MEP amplitude (Thickbroom et al. 2006). The authors postulated that the increase in excitability was a result of increased synaptic efficacy within I-wave generating circuits. While the exact mechanisms of iTMS are still unclear, it is generally accepted that iTMS modulates synaptic transmission through spike-timing-dependent (Hebbian) plasticity (Kidgell et al. 2016). Since their study, numerous other studies have demonstrated a positive effect of iTMS with durations as short as 10-15 minutes (Murray et al. 2011, Sewerin et al. 2011, Cash et al. 2013). While iTMS displays a level of variability that is common among TMS measures (Kiers et al. 1993, Cuypers et al. 2014), this plasticity-inducing TMS intervention has shown relatively promising outcomes with regards to facilitating excitability, with the majority of studies showing some degree of MEP facilitation after the iTMS intervention (Kidgell et al. 2016). Given the encouraging outcomes of recent iTMS research, this technique will be used in Chapter 4 to examine how regular exercise influences plasticity following an acute bout of exercise.

1.3.3 Motor learning and use-dependent plasticity

Motor learning is the process of improving the spatial and temporal accuracy of planned movements following practice (Willingham 1998). It has long been understood that skill acquisition involves mechanisms of cortical plasticity (Butefisch et al. 2000, Muellbacher et al. 2001, Ziemann et al. 2004). Early motor skill training studies in animals indicate that skill training is associated with both structural and functional changes within the cortex (Kleim et al. 1996, Kleim et al. 1998, Adkins et al. 2006). More accurately, initial phases of motor skill learning likely involve mechanisms of synaptic LTP (Rioult-Pedotti et al. 1998) or the unmasking of synaptic connections by reducing GABAergic intracortical inhibition (Jacobs and Donoghue 1991), while later stages of learning are likely mediated by synaptogenesis (Kleim et al. 2004).

1.3.3.1 Mechanisms of use-dependent plasticity in humans

Since early animal research, studies using TMS and various neuroimaging techniques have shown that humans share a similar response to skill training. Early TMS work demonstrated that skill acquisition in humans was associated with both structural and functional forms of plasticity. For example, enlargement of the cortical representation of trained muscles within the motor homunculus, as well as an increase in the excitability of the circuits within these cortical representations (Pascual-Leone et al. 1994, Pascual-Leone et al. 1995). Furthermore, human research has identified that modulation of outputs to the task-relevant muscles might result from either an increase in synaptic efficacy of pre-existing circuits or the unmasking of synaptic connections through disinhibition (Pascual-Leone et al. 1995). More recent research has expanded on these earlier findings to suggest that LTP- or LTD-like synaptic modulation is likely the driving mechanism behind the initial phases of motor learning (Rioult-Pedotti et al. 2000, Muellbacher et al. 2002, Ziemann et al. 2004). These findings were corroborated by Rosenkranz et al. (2007), who demonstrated that initial phases of motor practice are associated with mechanisms of synaptic plasticity, however, this was not the case with sustained motor practice over multiple days. They suggested that motor cortical reorganisation through synaptogenesis occurs with sustained motor practice, leading to enhanced corticospinal and intracortical recruitment. There is also significant evidence for modulation of intracortical excitability, such as a decrease in GABA-mediated inhibition (particularly GABA_A mediated SICI) during motor skill acquisition (Werhahn et al. 1999, Ziemann et al. 2001, Rosenkranz et al. 2007, Coxon et al. 2014, Berghuis et al. 2017). A use-dependent change in excitability (plasticity induction) within M1 is likely to result from a combination of mechanisms and is thought to be associated with improvements in motor function (see Sanes and Donoghue 2000, for review).

Based on the understanding that motor cortex plasticity is involved with skill acquisition, many TMS studies have since aimed to modulate use-dependent (skill training-induced) plasticity and motor skill training outcomes (acquisition and retention), with plasticity inducing TMS-paradigms. For example, work by Jung and Ziemann (2009) used PAS as a priming technique (applied prior to motor learning) to modify the response to a subsequent bout of learning using a thumb acceleration task. This study demonstrated that a PAS protocol known to induce LTD-like modulation of excitability enhanced motor skill acquisition (through homeostatic interactions across motor learning). Furthermore, when low-frequency rTMS is applied immediately following motor practice, motor skill retention is hindered compared with control groups (Muellbacher et al. 2002, Kantak et al. 2010). An increase in corticospinal excitability with motor training has now been demonstrated for training tasks involving both upper (Pascual-Leone et al. 1995, Garry et al. 2004, Jensen et al. 2005, Rogasch et al. 2009) and lower (Perez et al. 2004) limb muscles. Despite these examples, which demonstrate facilitation of corticospinal excitability with motor learning, there are numerous studies that fail to show such clear relationships, or present associations that are seemingly counterintuitive. For example, McDonnell and Ridding (2006) demonstrated a short term (i.e., < 10 minutes) decrease in corticospinal excitability following a sensorimotor training task, with no change in intracortical inhibition or facilitation. Furthermore, in contrast to previous work (Garry et al. 2004), McDonnell and colleagues failed to demonstrate any correlation between modulation of excitability and improvement of the learning task. This research suggests that the type of learning task and parameters of testing are important considerations for studies of use-dependent (training-induced) plasticity (see section 1.3.3.2).

1.3.3.2 Factors influencing use-dependent plasticity

There is now significant evidence that the association between use-dependent plasticity and motor skill acquisition is highly dependent on numerous factors, such as the type and duration of training conducted. For example, an increase in corticospinal excitability is common with visuomotor, but not ballistic training tasks in both young and older cohorts (Berghuis et al. 2017). That particular meta-analysis and review also revealed an effect of age for some measures of use-dependent plasticity, such as a decrease in SICI with visuomotor training in the older but not young cohort. The duration of the learning task may also influence use-dependent plasticity. For example, McDonnell and Ridding (2006) demonstrated downregulation of corticospinal excitability with a pegboard task, whereas Garry et al. (2004) demonstrated facilitation of the MEP with a similar pegboard task. In this case, task duration was the only clear differential factor (15 minutes vs 90 seconds total training time). Together, these findings demonstrate that a complex interaction of factors governs use-dependent plasticity and learning outcomes with motor training. Accordingly, it has recently been proposed that neuroplasticity may not be a necessary requirement of skill acquisition as was initially suggested, but instead a neurophysiological response to motor practice (Bestmann and Krakauer 2015, Berghuis et al. 2017).

1.3.4 Exercise and plasticity

There is now considerable evidence that exercise provides a myriad of advantages for brain function, including improvements in memory, cognition, mental health, and motor learning (Cotman and Berchtold 2002, Colcombe et al. 2004, Hillman et al. 2008, Deslandes et al. 2009, Baker et al. 2010, El-Sayes et al. 2019). Furthermore, exercise has been shown to slow the progression of various age-related deficiencies, such as the progressive decline in grey matter and cognitive function (Baker et al. 2010, Erickson et al. 2010), neurological diseases including dementia and mild cognitive impairment (Ahlskog et al. 2011), Alzheimer’s disease (Cass 2017, Meng et al. 2020), Parkinson’s disease (Oliveira de Carvalho et al. 2018, Steib et al. 2018) and stroke (Han et al. 2017, Nepveu et al. 2017). There are numerous mechanisms that may be responsible for the many benefits of exercise on brain function, including a combination of structural adaptations (e.g., angiogenesis, neurogenesis) as well as changes in synaptic efficacy involved with plasticity (Cotman and Berchtold 2002). The neurophysiological and functional consequences of physical activity differ between types of exerise completed. For example, a review by Adkins et al. (2006) summarises that strength training involves increased excitability of spinal motor neurons as well as synaptogenesis in the spinal cord, whereas endurance (aerobic based) exercise facilitates cortical modifications such as angiogenesis, which promotes cerebral blood flow and neurochemical delivery that promotes supraspinal plasticity. This thesis will focus on the effects of aerobic-based training.

The majority of the evidence surrounding structural adaptations from exercise stems from animal studies. Such research has demonstrated an exercise-induced upregulation of vascular endothelial growth factor (VEGF) supporting angiogenesis and vascular function, which facilitates higher cerebral blood flow and metabolic capacity (McCloskey et al. 2001, Adkins et al. 2006, Lou et al. 2008). Furthermore, aerobic exercise increases gene expression for cell proliferation and neurogenesis in the hippocampus of rodents, which is associated with improved memory and learning (van Praag et al. 1999, Christie et al. 2008, Wang and Van Praag 2012). Comparatively, structural adaptations in humans can be challenging to measure. Nonetheless, human work has shown aerobic exercise-induced increases in grey matter thickness (Erickson et al. 2010), vascular structure (Bullitt et al. 2009) and cerebral blood volume (Pereira et al. 2007). In addition to structural adaptations, there is evidence for numerous exercise-mediated synaptic changes. For example, aerobic activity has been shown to promote gene expression for brain-derived neurotrophic factor (BDNF), and upregulates N-methyl-D-aspartate (NMDA) receptor expression (Cotman and Berchtold 2002, Lou et al. 2008, Knaepen et al. 2010, Mang et al. 2014). Furthermore, exercise stimulates a critical increase in lactate concentration (Robergs et al. 2004), which is known to be a signalling molecule for neural growth factors such as BDNF, and provides an energy source for the brain (Taubert et al. 2015). These and numerous other factors are thought to act harmoniously to enhance synaptic efficacy and promote LTP (Kang and Schuman 1995, Patterson et al. 1996, Cotman and Berchtold 2002, Gottmann et al. 2009).

Exercise-mediated synaptic plasticity is also thought to contribute to altered behavioural outcomes. For example, animal research has demonstrated that changes in synaptic efficacy following exercise were correlated with improved familiar object recognition (Novkovic et al. 2015). In humans, Ostadan et al. (2016) demonstrated that high-intensity aerobic exercise facilitated corticospinal excitability during motor skill consolidation, which correlated with the level of improvement in procedural memory (see section 1.4). Furthermore, exercise-mediated changes in synaptic efficacy and the resultant functional benefits appear to differ between acute and regular forms of exercise. For example, there is considerable evidence to suggest that acute exercise downregulates intracortical inhibition, which is associated with motor skill consolidation (Stavrinos and Coxon 2017), whereas this is not the case with regular exercise (see section 1.3.4.1-2).

1.3.4.1 Influence of acute aerobic exercise on motor cortex excitability and plasticity

The last decade has seen a considerable number of studies investigate how acute exercise influences corticospinal and intracortical excitability, which have produced mixed results. Numerous studies now indicate that acute exercise does not modulate corticospinal excitability directly (McDonnell et al. 2013, Singh et al. 2014, Mooney et al. 2016, Smith et al. 2018). However, recent evidence suggests that experimental and other factors may influence this outcome. For example, Lulic et al. (2017) demonstrated that the exercise history of the participant cohort can be a determining factor, with greater corticospinal excitability following acute exercise in a high but not low physical activity group. Characteristics of the exercise bout, such as exercise intensity, also appear to be an important consideration. For example, while an increase in corticospinal excitability was apparent following moderate-intensity exercise, there was no change after rest or low-intensity exercise, and a decrease following high-intensity continuous exercise (Baltar et al. 2018). Certainly, a more consistent finding is that of reduced GABAergic inhibition following acute exercise. In particular, a reduction of SICI has been reported after both continuous (Yamaguchi et al. 2012, Smith et al. 2014) and interval (Stavrinos and Coxon 2017, Andrews et al. 2020) exercise, and is evident in both high and low-physical activity groups (Lulic et al. 2017). Although, other studies have shown no change in SICI following both continuous (Mooney et al. 2016) and interval (Nicolini et al. 2020) exercise. A recent study by Neva et al. (2021) showed an exercise-induced reduction of SICI, but only when TMS was applied with an anterior-posterior (AP) coil orientation, and not when a conventional PA-induced current was used. These findings suggest that an optimal approach for assessing SICI may be to utilise an AP coil orientation (which preferentially activates late I-waves that are modulated with SICI).

Perhaps a more exciting consequence of exercise is the promotion of neuroplasticity, particularly with exercise that is aerobic in nature. Numerous studies have now demonstrated that a single bout of acute exercise promotes neuroplasticity in response to various plasticity-inducing TMS (for review, see Mellow et al. 2020) and use-dependent (Ostadan et al. 2016, Stavrinos and Coxon 2017) interventions. However, the specific characteristics of exercise modality and intensity appear to be particularly important. For example, McDonnell et al. (2013) demonstrated that for continuous type exercise, a low exercise intensity promotes neuroplasticity with continuous TBS (cTBS), whereas rest and moderate-intensity activity conditions were ineffective for modulating plasticity. Furthermore, interval-based exercise paradigms may also be effective for promoting neuroplasticity, particularly high-intensity interval training (HIIT). In such paradigms, high-intensity work intervals (typically less than 4 minutes in duration at 80% of maximal or greater intensity) are interspersed with similar length recovery blocks that are approximately 50% of maximal intensity (Roy 2013). Acute HIIT exercise has been shown to upregulate corticospinal plasticity induced PAS_{LTP} (Mang et al. 2014). More recently, Andrews et al. (2020) demonstrated greater TMS-induced plasticity when intermittent TBS (iTBS) was preceded by acute HIIT exercise, compared with both a no-exercise control (rest) and moderate-intensity continuous exercise. A recent review by Mellow et al. (2020)

concluded that either longer bouts of low-intensity continuous exercise, or shorter bouts of HIIT, are most effective for promoting plasticity of the motor cortex.

1.3.4.2 Influence of regular aerobic exercise on excitability and plasticity

There is considerably less evidence surrounding the influence of regular physical exercise on cortical excitability and neuroplastic capacity, but the evidence that does exist is inconsistent. For example, while several studies have reported that corticospinal excitability is increased in physically active individuals (Cirillo et al. 2009, Dai et al. 2016), others have instead indicated a decrease (Hassanlouei et al. 2017, Rozand et al. 2019), or no change (Kumpulainen et al. 2015, Lulic et al. 2017). Interestingly, Dai et al. (2016) demonstrated that heightened corticospinal excitability in highly trained athletes coincided with increased intracortical inhibition (both SICI and LICI). They suggested that the increase in corticospinal excitability likely results from heightened synaptic efficacy through upregulation of glutamatergic transmission resulting in greater excitatory input to descending corticospinal neurons. However, no measures were taken to assess intracortical facilitation. They concluded that both cortical excitation and inhibition appear to increase in athletes and the balance between these factors is maintained during long-term training (Dai et al. 2016). As with measures of corticospinal excitability, there is currently limited evidence surrounding long-term exercise and plasticity. Cirillo et al. (2009) demonstrated that the response to PAS was significantly increased in non-exercised hand muscles of physically active participants (relative to sedentary controls), whereas Kumpulainen et al. (2015) demonstrated no effect of endurance training on plasticity in a task-relevant lower limb muscle. Despite these studies, many of the details about use-dependent and TMS-induced plasticity of exercised and non-exercised muscles remain unclear. Accordingly, Chapters 3 and 4 of this thesis aim to uncover further characteristics about how both use-dependent and TMS-induced plasticity are modulated with long-term endurance training.

One important consideration of the current literature regarding exercise and plasticity is that in nearly all cases, TMS is applied over the motor cortical representation for a hand muscle. This raises a potential confound as the exercise modalities (both acute and regular) are generally lower limb focussed (e.g., running and cycling). It is therefore possible that plasticity may be modulated differentially between exercised and non-exercised muscle groups. This possibility represents part of the motivation behind Chapter 3, where use-dependent plasticity and motor skill acquisition is assessed in both upper and lower limbs of endurance-trained and sedentary participants. Furthermore, it remains unclear if a history of endurance exercise, such as regular cycling, influences the plasticity response to acute exercise. This question is addressed in Chapter 4.

1.4 Influence of exercise on motor skill acquisition and retention

There is now considerable evidence that exercise not only influences plasticity within the motor cortex, but also has beneficial effects for motor skill learning in humans. For example, Statton et al. (2015) demonstrated that an acute bout of moderate-intensity exercise immediately preceding a motor learning task improves motor skill acquisition in both a single session and across multiple sessions over consecutive days. Furthermore, Roig et al. (2012) demonstrated that an acute bout of intense aerobic activity can improve retention of newly acquired motor skills. In addition, Mang and colleagues conducted a study that aimed to bridge the concepts of acute exercise mediated plasticity and motor skill acquisition. They demonstrated that an acute bout of HIIT cycling (utilised as a priming tool) improved the response to a plasticity-inducing TMS intervention (PAS) and increased skill acquisition and retention of a continuous tracking task (Mang et al. 2014). In response to these promising outcomes, Mang and colleagues suggested that aerobic exercise might be an effective priming tool within clinical rehabilitation settings, such as for promoting recovery of function in stroke patients. Ostadan et al. (2016) subsequently established that high-intensity aerobic exercise completed immediately following motor skill training facilitated corticospinal excitability during skill consolidation, which predicted the level of improvement in procedural memory. Furthermore, Stavrinos and Coxon (2017) demonstrated that acute HIIT cycling completed before skill training improved offline consolidation of a novel motor skill. Finally, work by Thomas and colleagues also revealed improved motor memory when skill training is followed by acute exercise, with the greatest outcomes present with high-intensity exercise (Thomas et al. 2016) that was completed immediately following motor training (Thomas et al. 2016). Despite these encouraging findings, not all research has demonstrated positive outcomes. One recent study aimed to investigate how aerobic fitness level influences motor skill retention when an acute bout of exercise is completed immediately following motor training. In contrast to earlier work (Roig et al. 2012), the authors reported that skill retention was not improved by exercise, and was not related to fitness level (Hung et al. 2021).

Compared with acute exercise, little is known about the influence of regular exercise on motor learning. Despite this, the evidence that does exist suggests that various forms of regular exercise may have some benefit for the promotion of motor skill acquisition. For example, Bakken et al. (2001) demonstrated that regular exercise administered through an 8-week aerobic exercise intervention could promote skill acquisition in a finger-tracking task. Interestingly, Seidel and colleagues provided evidence that endurance athletes show superior task performance in a static balance task compared with non-athletes; however, skill acquisition in a multimodal balance task was not different between groups (Seidel et al. 2017). Despite these findings, it remains unclear how regular exercise influences visuomotor skill acquisition, and whether there is a difference in motor learning between exercised and non-exercised muscles. Chapter 3 therefore aims to examine how skill acquisition differs between exercised (lower) and non-exercised (upper) muscle groups of endurance-trained cyclists and sedentary individuals.

1.5 Summary and aims

It is now understood that both acute and regular forms of exercise have the potential to modulate motor cortex plasticity in humans. Furthermore, plasticity is thought to be an essential factor for motor skill acquisition. Despite these developments, it is currently unknown how long-term (chronic) exercise influences M1 plasticity and motor skill acquisition, or whether long-term exercise alters the response to a single exercise session. Therefore, the overarching aim of this thesis is to investigate how M1 plasticity and skill acquisition are modified in endurance-trained cyclists, who have a history of long-term exercise.

Given that cycling exercise involves activity of lower limb muscles, it was necessary to examine M1 plasticity in cortical representations for the lower limb, and compare these with the untrained upper limb. However, different TMS techniques are often used to assess lower limb muscles, making comparisons between upper and lower limbs difficult. To address this, Chapter 2 compared the TMS responses in upper and lower limb muscles of the same participants using similar TMS parameters (TMS coil shape, coil orientation, and muscle activation state). This approach will demonstrate the optimal TMS parameters for upper and lower limb muscles, and will allow future studies (including Chapter 3) to appropriately compare the neurophysiological mechanisms associated with motor control of upper and lower limb muscle groups in the same participants.

Investigations utilising TMS have shown that regular exercise has the potential to modulate motor cortex plasticity, and that this is an important substrate of motor skill acquisition (see sections 1.3.3-4). However, it remains unclear how regular endurance exercise influences skill training-induced plasticity and skill acquisition, and whether these outcomes differ between exercised and non-exercised muscle groups. To address these issues, Chapter 3 examines skill training-induced M1 plasticity and visuomotor skill acquisition in endurance-trained (cyclists) in trained (lower limb) and untrained (upper limb) muscles. These outcomes will indicate whether the modulation of plasticity with regular exercise has measurable functional benefits (visuomotor skill acquisition) for both exercised and non-exercised muscle groups.

Evidence suggests that acute and long-term exercise may influence cortical plasticity through separate, although partially overlapping, physiological mechanisms (see section 1.3.4). The possibility, therefore, exists that participation in regular endurance exercise could enhance the effect of acute exercise for facilitating plasticity. However, it remains unknown whether long-term exercise influences M1 plasticity following an acute exercise session. Accordingly, Chapter 4 aims to determine whether regular endurance training (cycling) modifies TMS-induced plasticity of a non-exercised upper limb muscle following a bout of acute aerobic exercise involving the lower limbs. The outcomes of this study will determine whether long-term exercise offers beneficial effects for inducing short-term plasticity in non-exercised muscles.

Chapter II

TMS coil orientation and muscle activation influence lower limb intracortical excitability

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Signature		Date	16/11/2021

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate’s stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate to include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate’s stated contribution.

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2. TMS coil orientation and muscle activation influence lower limb intracortical excitability

2.1 Abstract

Introduction: Previous research with transcranial magnetic stimulation (TMS) indicates that coil orientation (TMS current direction) and muscle activation state (rest or active) modify corticospinal and intracortical excitability of upper limb muscles. However, the extent to which these factors influence corticospinal and intracortical excitability of lower limb muscles is unknown. This study aimed to examine how variations in coil orientation and muscle activation affect corticospinal and intracortical excitability of tibialis anterior (TA), a lower leg muscle.

Methods: In 21 young (21.6 ± 3.3 years, 11 female) adults, TMS was administered to the motor cortical representation of TA in posterior-anterior (PA) and mediolateral (ML) orientations at rest and during muscle activation. Single-pulse TMS measures of motor evoked potential amplitude, in addition to resting and active motor thresholds, were used to index corticospinal excitability, whereas paired-pulse TMS measures of short-interval intracortical inhibition (SICI) and facilitation (SICF), and long-interval intracortical inhibition (LICI), were used to assess excitability of intracortical circuits.

Results: For single-pulse TMS, motor thresholds and test TMS intensity were lower for ML stimulation (all $P < 0.05$). In a resting muscle, ML TMS produced greater SICI ($P < 0.001$) and less SICF (both $P < 0.05$) when compared with PA TMS. In contrast, ML TMS in an active muscle resulted in reduced SICI but increased SICF (both $P \leq 0.001$) when compared with PA TMS.

Conclusion: TMS coil orientation and muscle activation influence measurements of intracortical excitability recorded in the tibialis anterior, and are therefore important considerations in TMS studies of lower limb muscles.

2.2 Introduction

Transcranial magnetic stimulation (TMS) is a method of non-invasive brain stimulation that can be used to assess corticospinal and intracortical excitability, providing robust measures of excitatory and inhibitory activity within primary motor cortex (M1). TMS over M1 generates a complex descending volley in corticospinal neurons that consists of direct (D) waves, representing direct activation close to the intial axonal segment, and indirect (I) waves that result from trans-synaptic input from intracortical circuits. The D and I-waves of a descending volley summate at the spinal cord to produce a motor evoked potential (MEP) in the target muscle (Ziemann and Rothwell 2000, Ortu et al. 2008, Di Lazzaro et al. 2012). In addition, paired-pulse TMS is used to quantify intracortical excitability, which reflects the activity of complex inhibitory (GABAergic) and facilitatory (glutamatergic) networks in M1 (Kujirai et al. 1993, Di Lazzaro et al. 1998, Petroff 2002, Cash et al. 2017). Numerous factors related to the TMS approach that influence measures of corticospinal and intracortical excitability within M1 (e.g. coil type, orientation and TMS intensity) have been identified, but these have largely been established in upper limb muscles. In contrast, much less is known about how these methodological factors influence TMS measures of corticospinal and intracortical excitability in lower limb muscles.

Given their importance in posture, maintenance of balance and locomotion (Joseph 1985, Kleim and Jones 2008, Mille et al. 2014) there has been growing interest in assessing M1 characteristics for the neural control of lower limb muscles (Yamaguchi et al. 2012, Hirano et al. 2015, Ward et al. 2016, Tatemoto et al. 2019). However, it is more challenging to activate lower limb muscles with TMS (compared with upper limb muscles) due to a smaller cortical representation that lies deep within the interhemispheric fissure (Allison et al. 1996, Terao et al. 2000). Because of this, previous studies investigating lower limb muscles have used different TMS approaches which lack comparability with data obtained in upper limb muscles. For example, TMS studies on the lower limb have typically used double-cone coils, which can have a greater penetrating depth in the cortex (Deng et al. 2008) and are associated with greater discomfort than more commonly used circular or figure-of-eight coils (Deng et al. 2013, Panyakaew et al. 2016, Fernandez et al. 2018). Furthermore, the unique shape of the double-cone coil makes it challenging to use in orientations other than posterior-to-anterior (PA) or anterior-to-posterior direction (AP) (Deng et al. 2013, Fernandez et al. 2018). This provides a methodological limitation, given the recent evidence that mediolaterally directed (ML) induced current may be a more effective approach for targeting lower limb muscles (Terao et al. 2000, Smith et al. 2017, Kesar et al. 2018). However, the specific direction of TMS stimulation can influence the composition of the corticospinal descending volley, which may affect measurements of intracortical activity (Di Lazzaro et al. 2012, Di Lazzaro and Rothwell 2014, Opie et al. 2020). Accordingly, the effect of coil orientation on intracortical excitability for control of lower limb muscles is unknown, and is a major focus of the current study.

Another factor that influences measures of corticospinal and intracortical excitability is muscle activation. In general, voluntary contraction increases the excitability of corticospinal and spinal motor neurons to generate a facilitation in the motor evoked potential (MEP) of the target muscle. This decreases the threshold for TMS to generate a response in the muscle, which is why studies in the lower limb usually involve an active muscle (Roy 2009, Brownstein et al. 2018, Krishnan 2019). However, muscle activation in the upper limb has been shown to influence characteristics of the TMS-evoked descending volley by increasing the size of all I-waves (Di Lazzaro et al. 1998), which can influence measures of intracortical inhibition (Ridding et al. 1995, Abbruzzese et al. 1999, Fisher et al. 2002) and facilitation (Ilić et al. 2002, Ortu et al. 2008). It is currently unknown how muscle activation influences specific features of intracortical excitability in lower limb muscles, or how it interacts with the effects of TMS current direction that are induced by changing coil orientation.

The purpose of this study was therefore to examine the effect of coil orientation and muscle activation on corticospinal and intracortical excitability of a lower limb muscle. A figure-of-eight TMS coil was used in PA (conventional orientation) and ML (alternative) orientations (see Fig. 2.1), as PA TMS is more comparable to TMS in upper limb muscles, and ML current flow may produce a lower motor threshold for lower limb muscles (Terao et al. 2000, Smith et al. 2017). The cortical representation to the tibialis anterior muscle was used as it is commonly assessed with TMS, and is functionally relevant for processes like balance and locomotion (Perez et al. 2004, Cacchio et al. 2011, Smith et al. 2017). Based on previous studies (Terao et al. 2000, Smith et al. 2017), it is hypothesised that both the coil orientation and muscle activation will influence measurements of intracortical excitability in a lower limb muscle.

2.3 Methods

2.3.1 Subjects

Twenty-one young (mean ± SD; 21.6 ± 3.3 years, 11 female) adults were recruited from the university and broader community to participate in the current study. Exclusion criteria included a history of concussion, neurological disease, or ongoing use of psychoactive medication (antidepressants, sedatives etc.). All experimentation was approved by the University of Adelaide Human Research Ethics Committee and conducted in accordance with the Declaration of Helsinki. Each participant provided written, informed consent prior to inclusion in the study.

2.3.2 Experimental arrangement

Each participant attended two experimental sessions in a randomised order, one to collect neurophysiological measurements in a resting muscle, and another for measurements in an active muscle. Aside from the muscle activation state, the experimental protocol was consistent between sessions. During each session, participants were seated in a comfortable chair, with feet sitting flat on the floor. Surface electromyography (EMG) was recorded from the Tibialis Anterior (TA) muscle of the right leg using two Ag-AgCl electrodes with an inter-electrode distance of approximately 2 cm (Cacchio et al. 2011), placed 1 cm lateral to the Tibial Crest (Vieira et al. 2017) in accordance with the European recommendations of Surface electromyography (Hermens et al. 2000). An earth strap was fitted around the ankle to ground the electrodes. EMG signals were amplified (300×) and band-pass filtered (20 Hz high pass, 1 kHz low pass) using a CED1902 signal conditioner (Cambridge Electronic Design, Cambridge, UK) and digitized at 2 kHz using a CED1401 interface. Recordings were stored on a computer for offline analysis.

2.3.3 Experimental procedures

TMS was applied to the leg area of the left M1 using a figure-of-eight coil connected to two Magstim 200^2 magnetic stimulators through a Bistim module (Magstim, Dyfed, UK). The coil was held tangentially to the scalp in two specific coil orientations (Fig. 2.1): PA direction, in which the handle was orientated directly backwards (inducing an anteriorly directed current flow in the brain), and ML direction with the handle positioned over the contralateral hemisphere, perpendicular to the midsagittal line (inducing a laterally directed current flow within the brain)(Terao et al. 2000). For both orientations, the coil was positioned over the optimal location for producing MEPs in the resting TA of the right leg, as we operated on the assumption that the resting and active hotspot was likely to be in a similar location. To identify this location, the coil was positioned with its centre approximately 1 cm posterior and 1 cm lateral to the vertex. From this position, the coil was moved in small increments to identify the location that produced the largest and most consistent MEPs. This location was marked on the scalp for reference and checked throughout the experiment. TMS was delivered at 0.2 Hz with a 10% variation between trials in order to avoid anticipation of the stimulus.

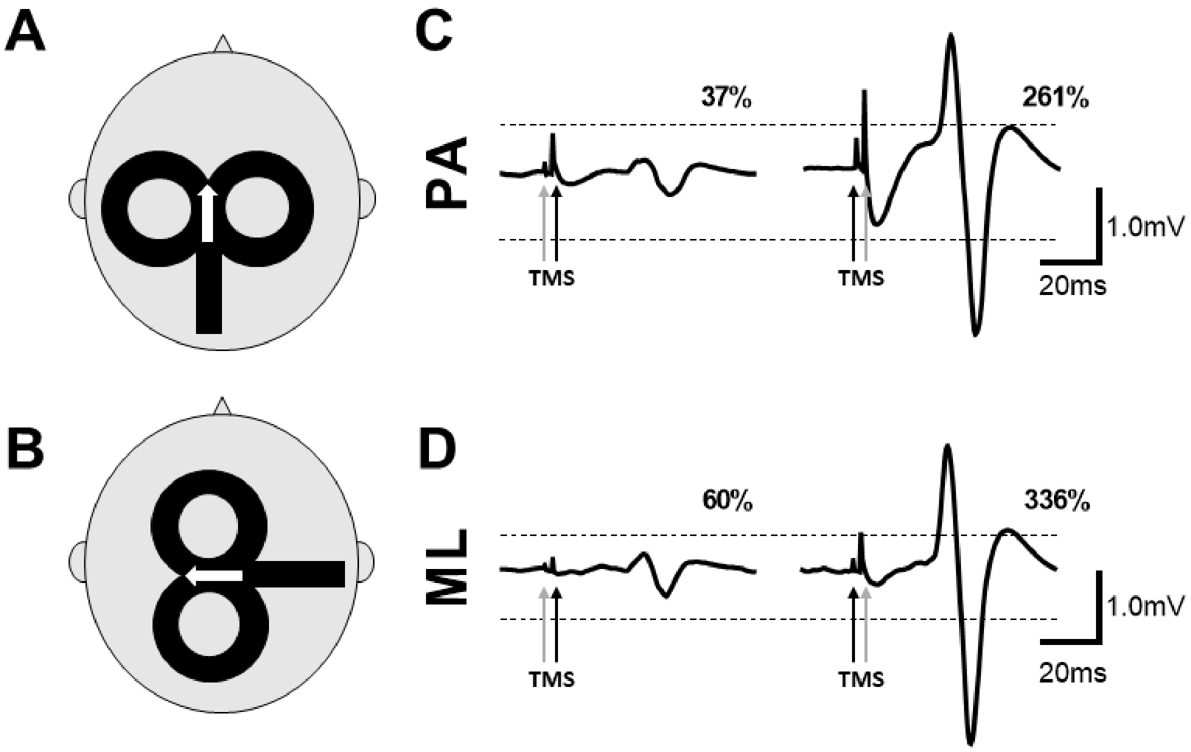

Figure 2.1: *TMS coil orientation to assess SICI and SICF. Coil was placed approximately 1 centimetre posterior and lateral of the vertex over the left hemisphere. The coil was orientated to induce either anterior (A) or lateral (B) current flow within the brain. The location was optimised to produce the largest response in the tibialis anterior (TA) of the right leg. Traces display data recorded in an active muscle of a single participant for PA (C) and ML (D) stimulation. The left column shows SICI recorded with a 3 ms ISI and conditioning intensity of 80% AMT; right column displays SICF recorded at 3 ms ISI with conditioning intensity of 90% RMT. Grey arrow, conditioning TMS; black arrow, test TMS.*

Resting motor threshold (RMT) was defined as the minimum stimulus intensity required to produce a motor evoked potential (MEP) with amplitude ≥ 50 µV in at least 5 out of 10 trials while the right TA was relaxed. In contrast, active motor threshold (AMT) was defined as the minimum stimulus intensity required to produce an MEP with an amplitude ≥ 200 µV in at least 5 out of 10 trials during a weak contraction of TA (sufficient to lift the forefoot while keeping the heal grounded). At baseline, a ‘test TMS’ intensity was defined as the stimulus intensity required to produce a peak-to-peak MEP amplitude of ~1 mV (range, 0.5 mV – 1.5 mV) when averaged over 20 trials (during a weak contraction for active session). The test TMS intensity was then used for all subsequent test blocks.

Short-interval intracortical facilitation (SICF) was tested with a test TMS followed by a subthreshold conditioning TMS at 90% RMT in both resting and active muscle (Ortu et al. 2008). The conditioning pulses occurred at ISIs of 1.5, 3.0 and 4.5 ms after the test pulse (Peurala et al. 2008). Short-interval intracortical inhibition (SICI) was tested using sub-threshold conditioning pulses set at 70, 80 and 90% of AMT (Yamaguchi et al. 2012) and inter-stimulus-intervals (ISIs) of 2 and 3 ms (Kujirai et al. 1993) prior to test pulse. Long-interval intracortical inhibition (LICI) was tested using conditioning and test stimuli set at the 1 mV intensity and an ISI of 100 ms.

Six test blocks were completed within each session (three test blocks for each coil orientation). Each test block consisted of eight single test TMS and four paired-pulse TMS for each condition (SICI, SICF, and LICI), resulting in a total of 24 total test TMS and 12 paired-pulse TMS for each condition, in both coil orientations and in each muscle activation state.

2.3.4 Data analysis

During offline analysis of EMG data from the resting session, trials containing EMG activity > 20 μV (peak-to-peak amplitude) in the 100 ms before TMS application were discarded. MEPs were measured peak-to-peak and expressed in mV. Individual MEPs recorded in response to paired-pulse stimulation were normalised to the mean MEP obtained from the test pulse. Accordingly, normalised values greater than 100% reflect facilitation, whereas normalised values of less than 100% reflect inhibition. In order to quantify how muscle activation influenced the activity of the investigated circuits, the difference between paired-pulse measures recorded in each activation state were expressed as a percentage of resting-state data. These delta values were only calculated for participants who completed both sessions.

2.3.5 Statistical analysis

The normality of data was assessed using Kolmogorov-Smirnov tests. Log transformations were applied in order to meet assumptions of normality where required. All data are displayed in the original form (non-transformed) for clarity. Single-pulse measures of RMT, AMT and test TMS intensities in resting and active muscle were compared between coil orientations (PA and ML) using paired t-tests. SICF was compared between coil orientations and ISIs (1.5, 3, 4.5 ms) using a two-factor linear mixed model (LMM). SICI was compared between coil orientations (PA, ML), ISIs (2, 3 ms) and conditioning intensities (70, 80, 90% AMT) using a three-factor LMM analysis whereas LICI was compared between coil orientations using a single factor LMM. These models were run separately for data recorded in resting and active muscle and included data from all participants. To examine the effect of muscle activation on paired-pulse TMS, these analyses were repeated for each dependent variable, but using the difference between rest and active muscle. For all models, subject was included as a random effect and significant main effects and interactions were further investigated using custom contrasts with Bonferroni correction. Unless otherwise stated, all data are shown as mean ± standard error of the mean (SEM).

2.4 Results

Due to the high TMS intensity required to produce an MEP response in lower limb muscles, mean test responses of > 0.5 mV were not achievable in a resting muscle in all subjects (not obtained in eight participants). Furthermore, one subject withdrew from the experiment after the first (resting) session. Therefore, 20 subjects completed the active session, while 12 completed testing in a resting state and 11 individuals completed both sessions.

2.4.1 Single-pulse TMS measures of corticospinal excitability.

Figure 2.2 displays the mean (and individual participant) TMS intensity required to produce motor threshold (RMT and AMT; Fig. 2.2a, b) and the test response (Fig. 2.2c, d) in a resting and active muscle for both coil orientations. TMS intensity was lower with ML TMS for all comparisons: RMT (7.8% difference, $t = 3.6$, $P = 0.002$), AMT (5.8% difference, $t = 2.1$, $P < 0.05$), resting test TMS intensity (5.6% difference, $t = 2.5$, $P = 0.03$) and active test TMS intensity (10.1% difference, $t = 4.7$, $P < 0.001$). For individual participant data, lower TMS intensity with ML orientation was shown in 18 (active test pulse), 12 (AMT) and 19 (RMT) of a possible 20 individuals. Furthermore, 9 out of a possible 12 participants had a lower test TMS intensity for ML orientation in a resting muscle. Within each activation state, the MEP amplitude produced by test alone stimulation was not different between coil orientations (t range = 1.4 - 1.7, all $P > 0.05$). Mean test TMS responses from randomised test blocks were 0.72 mV and 0.88 mV (resting muscle), and 1.31 mV and 1.39 mV (active muscle), for PA and ML stimulation, respectively.

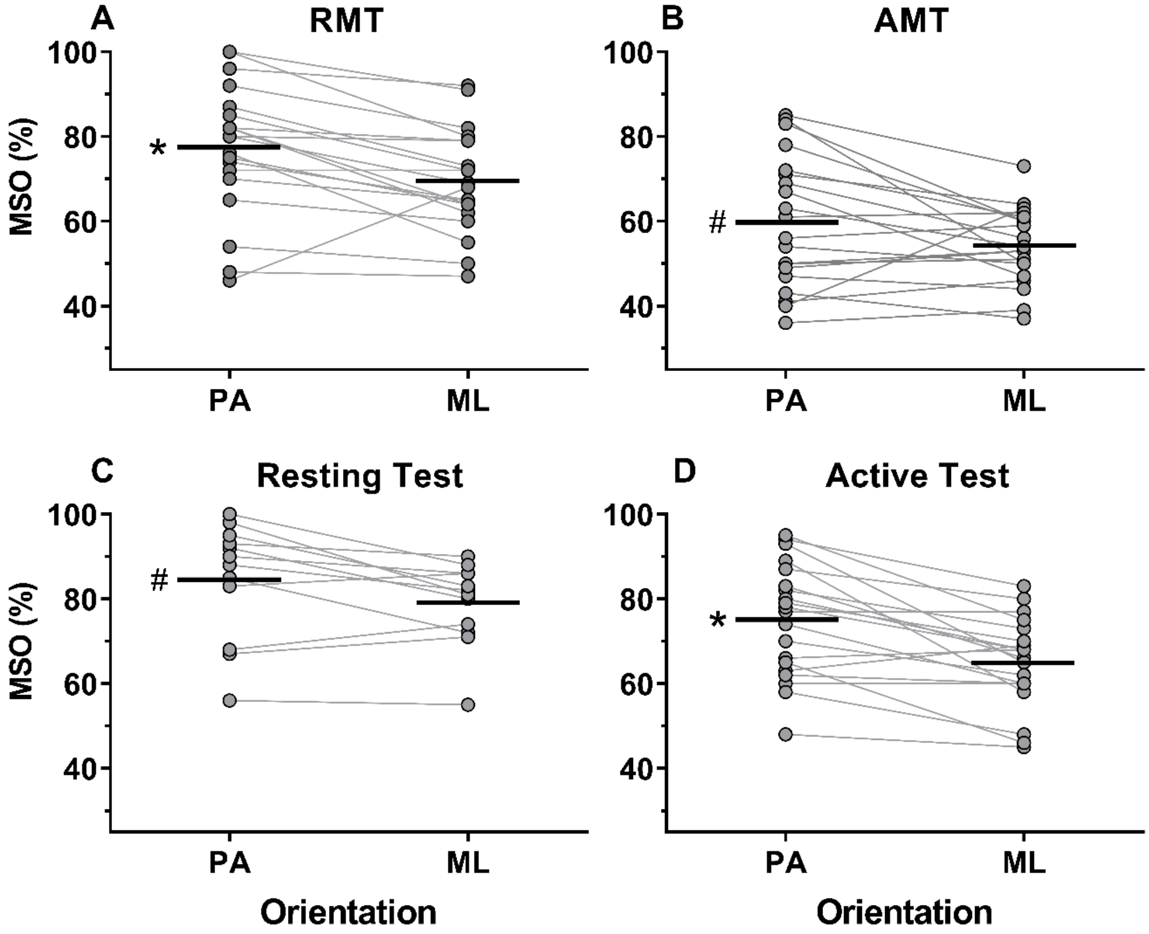

Figure 2.2: *Effect of coil orientation on corticospinal output. Data show the percentage of maximum stimulator output (% MSO) required to achieve RMT (A), AMT (B) test TMS at rest (C) and in an active muscle (D). Grey filled circles represent individual subject data. Solid black lines demonstrate mean values. * $P < 0.005$ compared with ML orientation; # $P < 0.05$ compared with ML orientation.*

2.4.2 Paired-pulse TMS measures of intracortical excitability

Measures of resting-state SICF did not vary between coil orientations ($F_{1, 661} = 2.9$, $P = 0.087$), but were different between ISIs ($F_{2, 421} = 22.8$, $P < 0.001$). Post hoc analysis showed that PA TMS elicited larger facilitation compared with ML orientation at 1.5 ms ($P = 0.02$) and 3.0 ms ($P = 0.01$) (Fig. 2.3a), with no difference at 4.5 ms ($P = 0.1$). Furthermore, there was a significant interaction between coil orientation and ISI ($F_{2, 421} = 5.4$, $P = 0.005$). Post hoc analysis indicated that an ISI of 4.5 ms produced less facilitation than both 1.5 ms and 3 ms ISIs during PA TMS (both $P < 0.001$), and less facilitation than 1.5 ms with ML TMS ($P = 0.02$). Analysis of active state SICF data indicated ML orientation produced greater facilitation than PA orientation ($F_{1,\,1087} = 8.8$, $P = 0.003$). A significant main effect was also shown for ISI ($F_{2,\,680} = 60.6$, $P < 0.001$). Post hoc analysis showed that facilitation with the 1.5 ms ISI was significantly greater than both 3 ms and 4.5 ms (both $P < 0.001$). Furthermore, facilitation with the 3 ms ISI was significantly greater than 4.5 ms ($P < 0.001$). There were no other significant main effects or interactions. A separate LMM analysis for the change in SICF with muscle activation showed an overall ISI effect ($F_{2,\,233} = 7.8$, $P = 0.001$), with post hoc tests indicating that a 1.5 ms ISI caused a greater change in facilitation during muscle activation than 4.5 ms ($P < 0.001$), but no difference between 1.5 ms and 3 ms ($P = 0.08$) or between 3.0 ms and 4.5 ms ($P = 0.2$).

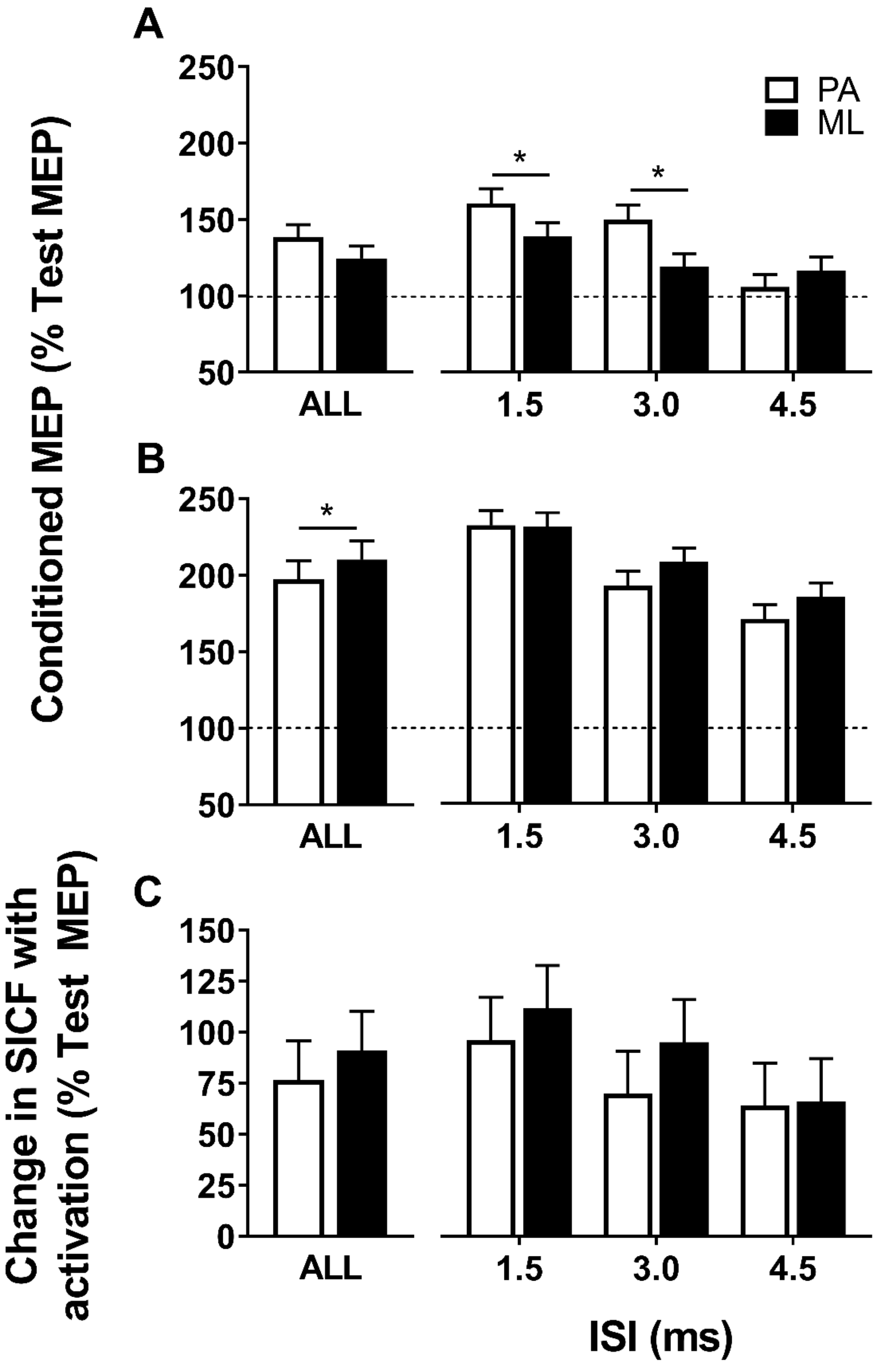

Figure 2.3: *Effect of coil orientation on SICF. Data show mean conditioned responses, normalised to the mean test response in a resting (A) and active (B) muscle state. Delta values (change in SICF between muscle states) are shown in plot (C). For clarity, only coil orientation effects are displayed on the figure. * P = < 0.05 between groups.*

LMM analysis of SICI at rest showed ML orientation produced greater inhibition ($F_{1,1343} = 66.3$, $P < 0.001$). Furthermore, an ISI of 2 ms produced greater inhibition than 3 ms (42.1% and 45.4% of mean test response respectively; $F_{1,1346} = 11.1$, $P = 0.001$). There was also a main effect of conditioning intensity ($F_{2,880} = 66.5$, $P < 0.001$). Post hoc analysis indicated that a conditioning intensity of 70% AMT produced less inhibition than 80% and 90% AMT (both $P < 0.001$), while there was no difference between 70% and 80% AMT ($P = 1.0$). An interaction between TMS orientation and conditioning intensity was also present ($F_{2,880} = 3.0$, $P < 0.05$). Post hoc analysis showed that ML stimulation produced greater inhibition than PA stimulation for conditioning intensities of 70% AMT ($P < 0.001$), 80% AMT ($P = 0.001$) and 90% AMT ($P < 0.001$; Fig. 2.4a). Furthermore, a conditioning intensity of 70% AMT produced less inhibition than other conditioning intensities (80% & 90% AMT) in both PA and ML coil orientations (all $P < 0.001$).

Analysis of SICI in active muscle showed that inhibition was greater with PA stimulation than ML stimulation ($F_{1,2457} = 21.9$, $P < 0.001$), and an ISI of 2 ms produced greater inhibition than 3 ms (63.5% and 68.3% of test mean respectively; $F_{1,2457} = 7.0$, $P = 0.008$). Furthermore, as conditioning intensity increased, inhibition decreased ($F_{2,1651} = 124.5$, all comparisons $P < 0.001$). The analysis also revealed interactions between TMS orientation and conditioning intensity ($F_{2,1651} = 14.3$, $P < 0.001$) as well as conditioning intensity and ISI ($F_{2,1651} = 6.2$, $P = 0.002$). For both ISIs combined, post hoc analysis showed that PA stimulation elicited greater inhibition than ML stimulation at a conditioning intensity of 80% AMT ($P = 0.04$) and 90% AMT ($P < 0.001$) (Fig. 2.4b). For both PA and ML coil orientations, a conditioning intensity of 90% AMT produced less inhibition than both 70% and 80% AMT (all $P < 0.001$). For ML stimulation, a conditioning intensity of 70% AMT elicited more inhibition than 80% and 90% AMT ($P < 0.001$). Post hoc analysis of the conditioning intensity and ISI interaction revealed that an ISI of 2 ms produced greater inhibition with a conditioning intensity of 70% AMT ($P = 0.006$) and 80% AMT ($P =$

0.002), but not 90% AMT ($P = 0.131$). A separate LMM analysis for the change in SICI with muscle activation showed that there was a greater reduction in inhibition in ML compared with PA orientation ($F_{1,\,321} = 24.4$, $P < 0.001$). Additionally, a main effect of conditioning intensity was present ($F_{2,\,379} = 107.4$, $P < 0.001$). Post hoc analysis revealed that all conditioning intensity comparisons were significant ($P < 0.001$) with the greatest reduction in inhibition during muscle activation occurring at 90% RMT, while 70% RMT increased inhibition during muscle activation.

Analysis of LICI at rest showed no overall coil orientation effect ($F_{1,\,74} = 0.03$, $P = 0.9$), where the mean normalised responses were 27% for PA and 25% for ML stimulation (Fig. 2.5). In contrast, LICI in an active muscle showed an overall coil orientation effect, where the PA orientation produced greater inhibition than the ML orientation with mean normalised responses of 33% and 41% respectively ($F_{1,\,132} = 8.4$, $P = 0.004$). A separate LMM analysis for the change in LICI with muscle activation showed no significant effects ($F_{1,\,129} = 3.2$, $P = 0.07$).

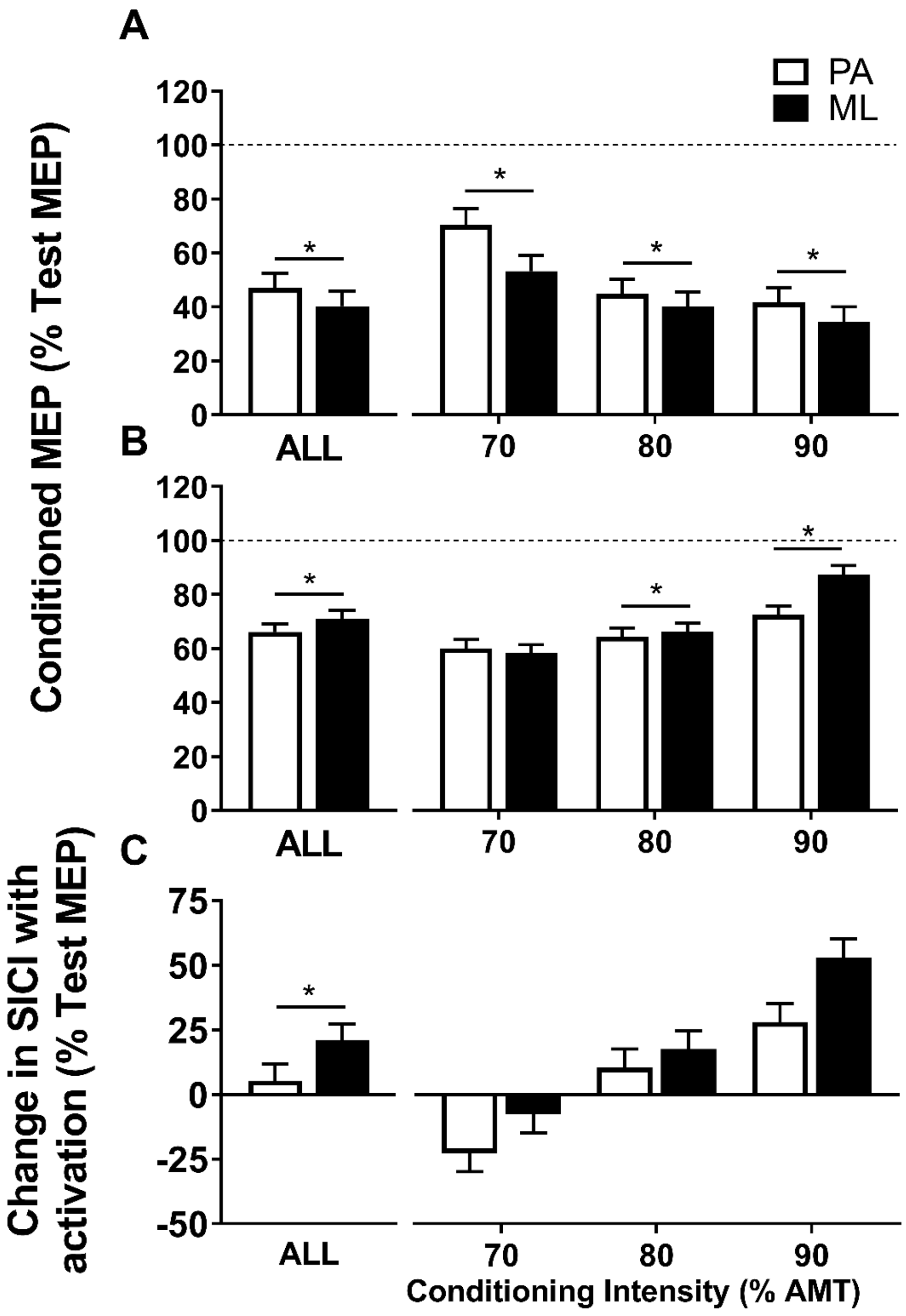

Figure 2.4: *Effect of coil orientation and conditioning intensity on SICI. Data are collapsed across ISIs and show mean conditioned responses, normalised to the mean test alone response in a resting (A) and active (B) muscle state. Delta values (change in SICF between muscle states) are shown in plot (C). For clarity, only coil orientation effects are displayed on the figure. * $P < 0.05$ between groups.*

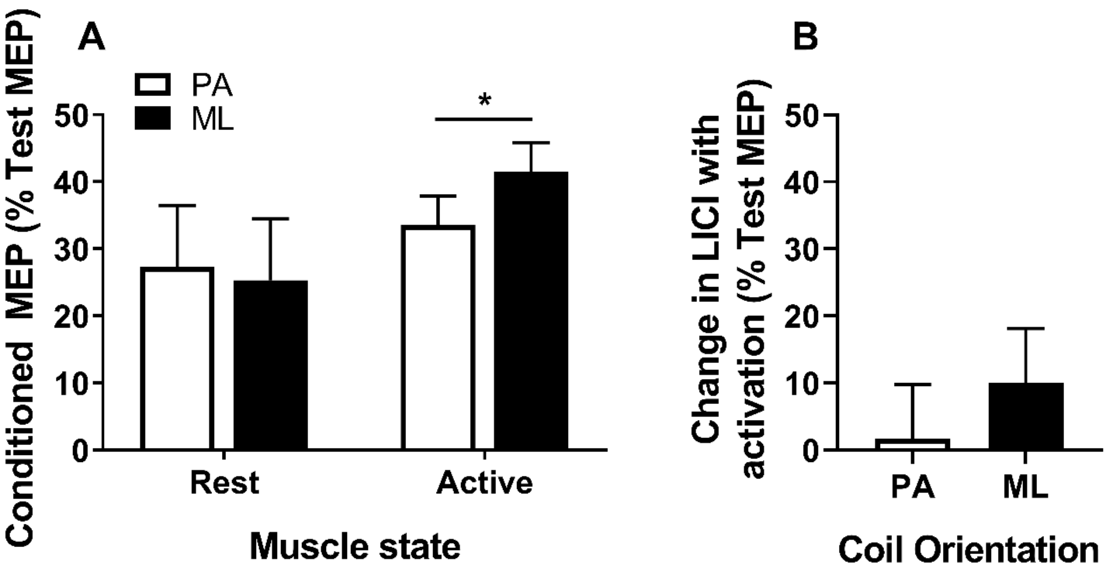

Figure 2.5: *Effect of coil orientation on LICI. Data show mean conditioned responses, normalised to the mean test alone response (A). Delta values (change in LICI between muscle states) are shown in plot (B). For clarity, only coil orientation effects are displayed on the figure. * P < 0.005 between groups.*

2.5 Discussion

This study investigated how coil orientation and muscle activation influence TMS measures of corticospinal and intracortical excitability recorded in a lower limb muscle. This was achieved by using a figure-of-eight coil to apply single and paired-pulse TMS in PA and ML orientations during both rest and activation of the TA muscle. There are two main findings in this study. First, TMS coil orientation influenced the magnitude of intracortical excitability for the TA cortical representation. Second, the effects of TMS coil orientation on intracortical excitability varied between muscle activation states.

2.5.1 Coil orientation influences corticospinal and intracortical excitability for tibialis anterior

Numerous studies in upper (Hanajima et al. 2002, Ni et al. 2011, Delvendahl et al. 2014, Di Lazzaro and Rothwell 2014, Opie et al. 2020), and relatively fewer studies in lower limb muscles (Terao et al. 2000, Smith et al. 2017), have indicated that TMS coil orientation affects the TMS descending volley, by preferential activation of specific interneuron (I-wave) circuits. For example, it has been demonstrated in resting upper limb muscles that single-pulse TMS with a PA orientation preferentially recruits I11-waves, whereas AP stimulation preferentially recruits I3 waves (Hanajima et al. 2002, Zoghi et al. 2003). Early single motor unit studies for the lower limb have shown that PA TMS activates D- and I1-waves (Priori et al. 1993), whereas more recent research has indicated that both PA and ML TMS predominantly activate I1-waves, with increased D-wave and late I-wave recruitment only at high TMS intensities (Terao et al. 2000). However, these latter studies are complicated by the need to activate the muscle to assess single motor unit activity, and a difference in the type of TMS coil used between studies, which makes it difficult to identify the physiological basis of the TMS-induced descending volley targeting lower limb muscles. Nonetheless, based on previous studies, it is reasonable to assume that differences in measures of corticospinal and intracortical excitability with different TMS coil orientations relate to differences in the relative combination of D- and I-waves that are activated by the TMS pulse. This is particularly evident for single-pulse TMS outcomes in the current study, where TMS intensities for motor thresholds and 1 mV test responses were lower for ML than PA coil orientation in resting and active muscle. This suggests that the ML coil orientation is able to more easily activate the corticospinal projections to TA, an observation that is consistent with previous findings. This finding may be related to the variable influence of the TMS-induced electric field on cortical neurons with different coil orientations (Priori et al. 1993, Richter et al. 2013). Multiple studies have demonstrated that the effective electric field is greatest when the induced current is perpendicular to the gyrus (Brasil-Neto et al. 1992, Fox et al. 2004, Gomez-Tames et al. 2018) and neurons are stimulated effectively only when their axons curve away from the direction of that induced current (Fox et al. 2004). However, recent evidence using realistic head models suggests it is likely to be a more complex interaction (Aberra et al. 2020, Gomez-Tames et al. 2020). For example, cortical activation may originate from sites superior or lateral of the gyrus, and may not exclusively occur from deep within the sulci where the induced current is perpendicular to the gyrus (Fox et al. 2004). Instead, neuronal activation may be related to the magnitude of the electric field, and originate close to the lip or crown of the gyrus (Aberra et al. 2020, Gomez-Tames et al. 2020). Nonetheless, given that the leg representation within M1 lies within the interhemispheric fissure, ML stimulation is expected to produce a higher effective electric field than PA stimulation for this region (Priori et al. 1993). Paired-pulse TMS was used in the current study to examine several different measures of intracortical excitability and how they are influenced by TMS coil orientation when targeting the cortical representation of lower limb muscles. One of the most commonly assessed measures of intracortical excitability is SICI, which involves a subthreshold conditioning stimulus that activates $GABA_A$ inhibitory circuits (Ziemann et al. 1996, Di Lazzaro et al. 2000), and is thought to be important for selective muscle activation (Zoghi et al. 2003). In resting upper limb muscles, these GABAergic circuits are known to influence I3 waves (Di Lazzaro et al. 2012) and preferential activation of these waves with AP TMS results in greater SICI (Zoghi et al. 2003). Our results show a greater SICI with ML stimulation in a resting TA at all three conditioning intensities (Fig. 2.4a). These results may, therefore, indicate the activation of a greater proportion of I3 waves with this coil orientation.

In contrast to SICI, LICI involves suprathreshold conditioning and test stimuli that activate $GABA_B$ inhibitory circuits (McDonnell et al. 2006). Despite this, both SICI and LICI are associated with modulation of I3 waves of the descending volley (Ni et al. 2011). While the functional consequences of LICI are not thoroughly understood, this process is known to be important for modulation of movement and accurate stopping (Sohn et al. 2002, Cowie et al. 2016) and is therefore relevant to locomotion and fatigue (Sidhu et al. 2018). Although the effect of coil orientation on LICI has not been extensively studied, de Goede et al. (2018) demonstrated that 10-degree adjustments to a 45 degree PA coil orientation have no effect on measurements of LICI in hand muscles. The outcomes of the present study support and build on these findings, showing that TMS coil orientation does not modulate measurements of LICI in a resting TA muscle. Given that LICI modulates I3 waves, and that I3 recruitment is modified by coil orientation, this outcome is perhaps unexpected. While the reason for this is currently unclear, it could be suggested that the suprathreshold intensity of the conditioning stimulus may have reduced the sensitivity of the MEP to changes in coil orientation. For example, higher intensity stimuli may result in greater recruitment of early descending volley components, reducing the relative contribution of the late I-waves to the generated MEP (Zoghi et al. 2003). Indeed, work in hand muscles has shown that coil orientation-dependent variations in I-wave recruitment require specific stimulation characteristics in order to preferentially activate late I-waves (Hannah and Rothwell 2017).

Finally, SICF involves a suprathreshold test pulse and a subthreshold conditioning pulse separated by short ISIs. It produces facilitation of the MEP that peaks at ~1.5, 3 and 4.5 ms, which matches the I-wave periodicity observed in direct recordings from corticospinal neurons (Ziemann and Rothwell 2000). It results from the interaction of I-wave generating circuits and is modulated by $GABA_A$ inhibitory circuits (Ziemann et al. 2015) and glutamatergic facilitatory circuits (Ortu et al. 2008). A significant coil orientation effect has been demonstrated for measurements of SICF in upper limb muscles (Hanajima et al. 2002, Delvendahl et al. 2014). Specifically, AP stimulation produces greater SICF than PA stimulation, most significantly with an ISI of approximately 1.5 ms (Delvendahl et al. 2014). Our results indicate that in a resting TA, ML stimulation fails to produce the same level of facilitation as PA stimulation at 1.5 and 3 ms ISIs. Given that direct measures of the corticospinal descending volley suggest that SICF application results in facilitation of all I-waves (Di Lazzaro et al. 2012), this orientation effect may seem counterintuitive. However, SICF is known to be contaminated by co-activated SICI circuits (Peurala et al. 2008). Therefore, it can be expected that application of SICF using a coil orientation that recruits greater proportions of late I-waves will have greater contamination by SICI, resulting in reduced facilitation relative to a coil orientation with less late I-wave recruitment. Our findings for SICF in resting TA muscle are therefore compatible with ML orientation recruiting greater proportions of I3 waves.

2.5.2 Muscle activation alters paired-pulse TMS measures of intracortical excitability

It is well known that muscle activation raises the excitability of corticospinal and spinal motor neurons, making it easier to activate the target muscle with TMS. Furthermore, numerous studies have shown that muscle activation also influences measures of intracortical excitability assessed with paired-pulse TMS. For example, there is a reduction in SICI during activation of both upper (Ridding et al. 1995, Zoghi et al. 2003, Hendy et al. 2019) and lower (Sidhu et al. 2013, Sidhu et al. 2013, Brownstein et al. 2018) limb muscles, which could be due to modulation of SICI circuitry by GABAergic presynaptic inhibition (Zoghi et al. 2003). Our findings largely support these previous studies, with a reduction in SICI during muscle activation seen predominantly at higher conditioning intensities. Furthermore, we find that muscle activation results in a greater reduction of SICI when TMS is delivered in the ML coil orientation (Figs. 2.4B, C). This differential effect of coil orientation in resting and active muscle could result from greater recruitment of I3 waves with ML stimulation, making this orientation more sensitive to reductions in SICI with muscle activation. Similar findings have been shown previously in hand muscles, where AP stimulation (which preferentially recruits I3 waves) is associated with a much greater decrease in SICI with muscular activation compared with I1 dominant PA stimulation (Zoghi et al. 2003).

Muscle contraction has been shown to influence measurements of LICI in hand muscles, however, the effect depends on the degree of activation. For example, McNeil et al. (2011) demonstrated a U-shaped curve of LICI across contraction strengths from 0-100% MVC, whereby inhibition was greatest at 10% MVC. To the best of our knowledge, no studies have investigated the combined effects of coil orientation and muscle activation on LICI in lower limb muscles. Results of the present study show PA stimulation produced greater inhibition for measurements of LICI in an active muscle, indicating greater $GABA_B$ transmission in this condition. Despite this, the change in muscle activation in resting and active muscle (delta analysis) showed no significant difference in LICI between coil orientations.

Evidence suggests that muscle activation increases SICF, but this occurs only at low activation levels (approximately 10% MVC). Ortu et al. (2008) suggested this is more likely due to subthreshold depolarisation of the glutamatergic interneurons that regulate SICF, as opposed to the increased excitability of cortical I-wave circuitry, given that the latter is most significant at higher contraction intensities, where SICF is no larger than at rest. However, this previous study only used a PA coil orientation to assess SICF, and we are unaware of any studies that have assessed the effect of coil orientation on the change in SICF with muscle activation, in upper or lower limb muscles. Nonetheless, the present study shows that SICF in an active muscle is greatest with ML stimulation, but there was no difference in the increase in facilitation between coil orientations with muscle activation. It is understood that activation increases the firing potential of the excitatory interneurons responsible for SICF (Ortu et al. 2008). Based on the present results, it is therefore possible that ML stimulation has a greater influence on these interneurons during volitional contraction (Ortu et al. 2008, Ni et al. 2011, Hamada et al. 2014, Di Lazzaro et al. 2018).

2.5.3 Effect of coil orientation in upper and lower limb muscles

In order to more easily compare the effect of coil orientation in upper and lower limb muscles, we have summarised the main outcomes from the tibialis anterior in the present study, along with some of the key findings obtained in previous studies involving upper limb muscles (Table 2.1). With these comparisons, it is important to note that PA and AP orientation for upper limb muscles are generally rotated clockwise by 45 degrees in the tangential plane so that the induced current is perpendicular to the central sulcus, which is optimal for activating the hand knob region (Brasil-Neto et al. 1992, Richter et al. 2013). In contrast, PA stimulation as used in this study for lower limb activation utilises a directly anterior induced current with the handle of the coil angled parallel to the midsagittal line (Terao et al. 1993, Smith et al. 2017). Using this latter approach for single-pulse TMS (motor threshold and 1 mV MEPs), we found a consistently lower intensity required for ML coil orientation in lower limb muscles, and this is comparable to the consistently lower intensity for PA coil orientation in upper limb muscles. Given that the optimal stimulation is achieved when the induced current is perpendicular to the axonal direction of corticospinal neurons, it seems likely that ML TMS for the lower limb is equivalent to PA TMS for the upper limb representations due to a different alignment of corticospinal neurons in these regions (Gomez-Tames et al. 2018). However, the comparison between upper and lower limb muscles for outcomes with paired-pulse TMS appears to be more complicated. For example, LICI seems to be minimally affected by coil orientation in both upper and lower limb muscles. However, SICF was greater with PA TMS in a resting muscle (1.5 and 3 ms) and greater with ML TMS in an active muscle of the lower limb, whereas it was greater with AP TMS in resting and active upper limb muscles. Furthermore, in contrast to the upper limb, SICI is differentially modulated by coil orientation in resting and active lower limb muscles, where there is greater SICI in resting TA for ML coil orientation but greater SICI in active TA muscle for PA coil orientation. This represents unique findings for the modulation of SICF and SICI with muscle activation in different coil orientations when targeting a lower limb cortical representation.

Table 2.1: *The effect of coil orientation and muscle activation on single and paired-pulse TMS in upper and lower limb muscles.*

Measured Variable	**Lower Limb (TA)**		**Upper Limb (APB or FDI)**		**Source for Upper Limb Studies**
	Rest	**Active**	**Rest**	**Active**	
MT	↓ML	↓ML	↓PA	↓PA	Brasil-Neto et al. (1992), Opie et al. (2018)
1 mV	↓ML	↓ML	↓PA	↓PA	Hannah and Rothwell (2017)
SICF	↑PA*	↑ML	↑AP	↑AP #	Hanajima et al. (2002), Delvendahl et al. (2014)
SICI	↑ML	↑PA	↑AP	↑AP	Zoghi et al. (2003), Sale et al. (2015)
LICI	No effect	↑PA	No effect	No data	de Goede et al. (2018)

*Comparisons of upper limb include PA and AP orientations. TA, tibialis anterior; APB, abductor pollicis brevis; FDI, first dorsal interosseous; MT, motor threshold. MT and 1 mV measures represent the lowest TMS intensity that generate these responses. *, 1.5 and 3 ms interstimulus interval. #, 3 ms interstimulus interval.*

In summary, although both PA and ML TMS coil orientations can be used to investigate corticospinal and intracortical excitability for a lower limb muscle, ML stimulation is likely to be a more optimal approach for the TA muscle due to a lower threshold for activation. When using paired-pulse TMS to assess intracortical excitability in a resting muscle, the main finding was greater SICI in the ML compared with the PA coil orientation. In contrast, muscle activation largely reversed the effect of TMS coil orientation on intracortical excitability, with reduced SICI and LICI and greater SICF in ML compared with the PA coil orientation. The results from this study indicate that TMS coil orientation and muscle activation influence measurements of intracortical excitability recorded in the tibialis anterior, and can therefore be used to inform the optimal stimulation parameters for TMS assessment of the tibialis anterior muscle.

Chapter III

Motor cortex plasticity and visuomotor skill learning in upper and lower limbs of endurance-trained cyclists

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Contribution to the Paper	Conceptualization, methodology, formal analysis, investigation, data curation, writing: original draft, writing: review & editing, visualization.		
Over percentage (%)	70		
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate to include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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3. Motor cortex plasticity and visuomotor skill learning in upper and lower limbs of endurance-trained cyclists

3.1 Abstract

Purpose Studies with transcranial magnetic stimulation (TMS) show that both acute and long-term exercise can influence TMS-induced plasticity within primary motor cortex (M1). However, it remains unclear how regular exercise influences skill training-induced M1 plasticity and motor skill acquisition. This study aimed to investigate whether skill training-induced plasticity and motor skill learning is modified in endurance-trained cyclists.

Methods In 16 endurance-trained cyclists (24.4 yrs; 4 female) and 17 sedentary individuals (23.9 yrs; 4 female), TMS was applied in two separate sessions: one targeting a hand muscle not directly involved in habitual exercise and one targeting a leg muscle that was regularly trained. Single- and paired-pulse TMS was used to assess M1 and intracortical excitability in both groups before and after learning a sequential visuomotor isometric task performed with the upper (pinch task) and lower (ankle dorsiflexion) limb.

Results Endurance-trained cyclists displayed greater movement times (slower movement) compared with the sedentary group for both upper and lower limbs (all $P < 0.05$), but there was no difference in visuomotor skill acquisition between groups ($P > 0.05$). Furthermore, endurance-trained cyclists demonstrated a greater increase in M1 excitability and reduced modulation of intracortical facilitation in resting muscles of upper and lower limbs after visuomotor skill learning (all $P < 0.005$).

Conclusion Under the present experimental conditions, these results indicate that a history of regular cycling exercise heightens skill training induced M1 plasticity in upper and lower limb muscles, but it does not facilitate visuomotor skill acquisition.

3.2 Introduction

There is now compelling evidence to suggest that regular physical exercise provides many benefits to brain health and function. For example, physically active individuals have demonstrated superior learning, memory and executive function, reduced age-related declines in mental capacity, and lower risk of brain-related disorders such as Alzheimer's disease (Colcombe et al. 2004, Larson et al. 2006, Kramer and Erickson 2007). At least for the central nervous system, it is understood that these benefits of exercise are related to the brain's capacity to modify neural connections, referred to as neuroplasticity (Cotman and Berchtold 2002, Müller-Dahlhaus and Ziemann 2015, Schättin et al. 2018, Opie et al. 2019). In addition, it is now commonly accepted that exercise generates an enriched cortical environment for neuroplasticity in motor areas of the brain, and that this can influence motor performance and learning (for reviews, see Taubert et al. 2015, Nicolini et al. 2021). However, our understanding of the contribution of regular physical activity in modulating neuroplasticity and motor behaviour in muscles that are used regularly in training, compared with those that are not directly involved in the exercise, is currently limited.

At the motor systems level in humans, transcranial magnetic stimulation (TMS) has previously been used to examine the role of regular physical activity and exercise in modulating excitability and plasticity in primary motor cortex (M1), but the outcomes vary depending on whether the target muscle was involved in chronic exercise training or not. For example, cross-sectional studies have shown that regular physical activity or endurance training (involving lower limbs) is associated with a decrease in M1 excitability of lower limb muscles (Hassanlouei et al. 2017, Rozand et al. 2019), but has no effect on TMS-induced M1 plasticity for the lower limb (Kumpulainen et al. 2015). Furthermore, several studies have examined the effect of regular exercise (lower limb muscles) on M1 excitability and plasticity of upper-limb muscles not directly involved in the exercise.

Using this approach, regular exercise involving lower limb muscles does not alter M1 excitability for upper limb muscles (Cirillo et al. 2009, Rozand et al. 2019), but results in greater TMS-induced M1 plasticity (Cirillo et al. 2009). These studies show that the effect of regular physical activity and exercise on M1 excitability and plasticity appear to be mixed, and may depend on whether the target muscle was directly involved with the exercise or not.

Neuroplasticity within cortical circuits is known to represent one of the key features of motor skill learning (Classen et al. 1998), which involves the acquisition of new patterns of muscle activation that result in improved task performance. The initial phases of motor learning involve mechanisms of long-term potentiation (LTP) or long-term depression in M1 circuits (Sanes and Donoghue 2000, Muellbacher et al. 2002, Ziemann et al. 2004) along with changes in intracortical excitability (Ziemann et al. 2001). These measures of use-dependent plasticity can be assessed with TMS in M1, which is considered a crucial site for motor learning (Sanes and Donoghue 2000). Although regular (long-term) physical activity and exercise is known to be beneficial for cognitive function (see McDonnell et al. 2011 for review), studies examining the effect of regular exercise on use-dependent M1 plasticity and motor behaviour are limited. For example, a single bout of lower limb exercise (Roig et al. 2012, Statton et al. 2015) or an 8-week aerobic exercise intervention (Bakken et al. 2001) can improve upper limb visuomotor skill performance. Furthermore, endurance athletes have been shown to have superior task performance in a static balance task compared with non-athletes, and this was accompanied by changes in M1 neuroplasticity assessed with functional near infrared spectroscopy (Seidel et al. 2017), although it is unclear whether this change was specific for the leg area of M1 or also involved upper limb M1 representations. Nonetheless, it is not currently known whether long-term physical activity involving the lower limbs influences use-dependent M1 plasticity or motor skill learning in upper and lower limb muscles of the same individuals.

Determining whether exercise influences M1 plasticity in a muscle-specific or global manner provides further evidence as to whether the modulation of synaptic communication within M1 circuits are specific to exercised muscle groups, or if the exercise-induced changes are due to systemic factors that are modulated with exercise, such as blood flow and circulating neurochemicals (for reviews, see Knaepen et al. 2010, Nicolini et al. 2021).

The purpose of this study was to examine use-dependent plasticity and motor skill learning in upper and lower limb muscles of physically active individuals compared with sedentary participants. The physically active group consisted of highly trained cyclists, who had a long history of exercise involving the lower limb muscles. We quantified motor skill learning by examining single session skill acquisition (referred to as online learning) using an isometric visuomotor task that required precise modulation of force (upper limb) or torque (lower limb) to control the movement of a cursor to various targets. This was accompanied by single-and paired-pulse TMS to M1 in order to examine changes in M1 (corticospinal) and intracortical excitability (facilitation and inhibition) after motor skill learning. Given that chronic physical activity and exercise is expected to result in widespread cortical adaptations (Adkins et al. 2006, Voss et al. 2010) and greater motor performance (Seidel et al. 2017), we hypothesized that trained cyclists would be characterized by increased use-dependent plasticity and motor skill learning in upper and lower limb muscles.

3.3 Methods

Thirty-three participants were recruited from the university and the broader community to participate in the current study. This study consisted of two participant groups that were recruited based on their physical activity levels, which were assessed using the International Physical Activity Questionnaire short form (Craig et al. 2003). An endurance-trained group, referred to as *END* group, consisted of 16 highly-trained cyclists (mean age

± SD: 24.4 ± 5.4; 4 female) who each had a total physical activity level of greater than 3000 MET-minutes of weekly exercise (McDonnell et al. 2013, Smith et al. 2014), including 5 or more hours of cycling but no formal upper body exercise, for at least 12 months. A sedentary group, referred to as *SED* group, included 17 (23.9 ± 3.6; 4 female) inactive individuals who had a total physical activity level of no more than 1500 MET-minutes per week (Craig et al. 2003) and did not participate in any regular exercise or aerobic training. Exclusion criteria for all participants included a history of concussion, neurological disease, or ongoing use of psychoactive medication (antidepressants, sedatives, etc.). All experimentation was approved by the University of Adelaide Human Research Ethics Committee and conducted according to the Declaration of Helsinki. Each participant provided written, informed consent to participate in the study.

3.3.1 Experimental arrangement and procedures

Each participant attended two randomized experimental sessions that were used for neurophysiological and functional assessments in each group (Fig. 3.1a). One session involved experimental testing of the first dorsal interosseous (FDI) muscle for control of the index finger (referred to as the upper limb session) as this muscle is not directly involved in lower limb cycling exercise. Another session (referred to as the lower limb session) involved an assessment of the tibialis anterior (TA) muscle for control of the ankle joint. The TA was selected due to its more prominent cortical representation and lower threshold for activation than other lower limb muscles in which resting TMS measurements are difficult to obtain (Groppa et al. 2012). Furthermore, TA plays a major role in dorsiflexion of the ankle joint (Petersen et al. 2003) and exhibits extensive activation during pedalling (Chapman et al. 2006, Hug and Dorel 2007). During the upper limb session, subjects were seated in a comfortable chair, with feet placed flat on the floor and the right hand on a benchtop positioned to apply force to a transducer (MLP-25; Transducer Techniques, USA) in a pinch grip arrangement (Fig. 3.1b). During the lower limb session, subjects were seated in a BIODEX System 4 isokinetic dynamometer (Biodex Medical Systems, USA) with the right foot secured on a footplate with ankle and knee flexion angles of approximately 90 degrees (Fig. 3.1d). Surface electromyography (EMG) was recorded from the FDI muscle of the right hand and the TA muscle of the right leg using two Ag-AgCl electrodes, in accordance with the European recommendations of surface electromyography (Hermens et al. 2000). An earth strap was fitted around the wrist or ankle to ground the electrodes. EMG signals were amplified (300×) and band-pass filtered (20 Hz high pass, 1 kHz low pass) using a CED1902 signal conditioner (Cambridge Electronic Design, Cambridge, UK) before being digitized at 2 kHz using a CED1401 interface. Recordings were stored on a computer for offline analysis. All measures within the experiment (i.e. EMG setup and TMS application) were conducted by the same experimenter to maintain consistency between sessions. By default, all sessions were completed exactly 1 week apart. In cases where this was not feasible, each participant completed the two sessions a minimum of 1 week and a maximum of 4 weeks apart. All participants were instructed not to complete any exercise on the day of testing.

3.3.2 Transcranial magnetic stimulation (TMS)

In both sessions, TMS was applied to the left M1 using a figure-of-eight coil connected to two Magstim 200^2 magnetic stimulators through a Bistim module (Magstim, Dyfed, UK). To optimize activation of the relevant motor cortical sites, coil orientation varied between upper and lower limbs in accordance with previous findings (Terao et al. 2000, Hand et al. 2020). For EMG measurements of FDI, the TMS coil was positioned to induce a posterior-anterior (PA) current within M1 by directing the handle posterior-laterally at an angle of approximately 45 degrees to the midline. For measurements of TA, the coil was instead positioned to induce a medio-laterally (ML) directed current in M1 by holding the coil handle 90 degrees to the midline and over the contralateral hemisphere, as this orientation is known to induce lower TMS thresholds than a more conventional PA orientation in TA

(Smith et al. 2017, Hand et al. 2020). Furthermore, a figure-of-eight coil was used to minimise discomfort that is commonly experienced with double-cone coils when activating lower limb muscles (Panyakaew et al. 2016, Fernandez et al. 2018). For both orientations, the coil was held tangentially to the scalp over the optimal location for producing MEPs in the target muscle. This location was marked on the scalp for reference and checked throughout the experiment. TMS was delivered at 0.2 Hz with a 10% variation between trials to avoid anticipation of the stimulus.

Resting motor threshold (RMT) was assessed at the beginning of the session, which was defined as the minimum stimulus intensity required to produce a motor evoked potential (MEP) with an amplitude of ≥ 50 μV in at least 5 out of 10 trials in the relaxed target muscle. For all active measurements, participants were instructed to maintain a contraction intensity of 10% MVC (maximal voluntary contraction), which was measured at the beginning of each session as the greatest force produced from 2 consecutive maximal contractions. 10% MVC was maintained via visual feedback of force provided on an oscilloscope. Active motor threshold (AMT) was defined as the minimum stimulus intensity required to produce an MEP with an amplitude of > 200 μV in at least 5 out of 10 trials in an active muscle. A 'test TMS' intensity was defined as the stimulus intensity required to produce a peak-to-peak MEP amplitude of ~1 mV (range, 0.5 mV – 1.5 mV) when averaged over 20 trials. This value was defined separately for both resting and active muscle states, allowing for analysis of corticospinal and intracortical excitability independently for each muscle state. Paired-pulse TMS was used to assess intracortical excitability with short-interval intracortical inhibition (SICI), short-interval intracortical facilitation (SICF) and intracortical facilitation (ICF) in resting and active muscles. SICI was assessed using a sub-threshold conditioning pulse set at 80% of AMT (Kujirai et al. 1993, Opie and Semmler 2014) and an interstimulus-interval between conditioning and test stimuli of 2 ms (Kujirai et al. 1993). SICF was tested with a subthreshold conditioning intensity of 90% RMT, which was applied 1.5 ms following the test pulse (Ziemann et al. 1998, Peurala et al. 2008, Lulic et al. 2017). ICF was tested using a subthreshold conditioning stimulus of 80% RMT and an interstimulus-interval of 10 ms prior to the test pulse (Wagle-Shukla et al. 2009). The test stimulus for all paired-pulse measures was set at the 1 mV intensity. Conditioning intensities did not vary between the resting and active test blocks; however, the test TMS intensity used within each block was muscle state specific.

To assess M1 excitability at baseline, two brief input-output (I/O) blocks were completed prior to learning: one at rest and another in an active muscle state. The resting I/O block included 3 intensities of 110, 120, and 130% RMT, whereas the active I/O block included 5 intensities from 110 – 150% AMT (10% increments). These I/O blocks included 10 stimuli at each intensity (resting block = 30 stimuli; active block = 50 stimuli). Subsequently, a single resting and a separate active test block were completed to assess M1 excitability as well as paired-pulse measurements of SICI, SICF, and ICF. These test blocks comprised 20 test TMS stimuli at either resting or active test TMS intensity, as well as 12 trials of each paired-pulse condition (SICI, SICF, and ICF) in a pseudorandomized order. To assess changes in both M1 and intracortical excitability across learning, these test blocks were completed at baseline, and then repeated 0 and 30 minutes following a motor skill acquisition task (Fig. 3.1).

3.3.3 Visuomotor learning task

In both sessions, a target-based visuomotor task was used as a platform to assess motor skill acquisition and induce use-dependent plasticity. The task used was a modified version of a sequential visual isometric pinch task (Reis et al. 2009), which is known to increase M1 excitability with learning (Opie et al. 2019, Opie et al. 2020), but is unlikely to involve changes in spinal excitability (Giesebrecht et al. 2012). For the upper limb session, the participant grasped a force transducer between the index finger and thumb in a pinch grip position (Fig. 3.1b). For the lower limb session, isometric dorsiflexion torque was measured with a Biodex isokinetic dynamometer (Biodex System 4) (Fig. 3.1d). For these tasks, participants were required to use isometric force or torque to control the movement of a cursor displayed on a computer screen to 5 colour target zones arranged vertically. The cursor position moved upwards with increasing isometric force (or torque). Reaching the highest target required application of force/torque approximately equal to 45% of the individual subject's MVC. Within each trial, the objective was to move the cursor to all 5 colour targets in a specific order (consistent within each session) while returning to baseline (0% MVC) between each colour (Fig. 3.1c, e). The colours would disappear at the end of each trial and reappear for the start of the next trial. There were 8 trials within each test block and 8 test blocks completed in total (approximately 16 – 20 minutes total training). Skill scores were calculated based on both speed and accuracy components of movement (see below) and were provided to the participants after each block. Participants were also encouraged to try to improve their speed and accuracy periodically. To increase the difficulty of the task, the transduction of force application to cursor movement was non-linear. Furthermore, different non-linear force transforms were used (sigmoidal for upper limb and exponential for lower limb) in order to avoid learning cross-over. Additionally, the vertical position of each colour target was varied between upper and lower limb sessions. The protocol design and testing parameters were consistent between the upper and lower limb sessions, which were randomized in order.

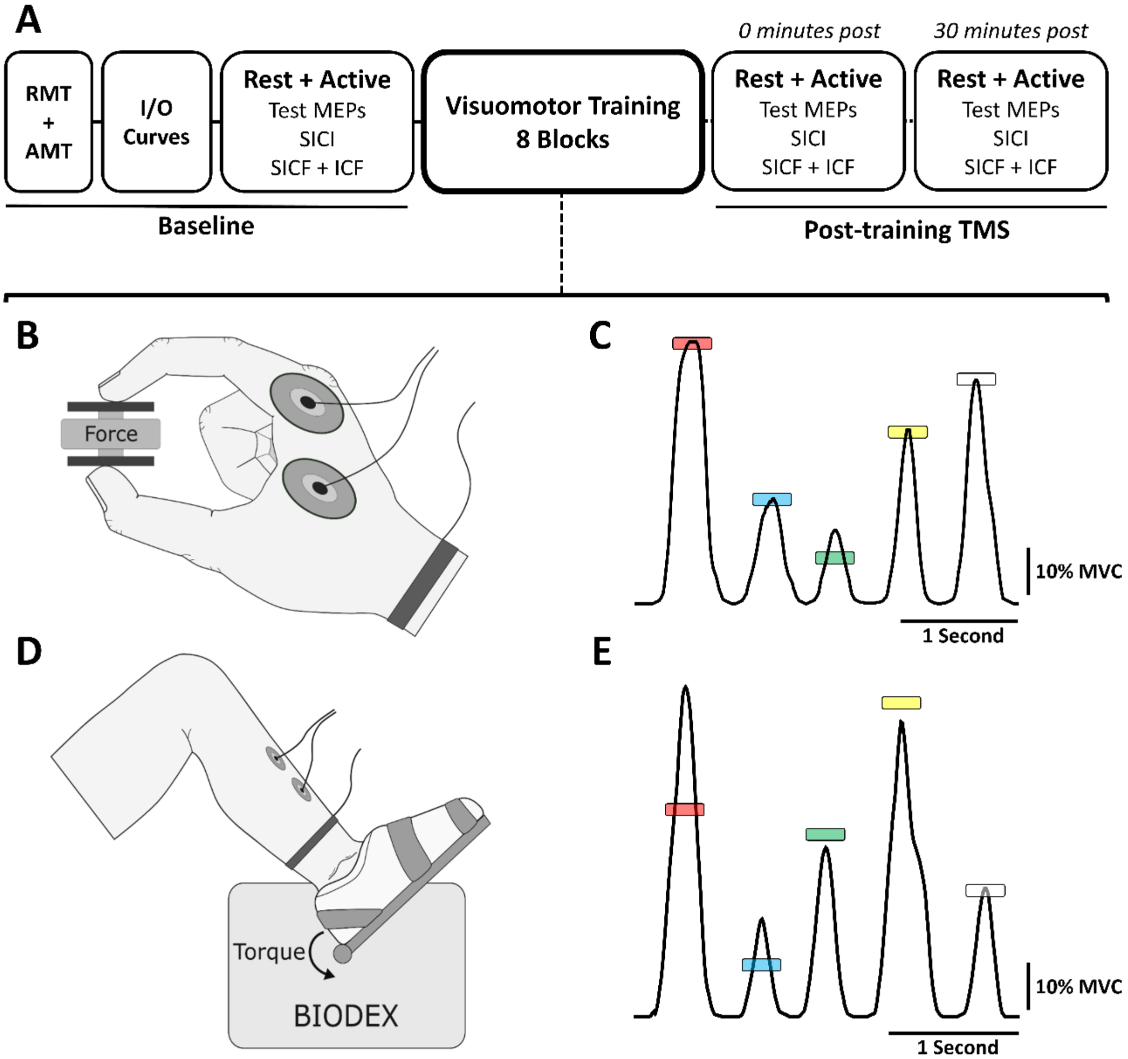

Figure 3.1: *Experimental protocol and visuomotor training setup. Experimental protocol for neurophysiological and behavioural assessments (A). Illustration of limb and electrode position for upper (B) and lower (D) limbs. Force (C) and torque (E) traces from a single visuomotor task trial for upper and lower limbs, respectively. Coloured blocks represent the location of the five colour targets.*

3.3.4 Data analysis

During offline analysis of EMG data from the resting session, trials containing EMG activity > 20 µV (peak-to-peak amplitude) in the 100 ms before TMS application were discarded, resulting in the removal of approximately 3% of all trials (END group, 3.2%; SED group, 2.9%). MEPs were measured peak-to-peak and expressed in mV. For I/O data, area under the recruitment curve (AURC) values were obtained for each participant and condition (limb, muscle state), using trapezoidal integration implemented in MATLAB with the *'trapz'* function (Carson et al. 2013, Fujiyama et al. 2017). To assess the influence of intracortical networks on MEP amplitude, individual MEPs recorded in response to paired-pulse stimulation were expressed as a percentage of the mean test MEP amplitude. Accordingly, normalized values greater than 100% reflect facilitation, whereas normalized values of less than 100% reflect inhibition. To assess the changes in M1 excitability following motor learning, post-training MEPs from test TMS stimuli were normalized to the average MEP amplitude recorded at baseline. To identify skill training-induced changes in intracortical excitability, normalized MEPs from paired-pulse TMS recorded after learning were expressed as a percentage of the mean values from baseline.

Skill scores were calculated for each block of 8 trials and were based on both speed and accuracy components of the movement. Speed was measured by the average movement time for each trial, while accuracy was quantified using the error values of each force peak, which were obtained from the Euclidean distance between the true force output and the force output required to reach the centre of the target (Stavrinos and Coxon 2017). A trial error value was then calculated by summing the error values for all 5 peaks within a trial. For assessment of motor performance, skill scores were calculated using the following formula, as proposed by Reis et al. (2009).

$$Skill = \frac{(1 - error)}{error(\ln(movement\ time)^{b})}$$

The dimensionless free b parameter has been shown to be insensitive to changes in performance and therefore, was set at a consistent 1.627 (Stavrinos and Coxon 2017). Visuomotor training data was grouped into quartiles (2 blocks each) for analysis. To assess motor skill acquisition, skill scores from the fourth quartile of learning (final 2 blocks) were normalized to the average skill score from the first quartile (baseline skill).

3.3.5 Statistical analysis

The normality of data was assessed using Shapiro-Wilk tests. Log transformations were applied in order to meet assumptions of normality where required, and all models were run separately for data recorded in upper and lower limb muscles. All graphical and tabulated data are displayed in their original form (i.e., non-transformed) for clarity. Unpaired t-tests were used to compare mean weekly exercise load (MET-minutes) between groups (END, SED), as well as baseline TMS intensities for RMT, AMT and test TMS between groups within each condition (limb and muscle state). Baseline I/O data (MEP amplitudes) were compared between groups and intensities (110–130% RMT for rest; 110–150% AMT for active) for each limb (upper, lower) and muscle state (active, resting) using separate two-factor linear mixed model (LMM) analyses. Furthermore, AURC values were compared between groups in separate single factor LMM analyses for each condition (limb, muscle state). Baseline paired-pulse measures of SICI, SICF and ICF were compared between groups and muscle states using a two-factor LMM. Baseline measures of error, movement time and skill from the visuomotor task were analyzed independently and compared between groups in single factor LMM analyses, using data from the first quartile (2 blocks). To analyze changes in error, movement time and skill with motor training, separate two-factor linear mixed model analysis with repeated measures ($\mathrm{LMM}_{\mathrm{RM}}$) were conducted with group and quartile (1 - 4) as factors. Post-intervention single-pulse measures of M1 excitability and paired-pulse measures of intracortical excitability (SICI, SICF & ICF) were compared between groups, muscle state and time points (0 & 30 minutes post-training) using a three-factor $\mathrm{LMM}_{\mathrm{RM}}$ analysis. Values for these tests were normalized to baseline, and for all models, subject was included as a random effect. Significant main effects and interactions were further investigated using custom contrasts with Bonferroni correction. For all significant effects and interactions, the estimated mean difference (EMD) and associated 95% confidence interval from post hoc pairwise comparisons have been presented as non-standardised measures of effect size. EMD's were exponentiated from the log values derived from individual pairwise comparisons, and therefore represent a ratio of the means being compared. Only minor differences were observed between the two time points (0 and 30 mins) after visuomotor learning, and therefore time points after learning were grouped in figures for display purposes. Significance was set at $P < 0.05$ for all comparisons and data are shown as mean ± 95% confidence interval, unless otherwise stated.

3.4 Results

All participants completed the study without any adverse effects. The International Physical Activity Questionnaire showed a significantly greater weekly exercise load of 6488 MET-minutes for the END group compared to 1209 MET-minutes for the SED group ($P < 0.001$). Furthermore, based on the self-reported activity levels, the END group accumulated 60% of their total weekly MET-minutes through *vigorous* intensity exercise (predominantly endurance-based cycling), compared to only 20% for the SED group, which was mostly everyday lifting/carrying tasks. Due to the high TMS intensity required to produce an MEP response in lower limb muscles, mean test responses of > 0.5 mV were not achievable in a resting TA muscle in 6 END and 7 SED participants. Furthermore, a mean test response of > 0.5 mV was not achievable in the resting FDI of 1 END participant. Therefore, 15 END and 17 SED participants completed all TMS measurements (rest and active muscle states) in the upper limb session and all active muscle state measurements in the lower limb. Resting TMS measures in the lower limb session were obtained for 10 participants in each group. The average time between sessions was 9.0 days for the END group and 10.7 days for the sedentary group.

3.4.1 Baseline measures of M1 and intracortical excitability

Table 3.1 displays the mean stimulator intensities for RMT, AMT and test TMS, as well as mean test TMS response amplitude for each condition (limb, muscle state, group). Mean baseline responses from paired-pulse measures of SICI, SICF and ICF are expressed normalized to the test response. There were no differences in mean stimulator intensity for RMT, AMT or test TMS and no difference in mean test TMS amplitude between groups for each limb and muscle state (all $P > 0.05$). For the upper limb, baseline SICI did not vary between groups ($F_{1,\,31} = 1.4$, $P = 0.2$), but was greater in the resting compared with the active muscle state (EMD = 269%, 95%CI [225, 322], $F_{1,\,186} = 119$, $P < 0.001$). Furthermore, there was a significant interaction between group and muscle state ($F_{1,\,186} = 8.2$, $P = 0.005$). Post hoc analysis showed that baseline SICI was greater in the END compared with the SED group in a resting muscle of the upper limb (EMD = 161%, 95%CI [106, 244], $P = 0.03$) and was not different between groups in an active muscle (EMD = 96%, 95%CI [64, 144], $P = 0.8$). Furthermore, SICI was greater in a resting muscle compared with an active muscle for both END (EMD = 349%, 95%CI [270, 452], $P < 0.001$) and SED (EMD = 207%, 95%CI [161, 266], $P < 0.001$) groups.

In the lower limb, baseline SICI showed no difference between groups ($F_{1,\,32} = 40.3$, $P = 0.6$), but was greater in a resting compared with an active muscle (EMD = 178%, 95%CI [146, 218], $F_{1,\,159} = 32$, $P < 0.001$). Baseline measures of SICF did not vary between groups for the upper ($F_{1,\,30} = 0.01$, $P = 0.9$) or lower ($F_{1,\,30} = 1.0$, $P = 0.3$) limb. However, SICF was greater in an active muscle state for both upper (EMD = 203%, 95%CI [184, 224], $F_{1,\,185} = 196$, $P < 0.001$) and lower (EMD = 158%, 95%CI [147, 171], $F_{1,\,160} = 135$, $P < 0.001$) limb muscles. Similarly, analysis of ICF showed no difference between groups for the upper ($F_{1,\,28} = 1.8$, $P = 0.2$) or lower ($F_{1,\,29} = 0.3$, $P = 0.6$) limbs, but greater ICF with a resting muscle state for both upper (EMD = 135%, 95%CI [117, 155], $F_{1,\,166} = 16.7$,

$P < 0.001$) and lower (EMD = 117%, 95%CI [104, 131], $F_{1, 158} = 6.7$, $P = 0.01$) limb muscles. There were no other significant main effects or interactions.

Figure 3.2 shows the mean MEP amplitudes with increasing TMS intensity in both END and SED groups obtained before the visuomotor task (baseline). For all conditions, MEP amplitude increased in response to increasing TMS intensity (all $P < 0.001$), but this was not different between groups, and there was no interaction between factors (all $P > 0.1$). Separate LMM of AURC values of each condition (limb, muscle state) revealed no significant differences between groups (all $P > 0.2$).

Table 3.1: *Baseline TMS measures.*

	Upper Limb (FDI)				**Lower Limb (TA)**			
	Resting		**Active**		**Resting**		**Active**	
	END	**SED**	**END**	**SED**	**END**	**SED**	**END**	**SED**
Motor Threshold (% MSO)	43.1 (39.7, 46.5)	41.5 (38.1, 44.9)	34.2 (30.9, 37.5)	33.7 (30.4, 37.0)	68.3 (62.0, 74.4)	69.2 (61.1, 77.4)	55.8 (49.8, 61.8)	55.2 (50.0, 60.4)
Test TMS Intensity (%MSO)	52.3 (46.2, 58.4)	49.0 (43.1, 54.9)	39.5 (35.0, 44.0)	38.9 (34.9, 43.0)	79.4 (72.4, 86.4)	75.9 (66.7, 85.1)	65.4 (58.1, 72.6)	66.2 (59.6, 72.9)
Test response (mV)	1.2 (1.1, 1.3)	1.2 (1.1, 1.3)	1.2 (1.1, 1.3)	1.1 (1.0, 1.2)	0.9 (0.8, 1.0)	1.0 (0.9, 1.1)	1.0 (0.9, 1.1)	1.1 (1.0, 1.2)
SICI (% test)	47.7* (40.7, 54.7)	61.5 (52.4, 70.5)	111.4 (98.2, 124.5)	98.8 (90.1, 107.5)	53.6 (44.7, 62.5)	43.8 (36.9, 50.7)	67.9 (63.1, 72.7)	79.2 (72.8, 85.6)
SICF (% test)	216.4 (196.8, 236.1)	210.3 (189.2, 231.4)	419.5 (374.5, 464.4)	433.0 (396.7, 469.2)	158.5 (144.7, 172.3)	148.7 (138.2, 159.3)	266.8 (247.0, 286.5)	219.1 (203.0, 235.3)
ICF (% test)	163.6 (139.8, 187.5)	161.9 (147.7, 176.1)	107.7 (97.8, 117.7)	145.5 (128.2, 162.8)	137.7 (124.6, 150.9)	132.9 (120.6, 145.3)	124.9 (113.0, 136.9)	159.4 (144.7, 174.1)

*Data represent mean (95% confidence interval). * $P < 0.05$ compared with SED group. Abbreviations: MSO, maximum stimulator output; END, endurance-trained; SED, sedentary.*

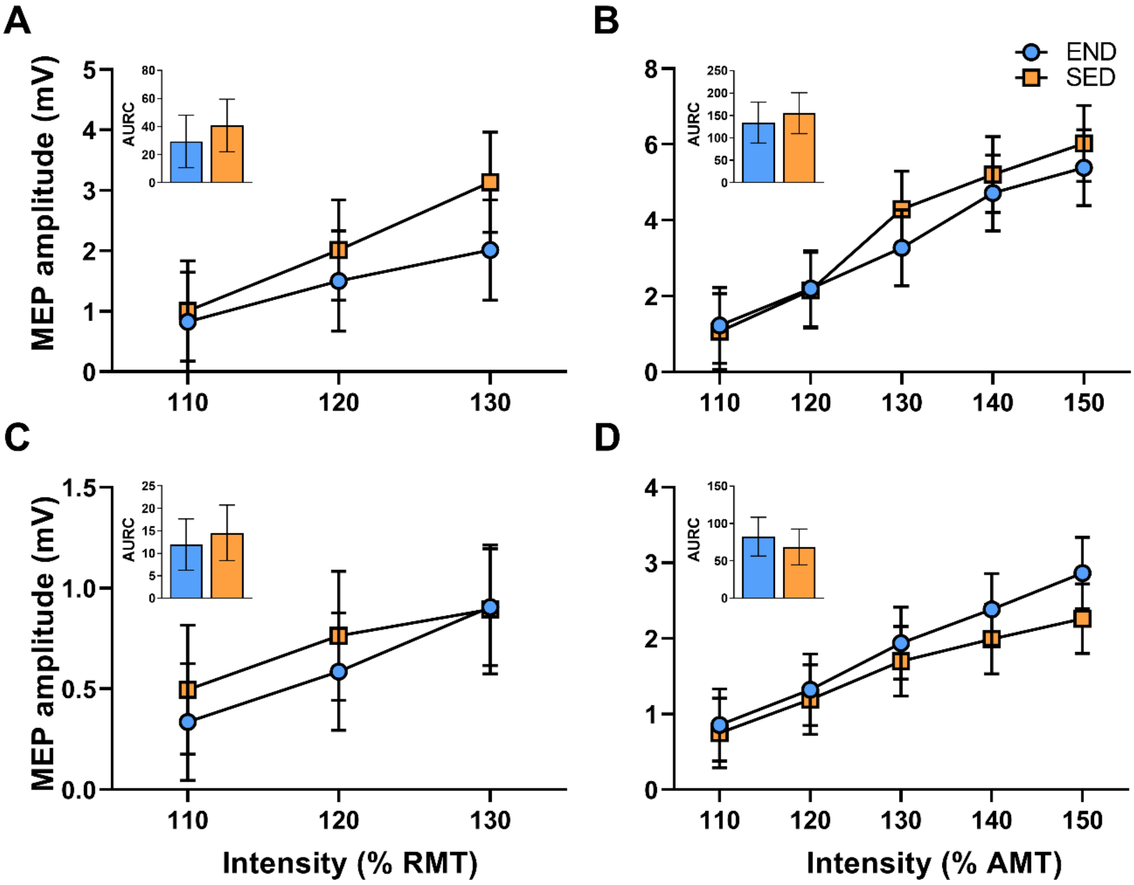

Figure 3.2: *Input-output curves. Data represent mean MEP amplitudes at different TMS intensities for resting (left column) and active (right column) muscles of the upper (A, B) and lower (C, D) limbs. Inset panels display the area under recruitment curve (AURC) for each group. Data were obtained at baseline before visuomotor skill learning. Abbreviations: END, endurance-trained; SED, sedentary*

3.4.2 Visuomotor skill training

For the upper limb at baseline (quartile 1), there was no difference between groups for error ($F_{1,31}$ = 0.006, P = 0.9) movement time ($F_{1,31}$ = 0.8, P = 0.8), or skill ($F_{1,31}$ = 0.4, P = 0.5). During training, error scores were not different between quartiles ($F_{3,663}$ = 1.4, P = 0.3) or groups ($F_{1,31}$ = 1.4, P = 0.9) and there was no interaction between factors ($F_{3,663}$ = 1.6, P = 0.2) (Fig. 3.3a). For movement time, there was also no difference between groups ($F_{1,31}$ = 2.3, P > 0.1). However, values differed between quartiles ($F_{3,448}$ = 105, P < 0.001; Fig. 3.3b), with post-hoc testing showing decreasing movement time (faster movement) for each consecutive quartile (all P < 0.001). Furthermore, a significant interaction between group and movement time was present ($F_{3,448}$ = 10.3, P < 0.001), with post hoc analysis revealing longer movement times (slower movement) for the END group in the 4th (final) quartile of training (EMD = 119%, 95%CI [104, 136], $P = 0.01$). For skill scores, values differed between quartiles ($F_{3,\,681} = 54$, $P < 0.001$; Fig. 3.3c), with post hoc testing showing increased skill scores with training: quartile 1 had lower skill scores than all other time points, quartile 3 had significantly greater skill than quartile 2 and quartile 4 showed greater skill than quartiles 1 and 2 (all $P < 0.001$). However, there was no difference between groups ($F_{1,\,31} = 2.6$, $P = 0.1$) and no interaction between factors ($F_{3,\,681} = 2.0$, $P = 0.1$).

For the lower limb at baseline, there were no differences between groups for error ($F_{1,\,30} = 0.1$, $P = 0.7$) or skill ($F_{1,\,30} = 2.2$, $P = 0.1$). However, movement times were significantly greater (slower movement) for the END group (EMD = 110%, 95%CI [101, 120], $F_{1,\,30} = 5.0$, $P < 0.05$). During training, error scores varied between quartiles ($F_{3,\,682} = 15.6$, $P < 0.001$; Fig. 3.3d), with post-hoc comparisons showing significantly greater error at the beginning of training (quartile 1) than all other time points (all $P < 0.001$). However, there was no difference between groups and no interaction between factors (all $P > 0.5$). Movement time was greater in the END group compared with the SED group (EMD = 117%, 95%CI [106, 130], $F_{1,\,30} = 10.3$, $P = 0.003$). Furthermore, movement time varied between quartiles ($F_{3,\,386} = 65.4$, $P < 0.001$), with post-hoc comparisons showing reduced movement time (faster movement) with each consecutive quartile (all $P < 0.02$). A significant interaction between group and quartile was also present ($F_{3,\,386} = 4.3$, $P = 0.006$), with post hoc tests revealing greater movement times from the END group at each quartile of learning (all $P < 0.05$; Fig. 3.3e). Skill scores also varied across quartiles ($F_{3,\,635} = 55$, $P < 0.001$; Fig. 3.3f), with post hoc testing showing increased skill scores with training: quartile 1 had lower skill scores than all other time points (all $P < 0.001$) and quartile 4 had greater skill than quartiles 1 and 2 (both $P < 0.001$). However, there was no difference between groups ($F_{1,\,30}$ = 4.1, $P > 0.05$) and no interaction between factors ($F_{3,\,635}$ = 1.0, $P = 0.4$).

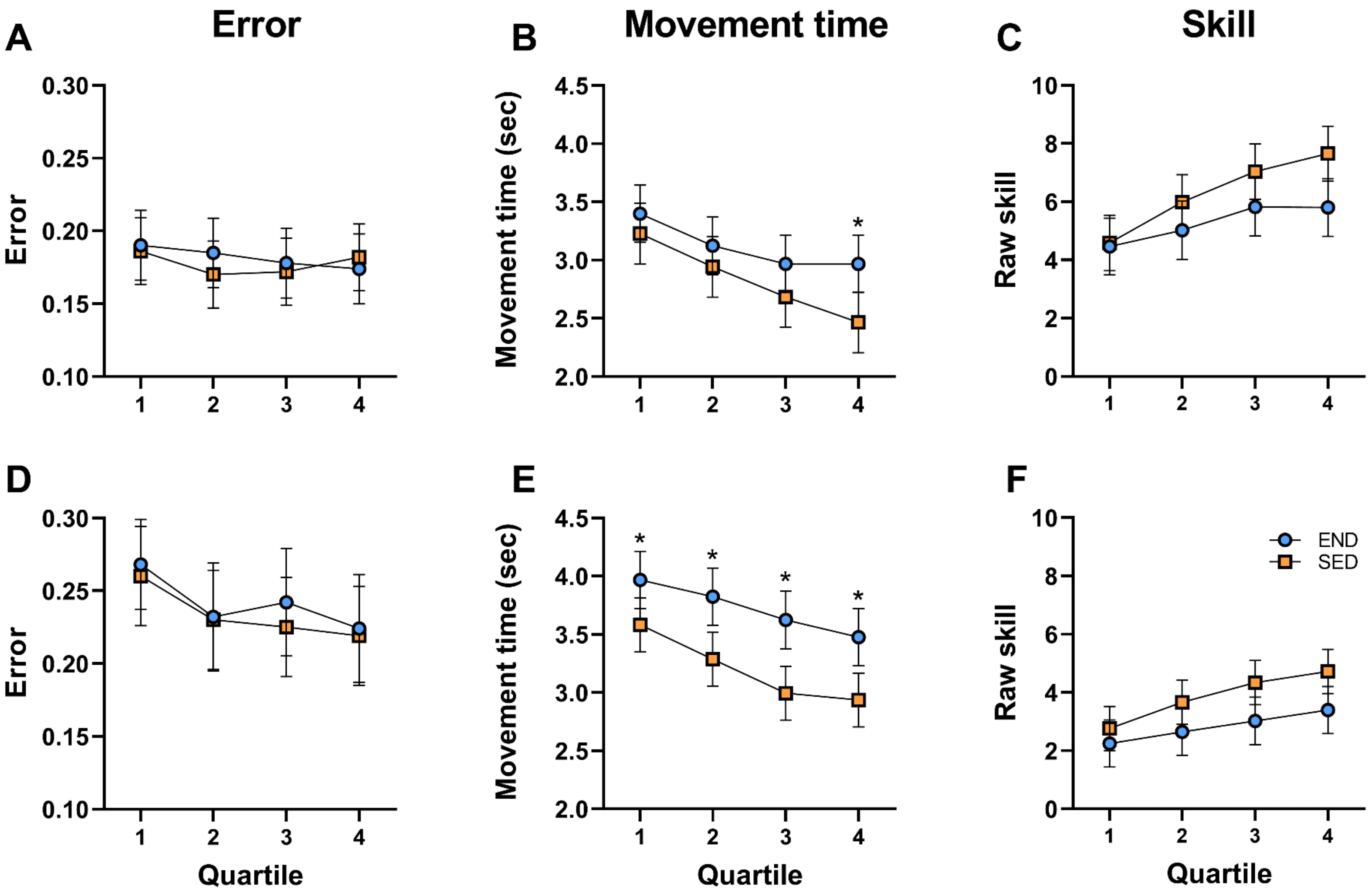

*__Figure 3.3:__ Visuomotor skill performance in active and sedentary groups. Data represent mean error scores, movement times and skill scores for the upper (A, B, C) and lower (D, E, F) limbs * $P < 0.05$ between groups. Abbreviations: END, endurance-trained; SED, sedentary.*

3.4.3 Change in MEP amplitude after visuomotor training

To compare skill training induced M1 plasticity, the change in MEP amplitude recorded 0 and 30 minutes after visuomotor training was expressed relative to baseline (Fig. 3.4). When comparing normalized MEP amplitude between groups for the upper limb (Fig. 3.4a), there was a greater training-related increase in MEP amplitude for the END group compared with the SED group (EMD = 121%, 95%CI [108, 134], $F_{1, 446} = 11$, $P = 0.001$). Furthermore, a significant interaction between group and muscle state was present ($F_{1, 446} = 7$, $P = 0.006$), with post-hoc comparisons showing that normalized MEP amplitude was greater in the END group compared with the SED group for measures in a resting (EMD = 140%, 95%CI [120, 164], $P < 0.001$) but not active (EMD = 104%, 95%CI [91, 121], $P = 0.7$) muscle. In addition, there was a significant interaction between muscle state and time point ($F_{1, 446} = 7$, $P = 0.001$), with post-hoc comparisons indicating that with a resting muscle, normalized MEPs were greater at 30 minutes than at 0 minutes (EMD = 142%, 95%CI [121, 166], $P < 0.001$). In contrast, normalized MEPs with an active muscle were greater at 0 minutes than 30 minutes (EMD = 117%, 95%CI [100, 136], $P = 0.04$).

For the lower limb, one outlier was present from the SED group in the active muscle state (> 2 standard deviations from mean). Accordingly, this condition was removed from relevant statistical tests. Lower limb analysis (Fig. 3.4b) revealed that there was a greater increase in MEP amplitude after visuomotor training in the END compared with the SED group (EMD = 123%, 95%CI [101, 149], $F_{1, 29} =4.8$, $P = 0.04$) and for measures in a resting compared with an active muscle state (EMD = 115%, 95%CI [108, 122], $F_{1, 450} = 18$, $P < 0.001$). In addition, normalised MEPs were larger at 30 minutes than at 0 minutes (EMD = 113%, 95%CI, 107, 120], $F_{1, 499} = 18$, $P < 0.001$). There was also a significant interaction between group and muscle state ($F_{1, 450} = 37.2$, $P < 0.001$), with post hoc analysis revealing that the END group demonstrated a greater increase in MEP amplitude compared with the SED group in a resting (EMD = 150%, 95%CI [121, 185], $P < 0.001$)

but not an active (EMD = 101%, 95%CI [83, 122], $P > 0.9$) muscle state. Furthermore, normalized MEP amplitude was significantly greater in a resting compared with an active muscle state for the END group (EMD = 141%, 95%CI [129,153], $P < 0.001$), however there was no difference between muscle states for the SED group (EMD 94%, 95%CI [86, 104], $P = 0.2$). In addition, there was a significant interaction between muscle state and time point ($F_{1,472} = 9$, $P = 0.004$). Post-hoc comparisons revealed greater normalized MEPs at 30 minutes than at 0 minutes with a resting muscle state (EMD = 123%, 95%CI [112, 135], $P < 0.001$), but no difference between timepoints with an active muscle state (EMD = 104%, 95%CI [97, 111], $P = 0.3$).

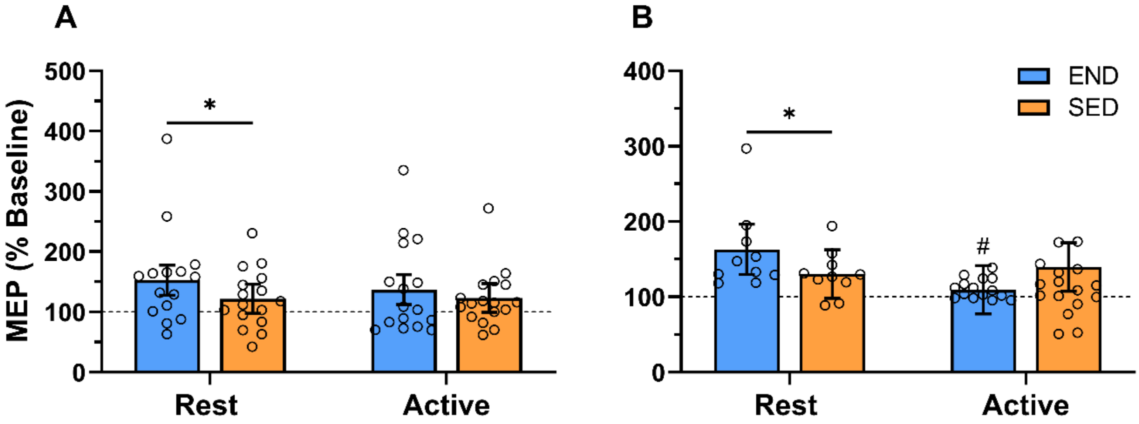

Figure 3.4: *Effect of motor learning on corticospinal output. Data represents mean MEP amplitude relative to baseline in a resting and active muscle state for the upper (A) and lower (B) limbs. Data are grouped for 0 and 30 min post time points. Unfilled symbols display subject means. *P < 0.001 between groups; # P < 0.001 compared with END group in a resting muscle state. Abbreviations: END, endurance-trained; SED, sedentary*

3.4.4 Paired-pulse measures of intracortical excitability after visuomotor training

In the upper limb, normalised SICI values (relative to baseline) showed that there was greater modulation of SICI with an active muscle compared with a resting muscle (EMD = 125%, 95%CI [112, 139], $F_{1,381} = 16$, $P < 0.001$; Fig. 3.5a). However, there was no difference between groups ($F_{1,31} = 0.2$, $P = 0.7$) or time points ($F_{1,476} = 1.2$, $P = 0.3$) and no interactions between factors (all $P > 0.1$). For SICF, there was no difference between groups ($F_{1,29} = 2.2$, $P = 0.1$), muscle states ($F_{1,359} = 0.6$, $P = 0.5$), or time points ($F_{1,458} =$

1.9, $P = 0.2$), however there was a significant interaction between group and muscle state ($F_{1,359} = 34$, $P < 0.001$). Post hoc tests revealed that normalised SICF was greater in the SED group for measures in resting (EMD = 137%, 95%CI [117, 160], $P < 0.001$), but not active (EMD = 90%, 95%CI [77, 104], $P < 0.2$; Fig. 3.5b) muscles. Furthermore, modulation of SICF in an active muscle was greater in the END group (EMD = 127%, 95%CI [114, 141], $P < 0.001$) and reduced in the SED group (EMD = 83%, 95%CI [76, 92], $P < 0.001$), compared with a resting muscle. There was also a significant interaction between muscle state and time point ($F_{1,402} = 7.1$, $P = 0.008$) with post hoc comparisons showing that normalised SICF was greater at 0 minutes than 30 minutes in a resting muscle (EMD = 115%, 95%CI [104, 127], $P = 0.005$), but not different between time points in an active muscle (EMD = 95%, 95%CI [87, 105], $P = 0.3$). LMM_{RM} analysis for upper limb ICF showed no significant effects or interactions of group, muscle state or time point (all $P > 0.05$; Fig. 3.5c).

Lower limb analysis of SICI modulation showed no difference between groups ($F_{1,33} = 0.1$, $P = 0.7$) or muscle states ($F_{1,307} = 2.6$, $P < 0.1$). However, there was a greater reduction in SICI at 30 minutes compared with 0 minutes post training (EMD = 111%, 95%CI [102,122], $F_{1,373} = 5.3$, $P = 0.02$). In addition, a significant interaction between muscle state and time point was present ($F_{1,337} = 24.9$, $P = 0.03$), with post hoc tests revealing SICI modulation is greater at 30 minutes than 0 minutes post training, for resting (EMD = 123%, 95%CI [107, 142], $P = 0.005$) but not active (EMD = 100, 95%CI [90, 112], $P = 0.9$) muscles. Lower limb SICF analysis revealed no difference between groups ($F_{1,30} = 2.3$, $P = 0.1$), muscle states ($F_{1,312} = 2.2$, $P = 0.1$), or time points ($F_{1,371} = 0.5$, $P = 0.5$). However, there was a significant interaction between group and muscle state ($F_{1,312} = 31$, $P < 0.000$). Similarly to the upper limb, post hoc tests revealed that normalised SICF was greater for the SED group compared with the END group in a resting (EMD = 133%, 95%CI [111, 160], $P = 0.003$) but not active (EMD = 97%, 95%CI [81, 115], $P = 0.7$; Fig.

3.5e) muscle. Additionally, normalised SICF with an active muscle was greater in the END group (EMD = 111%, 95%CI [105, 119], $P = 0.001$) and reduced in the SED group (EMD = 82%, 95%CI [76, 87], $P < 0.001$), compared with a resting muscle state. LMM_{RM} analysis for lower limb ICF showed no significant effects or interactions of group, muscle state or time point (all $P > 0.05$).

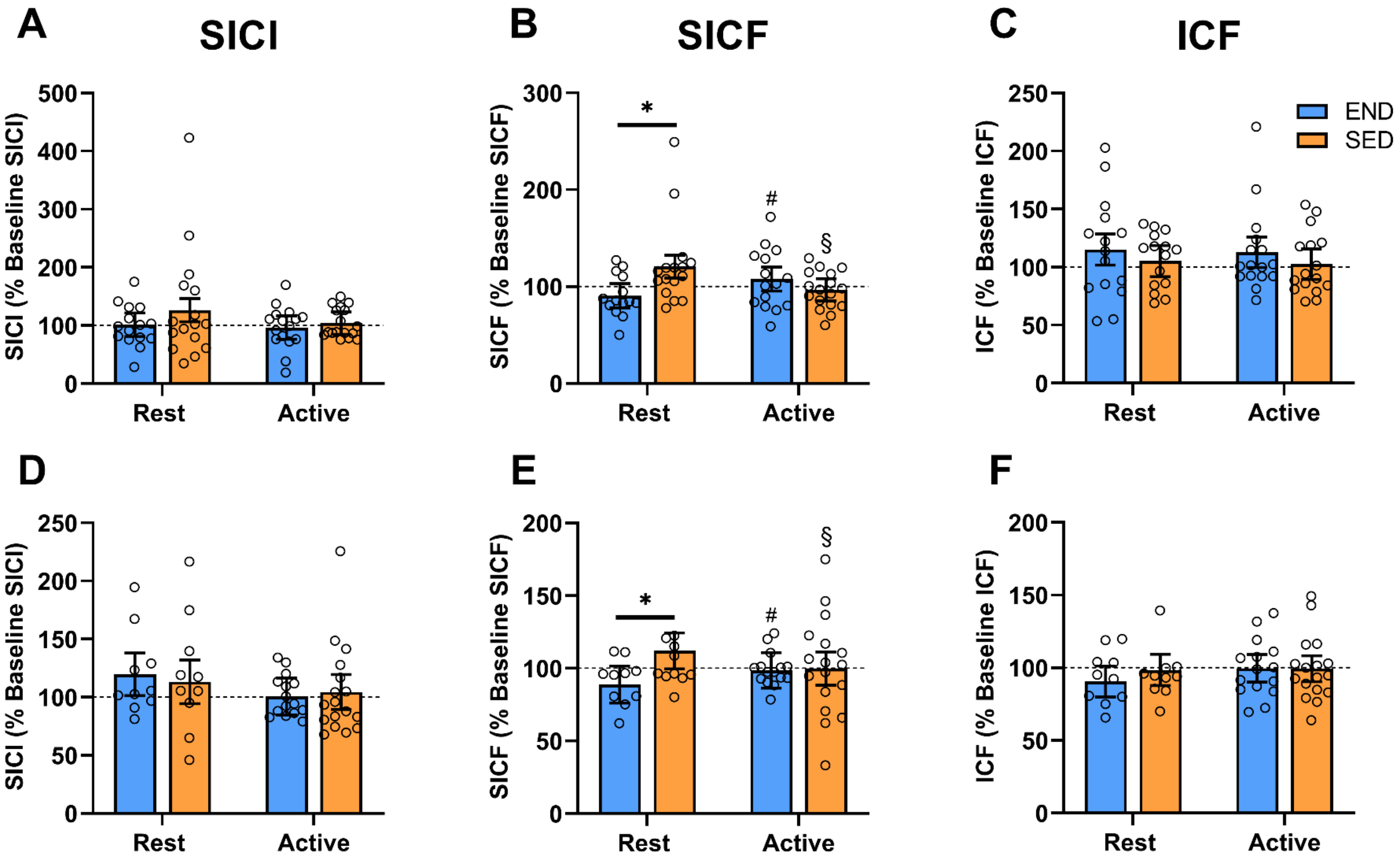

*__Figure 3.5:__ Effect of visuomotor skill learning on intracortical excitability. Data represent mean conditioned MEP amplitude relative to baseline for measures of SICI, SICF and ICF for the upper (A, B, C) and lower (D, E, F) limbs. Data are grouped for 0 and 30 post time points. Data points above 100% indicate a reduction of SICI (A, D) and greater SICF and ICF (B, C, E, F) after visuomotor skill training. Unfilled symbols display subject means. * $P < 0.005$ between groups; # $P < 0.005$ compared with END group in a resting muscle state; § $P < 0.001$ compared with SED group in a resting muscle state. Abbreviations: END, endurance-trained; SED, sedentary.*

3.5 Discussion

This study investigated how regular endurance training in experienced cyclists influences M1 plasticity and motor skill acquisition in both upper and lower limb muscles. The main outcomes of the study are: 1) there was a minimal difference in baseline M1 and intracortical excitability for upper and lower limbs in END and SED participants; 2) END individuals displayed greater M1 plasticity with visuomotor training; 3) there was an increase in SICF after visuomotor training in the SED but not the END group and 4) visuomotor skill learning was comparable between groups for both upper and lower limbs. These findings suggest that regular physical activity and exercise can influence skill training induced M1 plasticity, and these effects not only occur in the cortical representations of the trained muscles, but are also observed in muscles not directly involved with the exercise.

3.5.1 Corticospinal and intracortical excitability in END and SED participants

Prior research indicates that M1 excitability can be modulated by numerous factors such as advancing age (Pitcher et al. 2003, Sale and Semmler 2005, Oliviero et al. 2006, Rozand et al. 2019) and with specialized hand use, such as in musicians (Rosenkranz et al. 2007). In addition, several studies show that regular physical activity influences M1 excitability, but the results vary in upper and lower limb muscles. For example, physically active participants who complete at least 10,000 steps per day show reduced corticospinal excitability for lower limb muscles (Hassanlouei et al. 2017), but there is no difference in corticospinal excitability between active and sedentary participants for upper limb muscles (Cirillo et al. 2009, Rozand et al. 2019). In our study that assesses both upper and lower limb muscles, we show no difference in baseline corticospinal excitability between END and SED participants, which supports previous studies in upper but not lower limb muscles. A number of factors could contribute to this discrepancy between studies involving the lower limbs, such as differences in TMS coil type (figure-of-eight *vs.* double-cone), the maximum TMS intensities used (150% AMT *vs.* TMS intensity at MEP plateau) and the participant population tested (highly trained cyclists *vs.* increased habitual physical activity). In particular, Hassanlouei et al. (2017) showed that the largest differences in MEP amplitudes between groups (high vs. low activity) occurred at high TMS intensities (>150% AMT), which were above those used in the present study. Nonetheless, we show that participation in long-term cycling exercise does not influence corticospinal excitability in upper and lower limb muscles.

In an effort to examine changes in intracortical excitability with long-term exercise, we also used paired-pulse TMS to compare baseline differences in intracortical excitation (SICF, ICF) and inhibition (SICI) between END and SED participants. In support of our findings with single-pulse TMS, we show no differences in any measure of intracortical excitability between END and SED participants for the trained lower limb, suggesting that long-term cycling exercise does not result in chronic adaptations in M1 intracortical circuits targeting the trained muscle. Although regular exercise also failed to influence activity of intracortical excitatory circuits for the upper limb muscle, the activity of intracortical inhibitory circuits appears to be upregulated in the END group when assessed in the resting upper limb muscle.

3.5.2 Regular cycling exercise influences skill training-induced cortical plasticity

An increase in M1 excitability with skill training is thought to be a crucial first step in motor learning (Sanes and Donoghue 2000, Ziemann et al. 2004) and this has been demonstrated with TMS after both ballistic (Rogasch et al. 2009, Hinder et al. 2011) and visuomotor (Cirillo et al. 2011, Veldman et al. 2015, Mooney et al. 2019) learning tasks. Given that increased physical activity is associated with greater TMS-induced plasticity (Cirillo et al. 2009), and that TMS-induced and skill training-dependent plasticity involve overlapping mechanisms (Ziemann et al. 2004), we hypothesized that there would be greater M1 plasticity with visuomotor training in END participants. In support of this hypothesis, we found that the modulation of MEP amplitude with visuomotor skill training was greater in people who participated in regular cycling exercise. This MEP facilitation occurred with the performance of a visuomotor skill task, which is thought to involve increases in synaptic strength through LTP-like mechanisms (Muellbacher et al. 2002, Ziemann et al. 2004), and is likely to involve changes in M1 circuits, as similar visuomotor tasks do not alter spinal excitability (Giesebrecht et al. 2012). These findings suggest that regular exercise may upregulate LTP processes that contribute to increased M1 plasticity with visuomotor skill training. Interestingly, these findings were dependent on the muscle state, with increased M1 plasticity observed in a resting muscle but not when it was active. The reason for this difference is unclear, but it is possible that an increase in spinal excitability (Maertens de Noordhout et al. 1992, Weavil et al. 2015) and a decrease in intracortical inhibition (Ridding et al. 1995) that occurs during muscle activation may mask any change in M1 plasticity when measured in an active muscle.

A key feature of the current study is that the increase in M1 plasticity with visuomotor training was evident in both upper and lower limbs, suggesting that the effects of regular physical activity on M1 plasticity were not confined to the muscle directly involved in the exercise. Based on this, a plausible explanation for an increase in M1 plasticity in people who regularly exercise is an increase in cerebrovascular function coupled with consequent changes in the neurochemical environment within M1. For example, endurance exercise is known to result in increased angiogenesis in M1 (Kleim et al. 2002), with long-term training resulting in increased regional cerebral blood flow at rest and during task performance (Xiong et al. 2009). This heightened blood flow is expected to be accompanied by an increase in delivery of key neurochemicals that are important for synaptic plasticity. In particular, acute voluntary exercise is known to enhance levels of brain derived-neurotrophic factor (BDNF) (Ferris et al. 2007, Etnier et al. 2016), which is an important mediator of LTP (Schinder and Poo 2000, Gottmann et al. 2009) and can enhance use-dependent plasticity and motor learning (Klintsova et al. 2004, Skriver et al. 2014). Long-term exercise is also thought to alter circulating BDNF levels in humans, with some studies showing increases (Szuhany et al. 2015) and others showing decreases (Currie et al. 2009), although it has been suggested that this latter finding could reflect a beneficial increase in BDNF clearance and uptake in the brain with chronic exercise (Knaepen et al. 2010). Other factors that can contribute to an exercise-related upregulation of plasticity mechanisms include an increase in neuromodulatory transmitters (e.g. dopamine) and an increased energy supply to the brain (e.g. lactate) (see Taubert et al. 2015 for review), which is likely to produce widespread effects in M1 that can influence both upper and lower limb cortical representations.

To quantify the effect of regular exercise on the modulation of intracortical circuitry with visuomotor learning, we used paired-pulse TMS to examine the activity of GABAergic inhibitory (SICI) and glutamatergic facilitatory (SICF, ICF) intracortical circuits. Although there is some evidence that acute lower limb exercise can modulate upper limb SICI (Smith et al. 2014) and ICF (Singh et al. 2014, Neva et al. 2017) we found no difference in the modulation of these intracortical circuits between END and SED participants with visuomotor training in upper or lower limbs. In contrast, we found a reduced modulation of SICF in both upper and lower limbs after visuomotor training in END participants. This finding was surprising, given that SICF is not modulated by acute exercise in individuals with high or low physical activity levels (Lulic et al. 2017). However, increased SICF has been associated with improved bimanual dexterity in older adults (Clark et al. 2011), although decreased SICF in older adults has also been associated with increased unilateral pegboard performance (Clark et al. 2011, Opie et al. 2020). Additionally, SICF potentiation has been observed with action observation and movement planning when grasping different objects (Cattaneo et al. 2005, Cretu et al. 2020). Furthermore, a repetitive TMS intervention (I-wave TMS) known to modulate SICF can also influence motor performance in healthy young adults (Benwell et al. 2006, Teo et al. 2012), suggesting that its modulation may have some behavioural relevance. Nonetheless, we show that regular exercise reduces motor-training related modulation of glutamatergic SICF circuitry in upper and lower limbs, but the physiological and functional significance of this finding remains to be determined.

3.5.3 Visuomotor skill performance and learning in upper and lower limbs

To examine motor skill acquisition in upper and lower limbs, we used an isometric visuomotor task that relies on a combination of movement speed and accuracy for optimal task performance (Reis et al. 2009), where initial visuomotor skill (baseline) was similar between END and SED participants in upper and lower limbs. Although the same task was performed in each group, the END participants preferred to use a strategy that involved a slower movement time to achieve the task, particularly for the lower limbs. However, overall skill performance was not different between groups, indicating that task difficulty was likely comparable between END and SED participants. Therefore, we suggest that the different movement strategies used by each group is unlikely to contribute to the observed differences in plasticity, but further studies would be required to confirm this. Despite the differences in movement time, there were no differences between groups in skill acquisition over the four quartiles of visuomotor training in upper and lower limbs. This outcome was unexpected, given that the END group demonstrated enhanced skill training-induced M1 plasticity (enhanced corticospinal output after training), which has been demonstrated as an important factor of skill learning (Muellbacher et al. 2001, Ziemann et al. 2004). However, numerous studies have also shown that changes in M1 excitability do not align well with increased performance during motor learning (McDonnell and Ridding 2006, Gelli et al. 2007). Accordingly, it has recently been postulated that such changes are unlikely to be a necessary prerequisite of skill acquisition, but simply a neurophysiological response to motor practice (Bestmann and Krakauer 2015, Berghuis et al. 2017). Despite this, previous studies have shown that both acute (Roig et al. 2012, Statton et al. 2015) and long-term (Bakken et al. 2001, Seidel et al. 2017) exercise has the potential to improve some forms of motor skill learning. The reason for the differential outcomes remains unclear, but could be related to a range of factors, such as the type of task used (ballistic, balance, visuomotor etc.), duration of motor practice, or differential characteristics of the population groups that were not assessed (specialized hand use, i.e. playing a musical instrument or video gaming).

This study was the first to demonstrate that long-term cycling exercise involving the lower limbs alters skill training-induced M1 plasticity in both upper and lower limbs. This suggests that long-term, regular exercise produces widespread effects throughout M1 that influences use-dependent plasticity mechanisms in upper and lower limb cortical representations. However, under the present experimental conditions, this elevated skill training-dependent plasticity response did not result in improved learning, as the acquisition of a novel visuomotor skill was comparable between groups for both upper and lower limbs. Despite no benefit for motor skill acquisition, further studies are needed to determine whether long-term exercise may be advantageous for other aspects of motor learning, such as the long-term retention of the motor skill.

Chapter IV

Motor cortex plasticity is greater in endurance-trained cyclists following acute exercise

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Contribution to the Paper	Conceptualization, methodology, formal analysis, investigation, data curation, writing: original draft, writing: review & editing, visualization.		
Over percentage (%)	75		
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	16/11/2021

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate’s stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate to include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate’s stated contribution.

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4. Motor cortex plasticity is greater in endurance-trained cyclists following acute exercise.

4.1 Abstract

Introduction: Previous research using transcranial magnetic stimulation (TMS) has shown that motor cortex plasticity is greater in people who undertake regular exercise. Furthermore, there is increased corticospinal plasticity after acute exercise in untrained participants, with these effects greatest after high-intensity interval training (HIIT). This study aimed to examine the effect of regular endurance exercise on corticospinal plasticity following an acute bout of HIIT.

Methods: 14 endurance-trained (END) cyclists (mean ± SD; 23 ± 3.8 years) and 14 sedentary (SED) individuals (22 ± 1.8 years) performed two experimental sessions. One session included an acute bout of HIIT (stationary cycling), while another session involved no-exercise (control). Following exercise (or control), I-wave periodicity repetitive TMS (iTMS) was used (1.5 ms interval, 180 pairs for 15 mins) to induce plasticity within primary motor cortex. Motor evoked potential (MEP) amplitudes from a hand muscle were recorded at baseline, after HIIT (or control) exercise, and after iTMS.

Results: Following HIIT exercise, measures of corticospinal and intracortical excitability were unchanged from baseline in both groups (all $P = 0.05$). Following iTMS, END participants showed a greater increase in MEP amplitude when preceded by HIIT compared with the sedentary group ($P < 0.05$). Furthermore, with groups combined, the iTMS-induced increase in MEP amplitude was greater after HIIT exercise than control ($P < 0.001$). No difference was observed between groups after iTMS for the control session ($P > 0.9$).

Conclusion: Corticospinal plasticity induced by iTMS was greater in endurance-trained cyclists following HIIT. Physical activity levels are therefore an important consideration for understanding factors that contribute to exercise-induced plasticity.

4.2 Introduction

It is now well understood that exercise has the potential to modulate the excitability of various cortical networks and promote neuroplasticity, which refers to the ability of the nervous system to modify the strength of its neural connections (Sanes and Donoghue 2000). Increased plasticity with exercise has been demonstrated in numerous regions of the brain, including the hippocampus, cerebellum, and cerebral cortex (Colcombe and Kramer 2003, Kramer and Erickson 2007, Müller-Dahlhaus and Ziemann 2015, Schättin et al. 2018). Furthermore, it is now known that there are numerous physiological adaptations that may underpin the upregulation of neuroplasticity with exercise. For example, long-term endurance exercise has been shown to promote cerebral angiogenesis and resultant blood flow (Kleim et al. 2002, Xiong et al. 2009), as well as modulate circulating neurotrophins such as BDNF (Currie et al. 2009, Szuhany et al. 2015), which have been shown to promote neuroplasticity (Schinder and Poo 2000). In addition, experimental evidence suggests that the increased plasticity associated with exercise may have functional benefits, such as greater cognitive function (Sibley and Etnier 2002, McDonnell et al. 2011), and improved motor skill learning (for review, see Taubert et al. 2015) and retention (Roig et al. 2012). Despite these developments, it remains unclear if the effects of acute exercise on brain plasticity are modified in individuals that have a history of endurance activity.

Within the motor system, transcranial magnetic stimulation (TMS) has been regularly used to examine the role of acute exercise on excitability and plasticity of the corticospinal pathway. Although numerous studies indicate that an acute bout of exercise does not modify corticospinal excitability (McDonnell et al. 2013, Singh et al. 2014, Mooney et al. 2016, Smith et al. 2018), some recent evidence suggests that acute exercise does have the potential to acutely modify corticospinal excitability under some circumstances. For example, there is increased motor cortex excitability after acute exercise, but only in people with high physical activity levels (Lulic et al. 2017), or following exercise with specific intensity/duration (Baltar et al. 2018). Furthermore, a decrease in short-interval intracortical inhibition (SICI, a TMS measure indexing intracortical inhibition mediated by gamma aminobutyric acid type A receptors; Kujirai et al. 1993, Di Lazzaro et al. 1998) is more commonly observed after acute exercise (Yamaguchi et al. 2012, Smith et al. 2014, Stavrinos and Coxon 2017, Andrews et al. 2020), and this is evident in both highly active and inactive individuals (Lulic et al. 2017).

Along with changes in motor cortex excitability, it is commonly accepted that acute aerobic exercise has the potential to improve neuroplasticity induced by TMS. Numerous studies have now demonstrated that a single bout of acute exercise promotes neuroplasticity in response to various plasticity-inducing TMS interventions. For example, an acute bout of continuous exercise has been shown to increase the plastic response to continuous theta burst stimulation (McDonnell et al. 2013), whereas acute high-intensity interval training (HIIT) potentiates the plastic response to both paired associative stimulation (PAS) (Mang et al. 2014) and intermittent theta burst stimulation (iTBS) (Andrews et al. 2020). A recent review by Mellow et al. (2020) concluded that longer bouts of low-intensity continuous exercise, or shorter bouts of HIIT, are the most effective exercise protocols for promoting plasticity of the motor cortex.

In contrast to an acute bout of exercise, an active lifestyle that involves regular physical exercise may also result in alterations to motor cortex excitability and plasticity. For example, baseline corticospinal excitability is altered in physically active individuals under some circumstances (Cirillo et al. 2009, Hassanlouei et al. 2017, Rozand et al. 2019), but this is not always a consistent finding (Kumpulainen et al. 2015, Lulic et al. 2017). Similarly, a history of regular physical activity has also been shown to facilitate TMS-induced plasticity using PAS (Cirillo et al. 2009). However, it is unknown whether participation in long-term physical activity or exercise influences plasticity following an acute bout of exercise.

Therefore, the purpose of this study was to determine whether long-term endurance training modifies TMS-induced plasticity following a bout of acute aerobic exercise. The endurance-trained group consisted of highly trained cyclists with a history of endurance-based exercise, whereas a sedentary control group consisted of individuals who did not partake in regular exercise. Each participant underwent an exercise protocol that involved 20 minutes of aerobic exercise which has been suggested to acutely modulate the expression of separate I-waves (a series a descending waves resulting from trans-synaptic activation of corticospinal neurons from interneuronal circuits) (Di Lazzaro et al. 2012, Neva et al. 2021). Following exercise, I-wave periodicity repetitive TMS (iTMS) was utilised to induce plasticity, as this intervention has been developed to target synaptic events within the intracortical circuits activated by TMS (Thickbroom et al. 2006), producing robust effects (for review, see Kidgell et al. 2016) that are known to influence cortical circuits (Di Lazzaro et al. 2007). As a marker of M1 plasticity, we measured the change in corticospinal excitability and M1 intracortical facilitation and inhibition after HIIT exercise and after iTMS. Given that acute exercise (McDonnell et al. 2013, Mang et al. 2014, Andrews et al. 2020) and regular physical activity (Cirillo et al. 2009) is known to improve motor cortex plasticity, it was hypothesised that TMS-induced corticospinal plasticity after HIIT would be greater in endurance-trained participants.

4.3 Methods

Twenty-eight participants were recruited from the university and the broader community to participate in the current study. This study included two participant groups that were recruited based on their physical activity levels, which were assessed using the International Physical Activity Questionnaire (IPAQ) short form (Craig et al. 2003). An endurance-trained group, referred to as *END* group, consisted of 14 highly-trained cyclists (mean age ± SD: 23 ± 3.8, 3 female) who each had a total physical activity level of greater than 3000 MET-minutes of weekly exercise (McDonnell et al. 2013, Smith et al. 2014), including 6 or more hours of cycling but no formal upper body exercise. A sedentary group, referred to as *SED* group, included 14 (22 ± 1.8, 4 female) inactive individuals who had a total physical activity level of no more than 1500 MET-minutes per week (Craig et al. 2003) and did not participate in any regular aerobic training. Additionally, a physical activity readiness questionnaire (PAR-Q) was used to assess suitability to complete exercise with acceptable risk (Thomas et al. 1992). Exclusion criteria for all participants included a history of concussion, neurological disease, or ongoing use of psychoactive medication (antidepressants, sedatives, etc.). All experimentation was approved by the University of Adelaide Human Research Ethics Committee and conducted according to the Declaration of Helsinki. Each participant provided written, informed consent prior to inclusion in the study.

4.3.1 Experimental arrangement and procedures

Each participant attended two experimental sessions. One exercise session, referred to as the *HIIT* session, included a 20-minute bout of HIIT cycling. A separate session utilised a control exercise protocol in place of the cycling exercise and is referred to as the *control* session. The experimental protocol and all TMS measurements were consistent between the two sessions, with the exercise bout being the differential factor (Fig. 4.1). These sessions were randomised and conducted a minimum of 7 and a maximum of 30 days apart. A Polar M400 heart rate monitor (polar electro) was used for both sessions to assess resting heart rate at baseline, which was taken after 5 minutes of quiet sitting, and to monitor heart rate throughout the exercise or control block. For TMS measurements, subjects were seated in a comfortable chair, with feet placed flat on the floor and the right hand relaxed on a benchtop. Surface electromyography (EMG) was recorded from the first dorsal interosseous (FDI) muscle of the right hand using two Ag-AgCl electrodes, in accordance with the European recommendations of surface electromyography (Hermens et al. 2000). An earth strap was fitted around the wrist to ground the electrodes. EMG signals were amplified (300×) and band-pass filtered (20 Hz high pass, 1 kHz low pass) using a CED1902 signal conditioner (Cambridge Electronic Design, Cambridge, UK) before being digitised at 2 kHz using a CED1401 interface. Recordings were stored on a computer for offline analysis.

4.3.2 Transcranial magnetic stimulation (TMS)

In both sessions, TMS was applied to the left M1 using a figure-of-eight coil connected to two Magstim 200^2 magnetic stimulators through a Bistim module (Magstim, Dyfed, UK). The coil was positioned to induce a posterior-anterior (PA) current within M1 by directing the handle posterior-laterally at an angle of approximately 45 degrees to the midline. The coil was held tangentially to the scalp over the optimal location for producing MEPs in the target muscle. This location was marked on the scalp for reference and checked throughout the experiment. TMS was delivered at 0.2 Hz with a 10% variation between trials to avoid anticipation of the stimulus.

The resting motor threshold (RMT) was assessed at the beginning of the session, which was defined as the minimum stimulus intensity required to produce a motor evoked potential (MEP) with an amplitude of ≥ 50 µV in at least 5 out of 10 trials in the relaxed target muscle. Active motor threshold (AMT) was defined as the minimum stimulus intensity required to produce an MEP with an amplitude of > 200 µV in at least 5 out of 10 trials during a mild contraction of the FDI. A 'test TMS' intensity was defined as the stimulus intensity required to produce a peak-to-peak MEP amplitude of ~1 mV (range, 0.5 mV – 1.5 mV) when averaged over 20 trials. Paired-pulse TMS was used to assess intracortical excitability with measures of SICI, short-interval intracortical facilitation (SICF) and intracortical facilitation (ICF). Measures of SICI utilised a sub-threshold conditioning pulse set at 80% of AMT (Kujirai et al. 1993, Opie and Semmler 2014) and an interstimulus interval (ISI) between conditioning and test stimuli of 2 ms (Kujirai et al.

1993). Measurements of SICF were completed with a subthreshold conditioning intensity of 90% RMT, which was applied 1.5 ms following the test pulse (Ziemann et al. 1998, Peurala et al. 2008, Lulic et al. 2017). ICF was assessed using a subthreshold conditioning stimulus of 80% RMT applied 10 ms prior to the test pulse (Wagle-Shukla et al. 2009). The test stimulus for all paired-pulse measures was set at the 1 mV intensity. Conditioning intensities did not vary between test blocks. However, for paired-pulse blocks at post-exercise and post-iTMS time points, the test TMS intensity was adjusted to produce a mean response that was within 30% of the baseline mean, where necessary.

At baseline, a single-pulse test block (n=20 trials) was applied to measure corticospinal excitability at the predetermined test TMS intensity. Subsequently, a paired-pulse test block was completed to assess measurements of SICI, SICF, and ICF. These test blocks comprised 15 test TMS stimuli, as well as 15 trials of each paired-pulse condition (SICI, SICF, and ICF) in a pseudorandomised order. Following baseline TMS measurements, participants completed either the HIIT or control exercise block. Both the single- and paired-pulse test blocks were then repeated 10 minutes after the completion of exercise (or control), before iTMS was administered (~25 minutes after exercise completion). Single- and paired-pulse TMS blocks were repeated at 5 and 30 minutes following iTMS (Fig. 4.1)

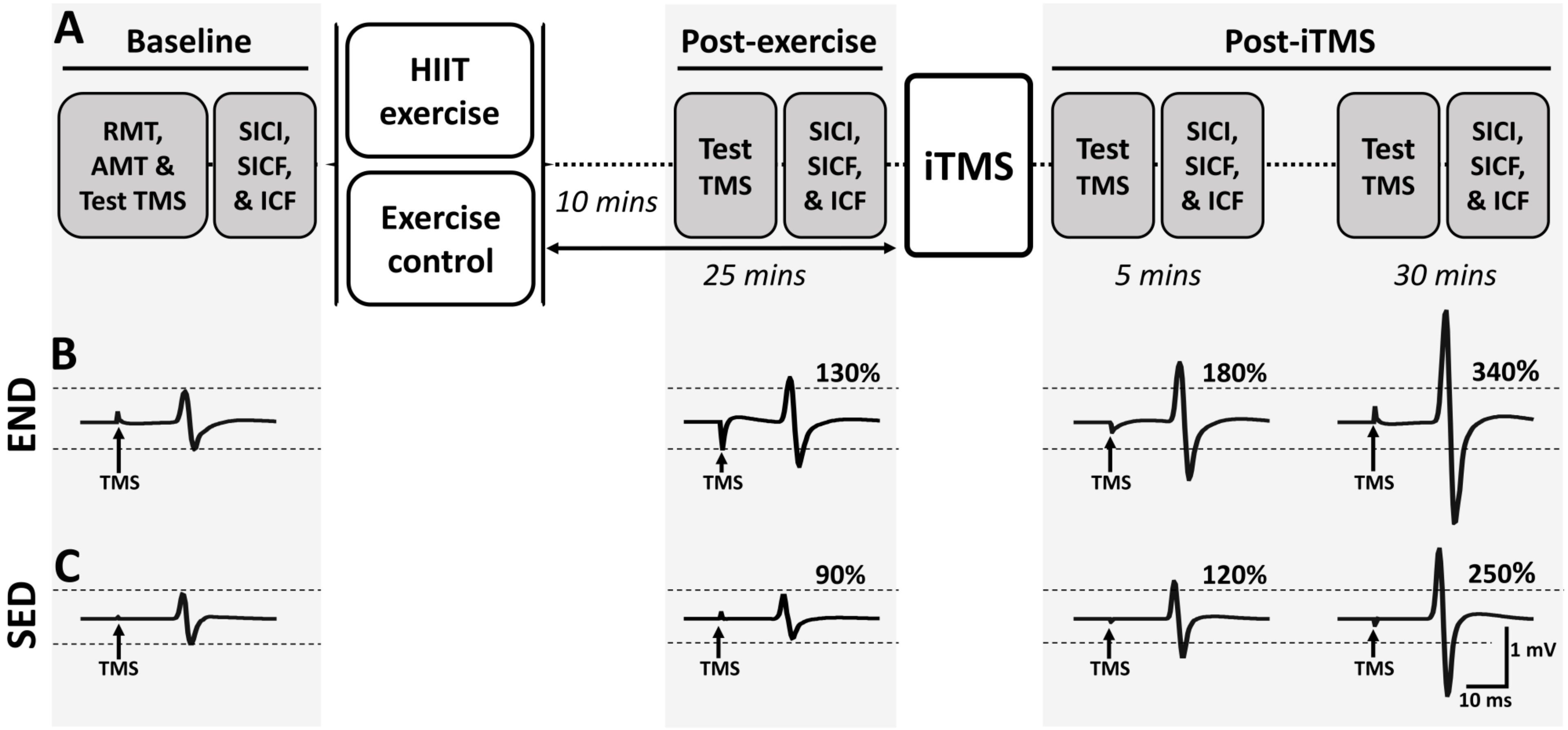

Figure 4.1: Experimental protocol. *Session protocol (A). Traces display test MEP responses recorded in a single END (B) and SED (C) participant at the four TMS testing time points. Dashed lines indicate the mean baseline MEP amplitude and inset percentage values represent the mean MEP amplitude relative to baseline.*

4.3.3 Exercise protocol

For the exercise session, participants pedalled on a stationary cycling ergometer (Wattbike, UK) for 20 minutes in a HIIT protocol (Stavrinos and Coxon 2017) as this exercise modality is thought to be optimal for promoting motor cortical plasticity (Mellow et al. 2020). To adjust for personal differences in age, cardiorespiratory function and other conditions, exercise intensity was personalised using each individual's maximum heart rate (HR_{max}) and resting heart rate (RHR), which were used to calculate heart rate reserve ($HRR = HR_{max} - RHR$). The HIIT cycling involved 3 minutes at low intensity (RHR + 50% HRR) followed by 2 minutes of high-intensity cycling (RHR + 90% HRR), repeated four times for a total duration of 20 minutes. At the completion of the exercise block, participants continued to pedal with low resistance for 2 minutes to cool down before sitting quietly for a further 8 minutes prior to application of TMS. During the control session, participants sat on the stationary bicycle in the same position as during the exercise session, but were instructed not to pedal. Prior to commencing exercise (or control), participants completed two maximal voluntary contractions (MVCs) of each hand via surface electromyography. During exercise and control blocks, participants were instructed to keep their hands relaxed on the handlebars to avoid activation of the FDI muscle. To assess activation of the FDI during both sessions, surface EMG was recorded from the FDI muscle of both hands with visual feedback provided to the participants. During HIIT and control exercise blocks, data was recorded for heart rate and average power, as well as a self-reported rating of perceived exertion (Borg 1970), and an affect score of feeling and emotion (Hardy and Rejeski 1989). These measures were taken at the end of each low or high-intensity block (or corresponding time points for the control session).

4.3.4 I-wave periodicity repetitive TMS (iTMS)

Plasticity within M1 circuits was induced using iTMS, which consisted of 180 pairs of stimuli (ISI of 1.5 ms) applied every 5 seconds for a period of 15 mins (Thickbroom et al. 2006). The intensity of TMS was the same for each stimulus in the pair, and was set to produce a paired-pulse MEP of 0.5 – 1.0 mV at the start of the block (Thickbroom et al. 2006). Small ice packs were applied to the TMS coil prior to the intervention to alleviate coil heating during iTMS.

4.3.5 Data analysis

During offline analysis of MEP data from the resting session, trials containing EMG activity > 20 μV (peak-to-peak amplitude) in the 100 ms before TMS application were discarded. MEPs were measured peak-to-peak for each trial and expressed in mV. To assess the influence of intracortical networks on MEP amplitude, individual MEPs recorded in response to paired-pulse stimulation were expressed as a percentage of the mean test TMS amplitude. Accordingly, normalised values greater than 100% reflect facilitation, whereas normalised values of less than 100% reflect inhibition. To identify exercise-induced changes in both corticospinal and intracortical excitability, normalised MEPs from single- and paired-pulse TMS blocks recorded after exercise were expressed as a percentage of the mean values from baseline. To identify iTMS-induced changes in excitability, normalised MEPs from single- and paired-pulse TMS blocks recorded 5- and 30-minutes post iTMS were expressed as a percentage of the mean values from the post-exercise time point.

To quantify FDI muscle activity during exercise, EMG recorded during these bouts was normalised to maximum (EMG_{max}), which was obtained from the MVCs recorded before exercise. Activity greater than 5% of EMG_{max} was categorised as a 'burst' of activity, with the total number of bursts and mean burst amplitude recorded for each session and group. Furthermore, cumulative burst time was expressed relative to the total recording time and referred to as total active time. As a measure of the net activity of the FDI during exercise, the intensity of the total burst activity was expressed relative to time (%MVC/h) and expressed as the summed area of activity.

For exercise-related measures, exercise intensity was expressed as %HRR (above RHR), and power was normalised to body weight and expressed as watts:kg. Exercise data (power: weight, exercise intensity [%HRR], RPE, and affect scores) from the HIIT block were grouped into low and high-intensity categories. Mean values were expressed for each group for the control session and for group and intensity separately for the HIIT session.

4.3.6 Statistical analysis

The normality of data was assessed using Shapiro-Wilk tests, and log transformations were applied when this test indicated deviation from a normal distribution. All data are displayed in the original form (non-transformed) for clarity. Unpaired t-tests were used to compare mean weekly exercise load (MET-minutes) between groups (END, SED). Baseline single-pulse measures of corticospinal excitability (test TMS) and paired-pulse measures of intracortical excitability (SICI, SICF and ICF) were compared between groups and sessions (HIIT, control) using separate two-factor repeated measures linear mixed model (LMM_{RM}) analyses. For analysis of EMG activity recorded during the exercise blocks, the number of bursts, mean burst amplitude, active time and summed area of activity were compared between groups and sessions using separate two-factor LMM_{RM} analyses. Furthermore, non-transformed data were used within Spearman's correlation analyses (which are known to be particularly robust against outliers, Mukaka 2012) to determine whether there was an association between activation of FDI during HIIT and the exercise- and iTMS-induced modulation of corticospinal excitability. Separate analyses were run for each of the exercise EMG variables (number of bursts, mean burst amplitude, active time and summed area of activity) against the normalised TMS measures of excitability recorded after exercise and iTMS. These correlation analyses were undertaken with both groups combined and separately. Outcomes from the HIIT exercise block (power: weight, exercise intensity [%HRR], RPE, and affect score) were compared independently between group and intensity (low, high) using a two-factor LMM. To compare overall outcomes between the HIIT exercise and control blocks, measures of exercise intensity (%HRR), RPE, and affect score were compared between groups and sessions in separate two-factor LMM_{RM} analyses. The change in corticospinal and intracortical excitability with exercise and iTMS were assessed with separate one-sample *t*-tests with Bonferroni correction, whereby normalised MEP responses from post-exercise and post-iTMS time points were compared to a 100% test value for each condition (group, session). Furthermore, normalised TMS measures (test TMS, SICI, SICF, ICF) from the post-exercise time point were compared between groups and sessions using separate two-factor LMM_{RM} analyses. TMS measures after iTMS (normalised to post-exercise) were compared between groups, sessions and time points (5-post, 30-post) using a three-factor linear mixed model with repeated measures (LMM_{RM}). Raw test TMS amplitudes from the paired-pulse test blocks were also compared between group, session and time point (baseline, post-exercise, 5 post- and 30 post-iTMS) using a three-factor LMM_{RM}. Spearman’s analyses were used to assess correlations between normalised TMS measures (test TMS, SICI, SICF, ICF) recorded after the exercise block and the change in test MEP amplitude with iTMS, as well as to compare each of the paired-pulse measures recorded after iTMS (normalised to post-exercise) to the test MEP recorded after iTMS. For tests of intracortical excitability, only participants who displayed the expected response at baseline (inhibited MEP for SICI and facilitated MEP for SICF and ICF) were included in analyses.

For all models, subject was included as a random effect, and significant main effects and interactions were further investigated using custom contrasts with Bonferroni correction. As the aims of this study were concerned with the effect of regular endurance training on plasticity, only interactions in which group was a factor were investigated. For all significant effects and interactions of LMM analyses, the estimated mean difference (EMD) and associated 95% confidence interval from post hoc pairwise comparisons have been presented as non-standardised measures of effect size (Opie et al. 2020). EMDs were exponentiated from the log values derived from individual pairwise comparisons of estimated marginal means, and therefore represent a ratio of the means being compared. Tables and figures display mean values of all participants included in the corresponding analysis. Data are displayed as mean ± 95% confidence interval unless stated otherwise. For clarity, 5- and 30-minute time points are grouped in figures.

4.4 Results

All participants completed the study in full and without any adverse effects. The IPAQ showed a significantly greater weekly exercise load of 5850 MET-minutes for the END group compared to 1139 MET-minutes for the SED group ($P < 0.001$). Furthermore, based on the self-reported activity levels within the IPAQ, the END group accumulated 54% of their total weekly MET-minutes through *vigorous*-intensity exercise (predominantly endurance-based cycling), compared to only 12% for the SED group, which was mostly obtained through normal daily activities (i.e. lifting/carrying).

4.4.1 Baseline measures of corticospinal and intracortical excitability

Table 4.1 displays the baseline TMS data for each participant group. There were no differences in mean stimulator intensity for RMT, AMT or test TMS, and no difference in mean test TMS amplitude between groups or sessions (all $P > 0.05$). For paired-pulse TMS, participants were excluded from the analysis if they did not show the expected response at baseline, so there were 11 END participants for SICI, 13 END participants for SICF, and 10 END and 10 SED participants for ICF from a total of 14 participants in each group. Mean baseline responses from paired-pulse measures of SICI, SICF and ICF were expressed normalised to the test MEP response. Baseline measures of SICI and ICF did not vary between groups or sessions and no significant interactions were obtained (all $P > 0.05$). While baseline SICF was not different between groups ($F_{1, 25} = 0.03$, $P = 0.9$), it was significantly greater in the control compared with the HIIT session (EMD = 112%, 95%CI [103, 123], $F_{1, 208} = 6.8$, $P = 0.01$). Differences between sessions also varied by group (group x session interaction, $F_{1, 208} = 5.8$, $P = 0.02$), with post hoc tests revealing that for the END group, baseline SICF was greater in the control session compared with the HIIT exercise session (EMD = 125%, 95%CI [110, 142], $P < 0.001$), whereas there was no difference between sessions for the SED group (EMD = 101%, 95%CI [89, 114], $P = 0.9$).

4.4.2 HIIT exercise

For recordings of FDI muscle activity during exercise, there was no difference between groups for measures of number of EMG bursts, mean burst amplitude, total active time or summed area of activity (all $P > 0.06$). Furthermore, values did not differ between sessions for mean burst amplitude ($P = 0.7$), but were greater in the HIIT exercise block compared with control for number of bursts, active time and summed area of activity (all $P < 0.005$; Table 4.2).

Table 4.1: *Baseline TMS data.*

	CONTROL		HIIT	
	END	**SED**	**END**	**SED**
TMS intensity - RMT (% MSO)	41.5 (37.9, 45.1)	41.5 (38.0, 45.0)	41.1 (38.1, 44.1)	40.7 (37.1, 44.3)
TMS intensity - AMT (% MSO)	34.3 (31.4, 37.2)	35.3 (31.9, 38.6)	35.3 (32.6, 38.0)	34.8 (31.6, 37.9)
TMS intensity - Test (% MSO)	51.4 (44.9, 57.9)	49.8 (44.8, 54.8)	51.8 (46.6, 57.0)	49.6 (44.4, 54.7)
Test response (mV)	0.8 (0.7, 0.9)	0.9 (0.8, 1.0)	0.9 (0.8, 1.0)	0.9 (0.8, 1.0)
SICI (% test)	48.1 (41.2, 55.1)	39.6 (34.1, 45.0)	45.3 (39.7, 50.9)	47.0 (41.2, 52.8)
SICF (% test)	219.8 (201.1, 238.6)	207.9 (190.0, 225.8)	178.2* (160.5, 196.0)	194.8 (180.6, 209.0)
ICF (% test)	170.4 (151.9, 189.0)	177.5 (158.8, 196.2)	218.0 (182.5, 253.6)	149.3 (136.7, 162.0)

*Data represent mean (95%CI). * $P < 0.005$ compared with control session (END group).*

Table 4.2: *Exercise EMG data.*

	CONTROL			**HIIT**		
	END	**SED**	**ALL**	**END**	**SED**	**ALL**
Number of bursts	52.2 (0.3, 104.2)	76.2 (-13.3, 165.6)	63.7 (16.8, 110.7)	145.7 (73.1, 218.2)	197.0 (96.2, 297.8)	171.3* (112.9, 229.8)
Mean Burst Amplitude (%MVC)	8.3 (5.5, 11.2)	9.8 (5.3, 14.3)	9.0 (6.6, 11.4)	10.6 (8.3, 13.0)	10.8 (9.0, 12.6)	10.7 (9.4, 12.1)
Active time (% total time)	2.2 (-0.1, 4.6)	3.0 (-0.7, 6.6)	2.6 (0.6, 4.6)	12.2 (3.2, 21.3)	15.1 (5.7, 24.6)	13.7* (7.6, 19.8)
Summed area of activity (%MVC/h)	0.2 (0.1, 0.4)	1.4 (-1.3, 4.1)	0.8 (-0.4, 2.0)	1.4 (0.2, 2.6)	1.9 (0.5, 3.2)	1.6* (0.8, 2.5)

*Data represent mean (95%CI). * $P < 0.005$ compared with control session.*

Table 4.3: *Exercise data for high-intensity interval training (HIIT) and control sessions.*

		CONTROL	HIIT		
		Rest	**Low intensity**	**High intensity**	**Total**
Power to weight ratio (watts:kg)	END	0.0 (0.0)	1.4 (1.2, 1.5)	4.6 (4.4, 4.8)	3.0 (2.7, 3.3)*
	SED	0.0 (0.0)	0.7 (0.6, 0.8)	2.3 (2.1, 2.4)	1.5 (1.3, 1.6)
	ALL	0.0 (0.0)	1.0 (0.9, 1.1)	3.4 (3.2, 3.7)+	2.2 (2.0, 2.4)
Exercise Intensity (%HRR)	END	13.2 (11.7, 14.6)	51.3 (50.0, 52.6)	88.5 (87.2, 89.8)+	69.9 (66.3, 73.5)
	SED	14.1 (12.2, 15.9)	53.5 (51.8, 55.2)	85.9 (83.2, 88.5)+	69.7 (66.3, 73.1)
	ALL	13.6 (12.5, 14.8)	52.4 (51.3, 53.5)	87.2 (85.7, 88.7)+	69.8 (67.3, 72.3)^
Rating of Perceived Exertion	END	6.6 (6.6, 6.7)	9.4 (8.8, 10.0)*	16.6 (16.2, 17.0)+	13.0 (12.2, 13.8)^
	SED	6.4 (6.3, 6.5)	10.5 (9.8, 11.2)	15.9 (15.4, 16.5)+	13.2 (12.6, 13.9)^
	ALL	6.5 (6.5, 6.6)	9.9 (9.5, 10.4)	16.3 (15.9, 16.6)+	13.1 (12.6, 13.6)^
Affect Score	END	4.4 (4.2, 4.5)	2.9 (2.5, 3.4)	1.7 (1.3, 2.2)	2.3 (2.0, 2.7)*
	SED	3.7 (3.5, 4.0)	2.0 (1.5, 2.5)	0.3 (-0.3, 1.0)	1.2 (0.7, 1.6)
	ALL	4.0 (3.9, 4.2)	2.5 (2.1, 2.8)	1.0 (0.6, 1.4)+	1.7 (1.5, 2.0)^

Data represent mean (95%CI). For HIIT sessions, data are grouped by intensity, with means for each displayed separately, as well as a 'total' column displaying values grouped by intensity. * $P < 0.05$ compared with SED group; $^{+}$ $P < 0.001$ compared with the low intensity; ^ $P < 0.001$ compared with control session.

Table 4.3 displays the outcomes of HIIT exercise and control sessions (power: weight, exercise intensity, RPE and affect score). Analysis of data from the HIIT session revealed significantly greater power to weight ratio for the END group compared with the SED group (EMD = 222%, 95%CI [183, 270], $F_{1, 26} = 70$, $P < 0.001$), and during high compared with low intensity phases of HIIT (EMD = 380%, 95%CI [343, 422], $F_{1, 64} = 653$, $P < 0.001$), but there was no interaction between factors ($F_{1, 64} = 1.3$, $P = 0.3$). For exercise intensity (%HRR), values did not differ between groups ($F_{1, 26} = 0.001$, $P > 0.9$), but were significantly greater during high compared with low intensity phases of HIIT (EMD = 166%, 95%CI [163, 169], $F_{1, 82} = 3156$, $P < 0.001$). Differences between high and low intensities also varied between groups (exercise intensity x group interaction, $F_{1, 82} = 18$, $P < 0.001$). Post hoc tests revealed that exercise intensity (%HRR) was greater for high compared with low intensity phases of exercise for each group (both $P < 0.001$). Analysis of RPE revealed no difference between groups ($F_{1, 26} = 0.5$, $P = 0.5$), however RPE scores were greater in the high compared with the low intensity phase of HIIT (EMD = 167%, 95%CI [160, 175], $F_{1, 65} = 533$, $P < 0.001$). Differences between intensities also varied by group ($F_{1, 65} = 12.1$, $P = 0.001$) with post hoc tests revealing that for the low intensity phase, RPE scores were lower in the END group compared with the SED group (EMD = 90%, 95%CI [81, 99], $P = 0.04$), however there was no difference between groups for the high intensity phase (EMD = 105%, 95%CI [95, 116], $P = 0.3$). Furthermore, RPE values were greater in the high compared with the low intensity phase of HIIT for each group (both $P < 0.001$). For affect scores, values were significantly greater for the END group compared with the SED group (EMD = 112%, 95%CI [100, 123], $F_{1, 26} = 4.4$, $P < 0.05$), and for the low compared with high intensity phase of HIIT (EMD = 114%, 95%CI [112, 116], $F_{1, 88} = 83$, $P < 0.001$), but there was no interaction between factors ($F_{1, 88} = 2.4$, $P = 0.1$). Comparison between HIIT and control exercise blocks revealed significantly greater values in the HIIT session compared with the control session for measures of exercise intensity (EMD = 569%, 95%CI [490, 662], $F_{1, 135}$ = 517, $P < 0.001$) and RPE (EMD = 192%, 95%CI [187, 196], $F_{1, 241}$ = 2769, $P < 0.001$), whereas affect score was lower in the HIIT session compared with the control session (EMD = 79%, 95%CI [77, 84], $F_{1, 111}$ = 102, $P < 0.001$). For RPE, differences between sessions also varied by group, with post hoc tests revealing that RPE values were significantly greater for the HIIT session in END and SED groups (both $P < 0.001$).

4.4.3 Corticospinal and intracortical excitability following HIIT exercise

Figure 4.2 displays mean MEP amplitudes and individual participant responses from TMS measures of corticospinal and intracortical excitability recorded at the post-exercise time point (expressed relative to baseline). MEP amplitudes did not differ from baseline for either group after HIIT or control (one-sample *t*-tests; all $P > 0.05$). $\mathrm{LMM_{RM}}$ analysis for normalised MEP amplitudes revealed no difference between groups ($F_{1, 26}$ = 1.1, $P = 0.3$), however values were significantly lower for the HIIT session compared with the control session (EMD = 72%, 95%CI [62, 83], $F_{1, 266}$ = 20, $P < 0.001$; Fig. 4.2a). Differences in normalised MEP amplitude between sessions also varied by group (group x session interaction, $F_{1, 266}$ = 8.7, $P = 0.003$), with post hoc tests showing that the normalised MEP amplitude was lower in the HIIT session compared with the control session (EMD = 58%, 95%CI [47, 71], $P = 0.001$) in the END group, whereas there was no difference between sessions for the SED group (EMD = 89%, 95%CI [73, 109], $P = 0.3$). Furthermore, END participants displayed greater normalised MEP amplitude compared with the SED group in the control session (EMD = 148%, 95%CI [103, 213], $P = 0.04$), but there was no difference between groups for the HIIT session (EMD = 96%, 95%CI [67, 138], $P = 0.8$).

Paired-pulse TMS measures of SICI, SICF and ICF did not differ from baseline in either group after the HIIT or control sessions (one-sample *t*-tests; all $P > 0.05$). $\mathrm{LMM_{RM}}$ analysis between groups and sessions showed no significant effects or interactions for SICI or SICF (all $P > 0.05$). For ICF, normalised values were not different between groups ($F_{1,\,18} = 0.3$, $P = 0.6$) or sessions ($F_{1,\,158} = 0.04$, $P = 0.8$), however a significant interaction between group and session was present ($F_{1,\,158} = 9.2$, $P = 0.003$; Fig. 4.2d). Post hoc tests revealed that normalised ICF values were significantly lower in the HIIT session compared with the control session in the END group (EMD = 82%, 95%CI [68, 100], $P < 0.05$), however normalised ICF was greater in the HIIT session compared with the control session in the SED group (EMD = 124%, 95%CI [103, 151], $P = 0.02$).

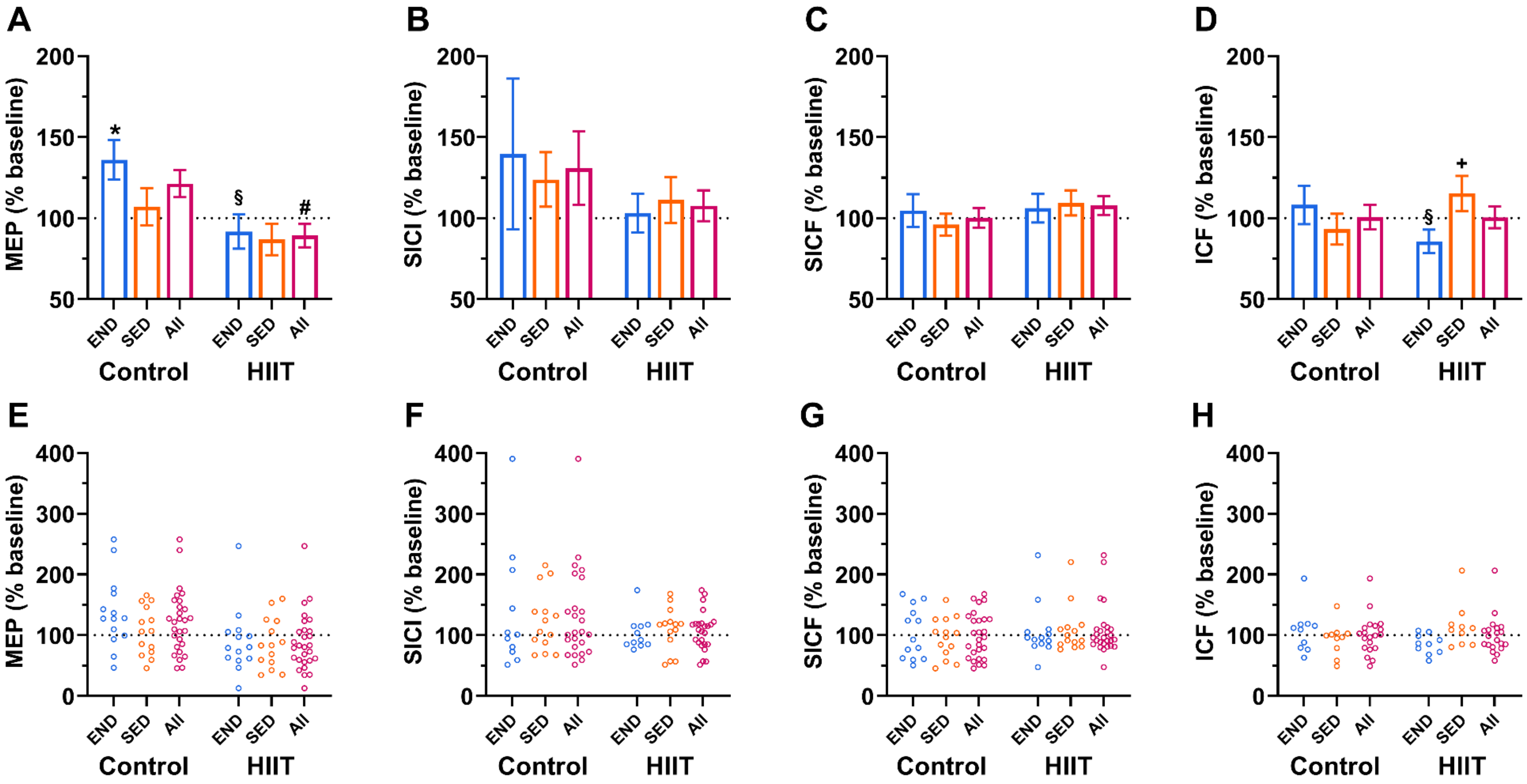

***Figure 4.2: Effect of high-intensity interval training (HIIT) and control on TMS measures**. Data are recorded at the post-exercise time point and represent mean response amplitudes (top row) and individual subject mean (bottom row) relative to baseline for test TMS (A, E), SICI (B, F), SICF (C, G) and ICF (D, H). Data points above 100% indicate a reduction of SICI (B, F) and greater test TMS (A, E), SICF (C, G), and ICF (D, H), compared with baseline. * $P < 0.05$ compared with SED group (control session); § $P < 0.05$ compared with control session (END group); # $P < 0.001$ compared with control session; + $P < 0.05$ compared with control session (SED group). Abbreviations: END, endurance-trained; SED, sedentary.*

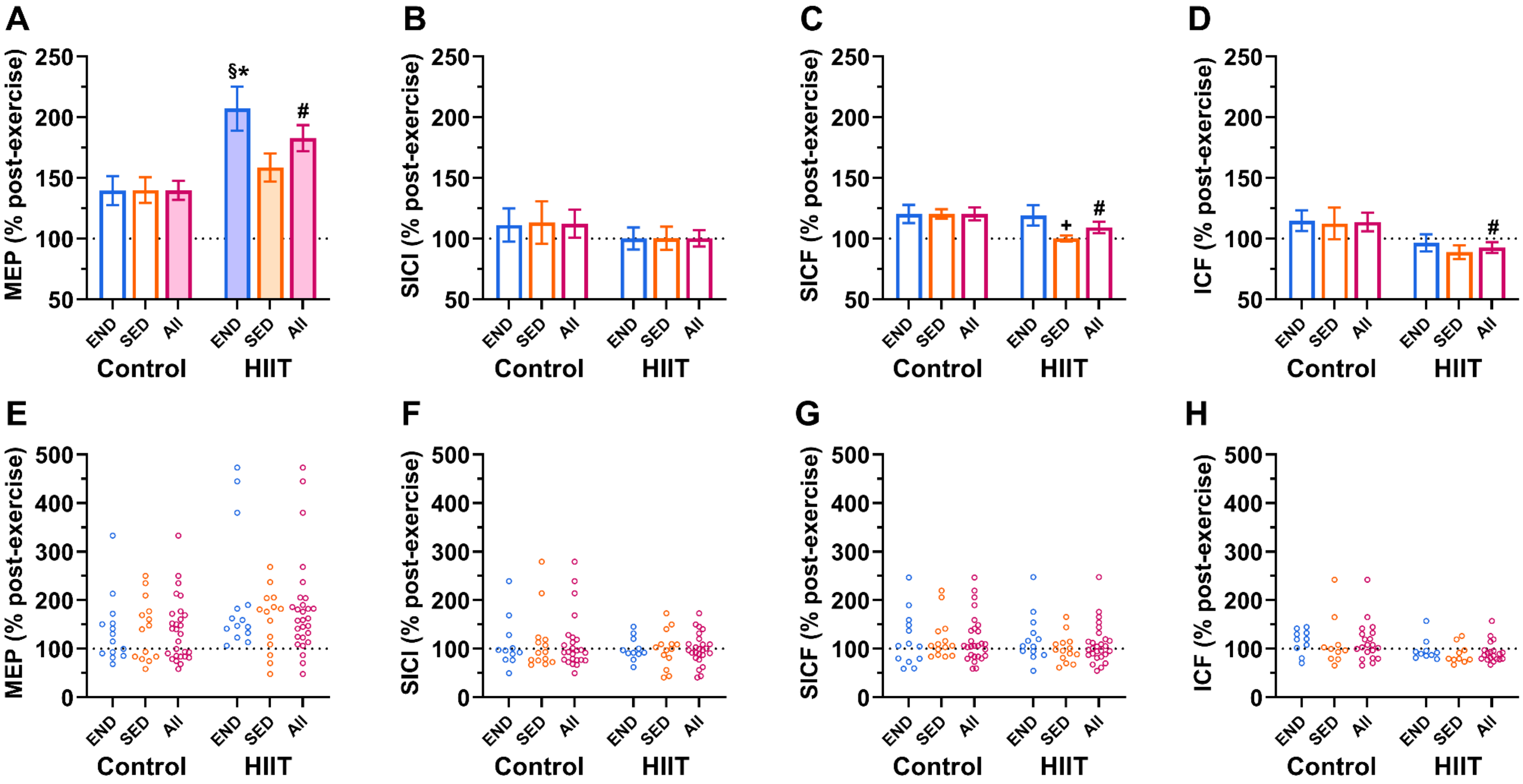

Figure 4.3: Effect of iTMS on TMS measures following HIIT or control*. Data are grouped across 5- and 30-minute time points and represent mean response amplitudes (top row) and individual subject means (bottom row) relative to the post-exercise time point for test TMS (A, E), SICI (B, F), SICF (C, G), and ICF (D, H). Data points above 100% indicate a reduction of SICI (B, F) and greater test TMS (A, E), SICF (C, G), and ICF (D, H), compared with the post-exercise time point. § P < 0.001 compared with control session (END group); * P < 0.05 compared with SED group (HIIT session); # P < 0.05 compared with control session (groups combined); + P < 0.005 compared with control session (SED group). Abbreviations: END, endurance-trained; SED, sedentary.*

4.4.4. Corticospinal and intracortical excitability following iTMS

The effect of iTMS on corticospinal and intracortical excitability is shown in Fig. 4.3, where the MEP amplitudes after iTMS have been normalised to the MEP amplitudes obtained after the HIIT or control session (referred to as the post-exercise time point). When compared with post-exercise, one-sample *t*-tests showed that test MEP amplitudes were not significantly modulated in the control session for either group (both $P > 0.05$), however the test MEP amplitudes increased in both the END ($P = 0.007$) and SED ($P = 0.004$) group in the HIIT session. Furthermore, with groups combined, test MEP amplitudes increased with iTMS in both the control ($P = 0.004$) and HIIT ($P < 0.001$) sessions. LMM_{RM} analysis of test MEP amplitude revealed there was no difference between groups ($F_{1,\,26} = 1.2$, $P = 0.3$), however normalised MEP amplitudes after iTMS were significantly greater in the HIIT exercise session compared with the control session (EMD = 133%, 95%CI [119, 148], $F_{1,\,484} = 26.3$, $P < 0.001$; Fig. 4.3a), and at the 30 minute time point compared with 5 minutes post iTMS (EMD = 114%, 95%CI [102, 127], $F_{1,\,504} = 5.6$, $P = 0.02$). Differences between sessions also varied by group (group x session interaction, $F_{1,\,484} = 6.9$, $P < 0.01$), with post hoc tests revealing that normalized MEP amplitudes after iTMS were greater in the END group compared with SED group after HIIT exercise (EMD = 134%, 95%CI [100, 179], $P < 0.05$). However, there was no difference between groups for the control session (EMD = 100%, 95%CI [75, 134], $P > 0.9$). Additionally, normalised MEP amplitudes for the END group were greater in the HIIT session compared with the control session (EMD = 153%, 95%CI [132, 179], $P < 0.001$), whereas there was no difference between sessions for the SED group (EMD = 115%, 95%CI [98, 134], $P = 0.07$). Importantly, individual participant data reveals that no participant in the END group showed a decrease in MEP amplitude after iTMS in the HIIT exercise session, whereas 6 END individuals had a decrease in MEP amplitude with iTMS following control exercise. For comparison, there were also 6 SED individuals to exhibit a decrease in MEP amplitude in the control session, which reduced to 3 in the HIIT session, a smaller shift than demonstrated by the END group.

For all paired-pulse blocks, test TMS intensity was adjusted to produce a mean response that was within 30% of the baseline, where necessary. Test MEP amplitudes recorded from the paired-pulse blocks did not vary between groups ($F_{1, 26} = 0.1$, $P = 0.8$) or sessions ($F_{1, 779} = 1.3$, $P = 0.2$) however differed over time point ($F_{3, 914} = 5.8$, $P = 0.001$), being greater than baseline (mean = 1.07 mV) at 5 post (mean = 1.33 mV, EMD = 117%, 95%CI [102, 135], $P = 0.01$) and 30 post (mean = 1.31 mV, EMD = 123%, 95%CI [107, 142], $P < 0.001$). When examining the change in paired-pulse TMS responses after iTMS (relative to post-exercise), there were no significant changes in SICI, SICF or ICF (one-sample *t*-tests; all $P > 0.05$). $\mathrm{LMM_{RM}}$ analysis of SICI after iTMS revealed no significant effects of group ($F_{1, 23} = 0.2$, $P = 0.7$), session ($F_{1, 385} = 0.008$, $P > 0.9$), or time point ($F_{1, 449} = 1.2$, $P = 0.3$), and no significant interactions were present (all $P > 0.09$). For SICF, there was no effect of group ($F_{1, 25} = 0.2$, $P = 0.6$) or time point ($F_{1, 432} = 1.7$, $P = 0.2$), however the change in SICF with iTMS was greater in the control session compared with the HIIT exercise session (EMD = 93%, 95%CI [86, 100], $F_{1, 339} = 4.4$, $P = 0.04$; Fig. 4.3c). Differences between sessions also varied by group (group x session interaction, $F_{1, 339} = 5.3$, $P = 0.02$) with post hoc tests revealing that for the SED group, the change in SICF after iTMS was greater in the control session compared with the HIIT session (EMD = 85%, 95%CI [77, 94], $P = 0.002$), whereas there was no difference between sessions for the END group (EMD = 101%, 95%CI [91, 112], $P = 0.9$). There was also a significant interaction between group and time point ($F_{1, 432} = 4.9$, $P = 0.03$) with post hoc tests showing that for the SED group, normalised SICF was significantly greater 30 mins after iTMS compared with 5 mins after iTMS (EMD = 113%, 95%CI [103, 124], $P = 0.01$), although there was no difference between time points for the END group (EMD = 97%, 95%CI [88, 107], $P = 0.5$). ICF analysis revealed no effect of group ($F_{1, 18} = 1.0$, $P = 0.3$)

or time point ($F_{1, 336}$ = 0.02, P = 0.9), but the change in ICF with iTMS for all participants (both groups combined) was greater in the control session compared with the HIIT exercise session (EMD = 84%, 95%CI [76, 94], $F_{1, 278}$ = 10, P = 0.002; Fig. 4.3d). No other effects or interactions were present.

4.4.5 Correlations between corticospinal and intracortical excitability after exercise and iTMS.

Spearman's analyses were used to assess correlations between normalised TMS measures (test TMS, SICI, SICF, ICF) recorded after the exercise block, and changes in test MEP amplitude with iTMS. For the control session, there was a significant negative correlation between normalised test MEP values recorded after control exercise, and test MEP values recorded after iTMS (r = -0.60, P = 0.002). On a group level, the same comparison was significant for the END (r = -0.60, P = 0.03), but not the SED (r = -0.50, P = 0.08) group. For the control session, all other comparisons were non-significant. For the HIIT session, there were no significant correlations between post-exercise and post-iTMS measures (all $P > 0.2$). Further correlation analyses were utilised to compare each of the paired-pulse measures recorded after iTMS (normalised to post-exercise) to the test MEP recorded after iTMS. The only significant correlation was between normalised measures of SICF and test MEP in the HIIT session, where there was a significant positive correlation in the END (r = 0.70, P = 0.02) but not the SED (r = -0.3, P = 0.2) group. No other significant correlations were present.

4.4.6 Correlation between muscle activity during exercise and TMS measures

Analysis revealed no significant correlations between any of the EMG measures of muscle activity (number of bursts, mean burst amplitude, active time and summed area of activity), and TMS measures of excitability recorded after exercise (all $P > 0.2$). Correlation analyses with test MEP amplitude recorded after iTMS (plasticity effect), revealed a weak but significant negative correlation between number of bursts and normalised MEP amplitude with groups combined (r = -0.41, *P* = 0.04), as well as between total burst time and normalised MEP amplitude for the SED (r = -0.60, *P* = 0.03) but not the END (r = -0.04, *P* = 0.9) group.

4.5 Discussion

This study investigated how regular endurance training in experienced cyclists influences TMS-induced plasticity following an acute bout of HIIT exercise. The main outcomes of the study were: 1) Measures of corticospinal and intracortical excitability did not vary between groups at baseline, 2) HIIT exercise did not significantly modulate corticospinal or intracortical excitability in either END or SED participants, 3) END participants displayed greater motor cortex plasticity following HIIT than SED participants, and 4) TMS-induced plasticity was greater following HIIT than control (no-exercise).

4.5.1 Changes in corticospinal and intracortical excitability with exercise

Numerous studies have examined the change in corticospinal excitability after an acute bout of exercise, with mixed results. The majority of studies have shown that an acute bout of exercise does not influence corticospinal excitability (McDonnell et al. 2013, Mang et al. 2014, Smith et al. 2014, Mooney et al. 2016, Neva et al. 2017, Opie and Semmler 2019, Andrews et al. 2020). However, several studies have shown an increase in corticospinal excitability following acute exercise of moderate or greater intensity (MacDonald et al. 2019, Opie and Semmler 2019, Nicolini et al. 2020). Furthermore, one recent study by Lulic et al. (2017) demonstrated that acute exercise upregulates corticospinal excitability in individuals who are highly active, but not in a low physical activity group. The present findings demonstrate no effect of acute HIIT exercise on corticospinal excitability regardless of exercise history. A possible reason for this discrepancy between the current study and that of Lulic and colleagues is that each implemented a different intensity of acute exercise. Lulic et al. (2017) utilised continuous moderate intensity aerobic activity, whereas the present study involved 20 minutes of HIIT cycling. Although there was no statistically significant change in MEP amplitude with exercise, normalised MEP amplitudes were significantly reduced after HIIT compared with control in END participants. This may indicate a potential lowering of motor cortical excitability after HIIT exercise. However, this is confounded by a larger MEP amplitude in END participants after the control exercise. The reason for this elevated MEP in the resting control session is heavily influenced by 2 END subjects who showed ~250% increase in MEP after 20 minutes of rest. The reason for such changes are unclear, however they could result from other circadian (chronotype), attentional (focussed/unfocussed), pharmacological (medications) or genetic (e.g. BDNF polymorphisms) factors that can influence plasticity in different subject populations (for review, see Ridding and Ziemann 2010).

There are now a considerable number of studies to indicate that acute exercise transiently downregulates $GABA_A$-mediated SICI (Takahashi et al. 2011, Yamaguchi et al. 2012, Singh et al. 2014, Smith et al. 2014, Stavrinos and Coxon 2017, Yamazaki et al. 2019). It is therefore surprising that the present results indicate no change in SICI with HIIT in END or SED participants. This outcome is also in contrast to previous findings showing that acute exercise decreases SICI in both highly and minimally active individuals (Lulic et al. 2017). Despite this, an insensitivity of SICI to exercise has also been reported following moderate-intensity continuous (Mooney et al. 2016) and HIIT (Nicolini et al. 2020) exercise. Interestingly, a recent study by Neva et al. (2021) showed exercise-induced disinhibition of SICI, but only when SICI circuits were selectively targeted with anterior-posterior (AP) induced current, and not when a conventional PA-induced current was used (as in the present study). These findings suggest that examining SICI with a PA current may be less sensitive for detecting changes in SICI circuits with exercise. However, it remains difficult to reason the contrasting results between the present study and those that have demonstrated SICI modulation with exercise when tested with PA TMS (Yamaguchi et al. 2012, Singh et al. 2014, Stavrinos and Coxon 2017, Andrews et al. 2020).

Compared with SICI, few studies have investigated the influence of acute exercise on intracortical facilitation (both SICF and ICF). Current evidence suggests that SICF may be either upregulated (Neva et al. 2017) or not modulated (Lulic et al. 2017, Yamazaki et al. 2019) with low-moderate intensity continuous exercise. Evidence surrounding the modulation of ICF with exercise is similarly variable. Singh et al. (2014) demonstrated an increase in ICF after exercise in a population of moderately active individuals, whereas Lulic et al. (2017) indicated a decrease in ICF with exercise in groups of both high and low levels of habitual physical activity. Here we demonstrate that neither SICF nor ICF are modulated following acute HIIT exercise in either END or SED participants. Our findings indicate that HIIT exercise does not influence the modulation of intracortical excitability, and that this effect is not modified by long-term endurance training in cyclists.

4.5.2 TMS-induced corticospinal plasticity is greater following HIIT

There is now considerable evidence that physical activity has the potential to modulate TMS-induced plasticity. An increase in plasticity following exercise has been demonstrated with various repetitive TMS paradigms such as PAS (Mang et al. 2014, Singh et al. 2014), iTBS (Andrews et al. 2020) and continuous theta burst stimulation (cTBS) (McDonnell et al. 2013). The plasticity-inducing TMS protocol used in the current study (iTMS) has been shown to upregulate corticospinal excitability through repetitive activation of interneuronal networks within M1. In particular, iTMS uses repetitive paired-pulse TMS at intervals of 1.5 ms, which correlate with the timing of indirect (I) waves present with the descending volley generated by a TMS pulse (Di Lazzaro et al. 2012), with early and late I waves representing the activation of separate interneuronal circuits (Opie et al. 2021). While the exact mechanisms of iTMS are unknown, it is thought to be a form of spike-timing-dependent (Hebbian) plasticity, that results from increased synaptic efficacy of these I-wave generating neural networks (Thickbroom et al. 2006, Kidgell et al. 2016). Interestingly, recent work by Neva and colleagues suggested that acute aerobic exercise can modulate the expression of I-waves independently (Neva et al. 2021), similarly to an iTMS intervention. Given this, results of the present study are unsurprising, as we provide new evidence showing that acute exercise enhances plasticity using iTMS, which specifically targets intracortical networks that contribute to the generation of the MEP.

With the present data, we cannot determine exactly which mechanisms are contributing to the increased TMS-induced plasticity with acute HIIT exercise. However, it may be associated with an acute increase in cerebral blood flow and modification of the neurochemical environment in a manner that promotes synaptic plasticity. For example, acute exercise is understood to enhance levels of lactate, brain derived-neurotrophic factor (BDNF) and uncarboxylated osteocalcin (Ferris et al. 2007, Saucedo Marquez et al. 2015, Etnier et al. 2016, Nicolini et al. 2020) which are important mediators of LTP/synaptic plasticity (Schinder and Poo 2000, Kuipers and Bramham 2006, Gottmann et al. 2009, Oury et al. 2013, Gundersen et al. 2015). Interestingly, evidence suggests that more than 70% of circulating BDNF originates from the brain, both at rest and during exercise, which has been shown to result in a two to threefold increase in circulating BDNF (Rasmussen et al. 2009). Another potential stimulant for plasticity with exercise is an increase in neuromodulatory transmitters, such as dopamine (for review, see Taubert et al. 2015). Further studies are needed to identify these mechanisms, however, such research must be designed and interpreted carefully, as peripheral markers (e.g., BDNF concentration) may not be reflective of acute changes occurring within the cortex (Piepmeier and Etnier 2015).

4.5.3 TMS-induced corticospinal plasticity following HIIT in endurance-trained participants

In addition to investigations of acute exercise and plasticity, there is some evidence to suggest that regular physical activity promotes the response to TMS-induced plasticity. For example, Cirillo et al. (2009) utilised PAS to demonstrate that TMS-induced plasticity is greater in physically active individuals. In contrast, Kumpulainen et al. (2015) found that PAS was only effective in upregulating MEP amplitudes in skill-trained participants and found PAS to be ineffective in an endurance-trained and control group. The disparity between these outcomes may stem from the nature of the subject groups in each study. Cirillo and colleagues utilised a cohort of active individuals (assessed with the IPAQ), whereas Kumpulainen et al. (2015) recruited highly trained endurance athletes, who completed on average 10 hours of formal endurance training per week. It is likely that the cumulative exercise load varies significantly between these groups.

In a novel finding and supporting our hypothesis, outcomes of the present study demonstrate that TMS-induced plasticity following acute HIIT is enhanced in endurance-trained participants. This finding suggests that regular physical activity upregulates the long-term potentiation (LTP)-like processes that are involved with neuroplasticity following acute exercise. The influence of regular endurance activity on plasticity with acute exercise likely results from partially unique, although overlapping physiological adaptations. For example, evidence suggests that long-term endurance exercise promotes angiogenesis in M1, which upregulates cerebral blood flow (Kleim et al. 2002, Xiong et al. 2009). Furthermore, regular physical exercise has been shown to modulate basal levels of BDNF. Interestingly, there is evidence for both increased (Zoladz et al. 2008, Szuhany et al. 2015) and decreased (Currie et al. 2009) BDNF levels with long-term exercise, which could be due to an increased blood volume in trained athletes or reflect heightened BDNF clearance and uptake in trained individuals (Knaepen et al. 2010). Nonetheless, the outcomes of the present study indicate that the effects of long-term endurance exercise influence the physiological response to acute HIIT exercise. While we are unable to determine exactly which mechanisms contribute to the increase in plasticity, previous research has demonstrated that acute and long-term forms of exercise can have combined physiological effects. For example, Zoladz et al. (2008) has demonstrated that acute exercise-induced upregulation of BDNF is greater in trained individuals.

A common limitation of exercise plasticity studies is that the lower limb exercise often involves activity of upper limb muscles that are the target of TMS. A standard approach is to instruct the participant not to activate the hand muscles during exercise (e.g., Andrews et al. 2020), but it is not known if this muscle activity can influence the subsequent response to TMS. Furthermore, it is not known if the characteristics of hand muscle activity might be different between END and SED participants during exercise. To address this confound, we recorded EMG activity of the FDI in each participant during HIIT. The present results show a significant negative correlation between the number of bursts of EMG activity in FDI during lower limb exercise, and the magnitude of plasticity (change in test MEP amplitude) after iTMS. Similarly, a negative correlation was present between total burst time and test MEP modulation in SED but not END participants. These findings indicate that ensuring the FDI (or equivalent tested muscle group) is relaxed during an acute exercise bout is an important consideration for future exercise plasticity studies. However, as there was no difference between groups for any exercise EMG measures, muscle activation during exercise is unlikely to have contributed to the increased plasticity in END participants observed here.

4.5.4 Modulation of intracortical excitability with iTMS following HIIT

The majority of TMS research utilising iTMS as a plasticity-inducing intervention has focussed primarily on the upregulation of corticospinal excitability (for review, see Kidgell et al. 2016). However, evidence suggests that iTMS can modify the activity of both inhibitory and excitatory intracortical networks, which is likely to occur through modulation of GABA-mediated neurotransmission (Ziemann et al. 1998, Di Lazzaro et al. 1999, Di Lazzaro et al. 2000, Cash et al. 2009, Long et al. 2017). The present study is the first to utilise iTMS to examine the influence of exercise on the plasticity of intracortical networks. These outcomes show that SICI is not significantly modulated with iTMS, with no difference between exercise groups or sessions. These findings contrast previous evidence which suggests that plasticity-inducing TMS protocols are more effective for reducing SICI when preceded by exercise. For example, Singh et al. (2014) demonstrated a greater reduction of SICI with PAS following moderate-intensity continuous exercise compared with a no-exercise control session. Furthermore, Andrews et al. (2020) demonstrated a greater reduction of SICI with iTBS following HIIT compared with a rest condition. However, these findings must be interpreted with caution, as the present study assessed the change in SICI after iTMS when expressed relative to after exercise (post-exercise time point), whereas the previous studies assessed the change in SICI relative to before exercise (baseline). Furthermore, Andrews et al. (2020) analysed paired-pulse ratios (conditioned/nonconditioned) that had not been normalised to a particular timepoint and therefore varied (albeit non-significantly) at the post-exercise timepoint. Nonetheless, the results of the present study indicate that neither acute nor regular forms of exercise influence modulation of SICI with iTMS.

Normalised measures of intracortical facilitation (both SICF and ICF) were significantly greater when iTMS is preceded by the no-exercise control than when preceded by HIIT exercise. These findings may indicate that intracortical facilitation reduces the TMS-induced corticospinal plasticity present after HIIT exercise. However, this effect was not consistent in END and SED groups, where the upregulation of SICF with iTMS was reduced after HIIT exercise for the SED group, but not for the END group. Interestingly, a significant positive correlation was present between the upregulation of SICF and test MEP amplitude with iTMS for the END group. These findings indicate that SICF may play a role in driving the increased corticospinal plasticity present in endurance-trained participants after HIIT exercise.

This study is the first to demonstrate that TMS-induced corticospinal plasticity is greater in endurance-trained cyclists after an acute bout of exercise. Further, we support previous findings that TMS-induced plasticity is greater following acute HIIT exercise. These findings add to the extensive body of literature surrounding the positive effect of exercise on neuroplasticity and indicate that exercise history is an important consideration for understanding neuroplasticity.

5. General Discussion

Extensive TMS research has demonstrated that both regular and acute forms of exercise have the potential to upregulate plasticity within the motor cortex (Cirillo et al. 2009, Mang et al. 2014, Singh et al. 2014, Andrews et al. 2020, Mellow et al. 2020), which is thought to be a critical component of motor skill acquisition (Classen et al. 1998, Sanes and Donoghue 2000, Muellbacher et al. 2001, Ziemann et al. 2004). Despite these developments, the effect of long-term exercise on motor cortex plasticity and learning is not well understood. The overarching aim of this thesis was, therefore to investigate how motor cortex plasticity and visuomotor skill acquisition is modified for both exercised (lower limb) and non-exercised (upper limb) muscle groups of endurance-trained individuals. An initial study identified the optimal approach for stimulating lower limb areas of the cortex with NIBS, particularly with respect to examining activity within intracortical circuits (Chapter 2). This allowed for the investigation of use-dependent plasticity and motor learning in upper and lower limb muscles (Chapter 3). Subsequently, Chapter 4 examined the effect of acute exercise on plasticity. Importantly, these effects were examined in a unique population of highly trained cyclists with an extensive history of aerobic training, primarily involving lower limb muscles. Data were compared with people who did not participate in planned physical activity, in an attempt to identify the role of long-term exercise on M1 plasticity in exercised (lower limb) and non-exercised (upper limb) muscles. These participant groups represent physical activity levels on the opposite ends of the spectrum, and provide unique insight into the role of long-term physical activity on M1 plasticity and motor function.

5.1 Optimising TMS methods for assessment of plasticity in lower limbs

Since its introduction, TMS has undergone many technical advancements (e.g., coil design for optimised depth-focality trade-off), and has facilitated the development of various methods for assessing motor cortex excitability and plasticity (e.g., plasticity-inducing brain stimulation paradigms such as TBS and iTMS). However, due to the lower stimulation intensity required for activation, TMS is still most commonly utilised to assess muscles of the hand or upper limb (Roux et al. 2020). In contrast, cortical representations for lower limb muscles are much harder to stimulate with TMS (Terao et al. 2000), and the application of TMS to these areas has therefore received substantially less attention. Given the involvement of lower limb muscles in many aspects of motor function (e.g., balance, posture and locomotion) (Mann and Hagy 1980, Joseph 1985, Mille et al. 2014), this limited understanding of how NIBS interacts with lower limb areas of the cortex represents a significant limitation of the field.

Among the comparatively few studies that have applied TMS within lower limb representations, there are some characteristic methodological deviations from the way in which stimulation is generally applied in upper limb muscles. For example, a double-cone coil is often utilised due to the greater penetrating depth (in contrast to a figure-of-eight coil in hand muscles), and stimulation is often applied in an active muscle in order to reduce the threshold intensity (Deng et al. 2013, Brownstein et al. 2018, Krishnan 2019). However, these methodological distinctions may alter the specific neuronal elements activated by TMS, and the composition of the descending volley (Brasil-Neto et al. 1992, Mills et al. 1992, Werhahn et al. 1994, Terao et al. 2000, Richter et al. 2013, Laakso et al. 2014, Janssen et al. 2015, Lu and Ueno 2017, Gomez-Tames et al. 2018), which influences the interpretation of the findings in upper and lower limbs. Furthermore, research investigating the influence of aerobic exercise on neuroplasticity commonly assesses muscles of the hand, yet the training itself generally involves lower limb muscles. This research, therefore, assumes that exercise-induced modulation of neuroplasticity extends beyond the bounds of the cortical representation of exercised muscle groups. Despite this, it has remained unclear whether training of particular muscle groups (e.g., lower limb focussed cycling) elicits neurophysiological changes that are specific to exercised muscles, or result in global changes within the motor cortex. Therefore, an optimal approach to investigating the influence of regular lower limb exercise on neuroplasticity is to assess and compare characteristics between exercised and non-exercised muscles. Unfortunately, it had not been determined whether comparable TMS parameters (e.g., TMS coil type and stimulus intensities) can be utilised appropriately for the assessment of both upper and lower limb muscles. This comparison has been performed in Chapter 2, in which TMS parameters were optimised for neurophysiological assessment of both upper and lower limb muscles.

Novel outcomes of Chapter 2 indicate that a figure-of-eight coil can be used effectively for the assessment of both corticospinal and intracortical circuits for control of lower limb muscles. However, for the most efficient cortical activation, an ML orientation must be utilised for the lower limb (as opposed to the PA orientation typically used for upper limb TMS). These findings facilitate more methodologically rigorous comparisons of cortical control between upper and lower limb muscles, allowing subsequent studies to investigate how neurophysiological characteristics and plasticity vary between cortical regions. Utilising a figure-of-eight coil provides an additional benefit of reduced discomfort to the participant compared with a double-cone coil (Panyakaew et al. 2016, Fernandez et al. 2018). This approach was used in Chapter 3, where an optimised stimulation approach was used to assess if exercise-mediated changes in skill training-induced plasticity are specific to the exercised muscle groups (lower limbs), or result in more global changes within the motor cortex (as shown in upper limb muscles). Outcomes from further research implementing this approach may have positive implications for clinical applications of TMS. For example, stroke often results in functional deficits that are limb/area-specific, and it would be valuable to understand to what extent aerobic activity of functionally sound locations can promote the plasticity response to motor training of the affected regions.

While outcomes of Chapter 2 have positive implications for TMS assessment for lower limbs, a logical extension of this research is to assess the TMS characteristics of other lower limb muscles such as quadriceps and hamstrings. These muscle groups are often the target for TMS research as they are functionally important for daily activities such as locomotion and play a major role in exercise activities such as cycling (Mann and Hagy 1980, Begalle et al. 2012, da Silva et al. 2016). However, the outcomes of this approach are more difficult to interpret in the quadriceps and hamstring muscle groups, as they are comprised of several muscles that each make a different contribution to motor function at the knee joint. Nonetheless, given the evidence that the cortical representations differ among lower limb muscle groups (Davies 2020), there is merit for future studies to investigate the optimal TMS parameters for activation of quadriceps and hamstring muscle groups, as has been identified for the tibialis anterior muscle in Chapter 2.

5.2 Increased cortical plasticity in endurance-trained cyclists

There is now considerable evidence showing that plasticity occurs within the motor cortex in response to repetitive movement or motor learning (use-dependent plasticity) (Classen et al. 1998, Muellbacher et al. 2002, Perez et al. 2004, Ziemann et al. 2004) as well as following the application of various TMS paradigms (Chen et al. 1997, Berardelli et al. 1998, Muellbacher et al. 2000, Huang et al. 2005, Thickbroom et al. 2006). Furthermore, both acute (McDonnell et al. 2013, Mang et al. 2014, Andrews et al. 2020) and regular (Cirillo et al. 2009) forms of exercise have been shown to modulate TMS-induced neuroplasticity. This thesis builds upon this previous literature by combining these concepts to demonstrate that regular physical activity promotes skill training-induced corticospinal plasticity in the cortical representations for both exercised and non-exercised muscle groups (Chapter 3). A positive influence of regular exercise is also demonstrated in Chapter 4, which provides new evidence that acute and regular forms of aerobic exercise can have a combined physiological effect, with the greatest motor cortex plasticity demonstrated in endurance-trained individuals after an acute bout of HIIT. While the findings of Chapters 3 and 4 provide novel evidence that regular exercise promotes use-dependent and TMS-induced plasticity within the motor cortex, it remains unclear which physiological processes underpin these outcomes. Identifying such mechanisms represents a valuable direction for future research. Studies could assess peripheral concentrations of the various chemical factors that are known to be modulated with exercise and are important for plasticity induction. For example, signalling molecules (e.g., lactate) and plasticity-inducing neurotrophins (e.g., BDNF). However, as indicated previously, findings from such research must be considered carefully as it remains unclear how accurately peripheral measures reflect acute changes within the cortex.

Another key factor that remains to be determined is whether the enhanced plasticity demonstrated by the endurance-trained groups (cyclists) in Chapters 3 and 4 is specific to the type of exercise performed. For example, while Chapter 3 investigated the neurophysiological effects of long-term cycling exercise, it is unclear whether use-dependent plasticity would be similarly upregulated with another form of endurance activity, such as running. Given that many of the physiological adaptations that result from endurance exercise are related to the aerobic component of the training (Colcombe et al. 2004), it is likely that similar outcomes would result from other forms of long-term aerobic endurance activity. In support of this, enhanced plasticity has been demonstrated in aerobic athletes of various sports. For example, Cirillo et al. (2009) demonstrated enhanced TMS-induced plasticity in a cohort of mixed endurance-trained athletes of running, cycling, and swimming backgrounds. Within Chapter 4, the question of exercise specificity introduces an additional confound, as this study incorporated an acute bout of HIIT cycling within elite cyclists. It is, therefore possible that the increased plasticity response in this group stemmed from the use of an exercise modality within which they are highly-trained, and it is unclear if similar benefits would be apparent following a bout of exercise using a non-trained modality. Taken together, it is apparent that further research is required to determine how the neuroplastic benefits of exercise relate to the type of exercise being performed.

Together, the outcomes of Chapters 3 and 4 expand the current understanding of exercise and brain function and may have implications for motor rehabilitation. Specifically, these findings support those of Nepveu et al. (2017) and Yang et al. (2020) by indicating that a combined intervention strategy utilising both exercise and plasticity-inducing TMS may be optimal for promoting plasticity, which is a critical aspect of motor restoration. This being the case, a potential limitation of the present research is that only one form of acute exercise (HIIT) was utilised as a plasticity priming paradigm. Evidence suggests that low-moderate continuous exercise is also effective for the promotion of plasticity (Mellow et al. 2020). Therefore, future studies could seek to determine whether lower-intensity continuous-type exercise promotes plasticity in endurance-trained individuals with similar efficacy to HIIT exercise.

In addition to motor cortex plasticity, exercise has been shown to have positive implications for non-motor areas. For example, exercise has the potential to upregulate cerebral blood volume in the hippocampus (Pereira et al. 2007) and increase cortical thickness in the prefrontal regions (Stern et al. 2019). These adaptations and others appear to provide benefits for cognitive domains, such as executive function and memory (Kramer and Erickson 2007, Hillman et al. 2008, Schättin et al. 2018, Stern et al. 2019). Future research could investigate whether regular and acute forms of exercise have a robust combined effect on plasticity and function of non-motor regions.

5.3 Is increased plasticity important for motor function?

Induction of neuroplasticity (particularly an increase in corticospinal excitability) has been linked with enhanced motor skill acquisition of various forms, including ballistic (Rogasch et al. 2009), fine motor (target based visuomotor, Pascual-Leone et al. 1995) and precision tracking (Perez et al. 2004, Jensen et al. 2005) tasks. In contrast to our expectations, Chapter 3 failed to demonstrate that the heightened skill training-induced plasticity in endurance-trained participants results in benefits to skill acquisition with a visuomotor task. Given the evidence that exercise promotes motor skill acquisition and retention (Roig et al. 2012, Statton et al. 2015, Seidel et al. 2017, Stavrinos and Coxon 2017), and that corticospinal plasticity is thought to be important for skill acquisition (Pascual-Leone et al. 1995, Perez et al. 2004, Jensen et al. 2005), these outcomes could be considered counterintuitive. However, a lack of association between motor cortex plasticity and complex motor task acquisition has also been demonstrated previously (McDonnell and Ridding 2006, Opie et al. 2020). This unexpected outcome may reflect a fundamental limitation of TMS as a tool to understand motor function: while TMS is effective for activating corticospinal and intracortical circuits that generate responses in peripheral muscles, the neuronal populations recruited with TMS are not identical to those activated physiologically during voluntary movement. Furthermore, evidence suggests that there is not always a clear relationship between MEPs and motor output following motor training (Gelli et al. 2007). Accordingly, Bestmann and colleagues have postulated that MEPs may be used to demonstrate a state-dependent change in the motor system, but may not have a causal relationship with changes in motor function (Bestmann and Krakauer 2015). This may be especially relevant to visuomotor learning, which involves a higher degree of memory and error correction components, and therefore involves input from a widespread cortical network involving a number of brain areas (Floyer-Lea and Matthews 2004). For example, the cerebellum is known to have roles in error correction and movement prediction for target/error-based motor tasks (for review, see Shadmehr et al. 2010), while the hippocampus is critical for motor sequence learning (Gheysen et al. 2010, Jacobacci et al. 2020). Therefore, skill training-induced plasticity with visuomotor tasks is likely to involve cortical regions that are not assessed directly with TMS. Consequently, there is significant merit for future investigations of exercise-mediated plasticity with skill acquisition in regions outside M1. As a means to provide further information than what is available with TMS measurements, further investigations of exercise and brain structure/function could include measures of TMS-EEG and MRI, as these imaging techniques are able to assess more global changes in cortical excitability and connectivity that are more likely to predict performance.

Another avenue for future research stemming from the findings presented in this thesis is to investigate the influence of regular exercise on neuroplasticity and skill retention (as opposed to just skill acquisition). This is an important consideration as learning is thought to continue after motor practice has been completed (a concept known as ‘offline’ learning) and this is thought to underpin skill consolidation and retention (Dayan and Cohen 2011). Previous research has shown that online skill acquisition and motor skill retention (tested on following days) is not always positively associated. An example relevant to exercise is a study by Roig and colleagues, which demonstrated that although acute exercise prior to training failed to influence visuomotor skill acquisition, it did enhance skill retention 24 hours and 7 days after practice (Roig et al. 2012). The possibility therefore exists that including retention tests in the present work may have identified important additional information about the influence of exercise on skill consolidation and retention, despite there being no between-group differences in single session skill acquisition.

A final point of consideration for these findings is whether the motor task utilised was suitable for the aims of the study. This visuomotor task was based on a target-based motor task initially created by Reis et al. (2009), and has since been implemented in numerous motor control and skill acquisition studies (Fujiyama et al. 2017, Stavrinos and Coxon 2017, Opie et al. 2019). This task provides some notable advantages, such as the potential for analysis of speed and accuracy components separately. However, one possible advancement that could be applied in future research is to structure the learning task so that it is progressive in difficulty. This strategy may be especially effective if the study design includes numerous motor learning sessions (over several days). Recent evidence suggests that utilising a learning task that becomes progressively more difficult with practice results in superior learning outcomes and more reliable modulation of corticospinal excitability (Christiansen et al. 2018). Together, implementing progressive difficulty and retention measures in the applied visuomotor task has the potential to more effectively characterise the associations between long-term endurance exercise and motor skill acquisition.

5.4 Concluding remarks

This thesis provides new evidence for an optimised method of utilising TMS for measures of lower limb corticospinal and intracortical excitability (Chapter 2). This outcome opens the door for methodologically rigorous comparisons of upper and lower limb plasticity; however, it requires further studies on other lower limb muscles to be more widely applicable to other muscle groups and movement strategies. I have also provided evidence that regular endurance-based cycling promotes use-dependent (skill training-induced) plasticity, which is not restricted to the exercised limb. However, greater skill training-induced corticospinal plasticity did not promote motor skill acquisition with a visuomotor training task (Chapter 3). Finally, this research has demonstrated that acute and regular forms of exercise may have a harmonious influence on motor cortex plasticity, whereby acute exercise itself upregulates plasticity, and this effect is greater in endurance-trained cyclists (Chapter 4). These outcomes provide valuable insight into the potential benefits of exercise for cortical plasticity by demonstrating upregulated motor cortex plasticity in endurance-trained cyclists. However, further research is required to determine whether these exercise-induced neurophysiological changes are specific to cycling exercise and to determine whether these effects can promote skill acquisition or retention. Understanding these implications may have consequences for highly trained endurance athletes, such as the potential for superior motor skill acquisition or retention when skill training is coupled with acute aerobic exercise. However, further research is required to reveal the nature of these possibilities. Furthermore, these findings may help facilitate the development of TMS/exercise interventional strategies to improve motor planning and function in clinical populations (e.g., in stroke patients, Nepveu et al. 2017, Yang et al. 2020). Together, outcomes from this thesis highlight the powerful potential of long-term cycling exercise for brain function. These benefits are likely to include motor function and recovery from injury; however, they may also involve upregulated plasticity and function in motor and non-motor areas for neurophysiologically healthy individuals.

6. Appendices

6.1 Presentations arising from thesis

Title: Cortical plasticity, connectivity and motor skill learning in highly trained athletes 2018

Event: HDR Seminar Series, University of Adelaide

Title: TMS coil orientation and muscle activation influence lower limb corticospinal and intracortical excitability 2019

Event: Sensorimotor Satellite Meeting, Australasian Neuroscience Society

Title: Long-term physical activity influences motor cortical plasticity following lower limb visuomotor skill learning. 2020

Event: Florey Postgraduate Research Conference, University of Adelaide

Title: TMS-induced corticomotor plasticity is greater in endurance-trained cyclists following acute exercise. 2021

Event: 2021 Scientific Meeting, Australasian Brain Stimulation Society

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