Neurophysiological responses and adaptation to muscle shortening and lengthening in young and older adults

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by

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ABSTRACT

Healthy aging is characterised by alterations in the nervous system, leading to decrements in neuromuscular performance, particularly during dynamic contractions. Muscle shortening and lengthening differently modulate the corticospinal output, with the possibility of this modulation being altered in aging adults, which might affect the adaptability of an aging neuromuscular system to maximal lengthening contractions. The aim of this thesis was to elucidate the differences in neurophysiological responses and adaptation to muscle shortening and lengthening between young and older adults. It was hypothesised that the age-related alterations in the nervous system will lead to impaired sensorimotor integration with muscle length changes and reduced corticospinal responses during dynamic contractions, impairing the adaptability of older adults to maximal lengthening contractions. In Study 1, a novel method for assessment of subcortical excitability of descending tracts was developed, followed by investigation of corticospinal responses during passive muscle shortening and lengthening. Corticospinal excitability was modulated by muscle length changes in young adults, likely through inhibitory input of muscle spindle afferents on cortical areas. In contrast, older adults showed no modulation, which may be linked to altered sensorimotor integration. In Study 2, a method for normalising torque outputs during submaximal dynamic contractions was developed, followed by assessment of muscle fascicle behaviour. Subsequently, evoked responses were assessed during submaximal contractions of different types in young and older individuals. Despite preserved maximal torque producing capacity, corticospinal responses were reduced in older compared with younger adults across contraction types, along with increased torque variability during dynamic contractions. Study 3 assessed the contribution of spinal and supraspinal properties in adaptation to repeated bouts of maximal lengthening contractions in young and older adults. Less damage was incurred in older individuals, but the rate of adaptation was similar between young and older adults. However, the corticospinal processes played a limited role in the adaptive response. This work extends the understanding of the modulation of corticospinal networks with muscle shortening and lengthening and age-related alterations in corticospinal pathway during dynamic contractions. It also suggests that the adaptability of an aging neuromuscular system to maximal dynamic contractions remains preserved.

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LIST OF SYMBOLS AND ABBREVIATIONS

AMT	Active motor threshold
CK	Creatine kinase
CMCT	Central motor conduction time
CMEP	Cervicomedullary evoked potential
CNS	Central nervous system
CS	Conditioning stimulus
CTS	Contraction type specific normalisation
CV	Coefficient of variation
CV_{torque}	Coefficient of variation of torque
DOMS	Delayed onset muscle soreness
D-wave	Direct wave
EMG	Electromyography
GABA	Gamma-aminobutyric acid
H-reflex	The Hoffman reflex
ICC	Intraclass correlation coefficient
ICF	Intracortical facilitation
ISI	Interstimulus interval
I-wave	Indirect wave
LEP	Lumbar evoked potential
LS	Electrical stimulation of the lumbar spinal segments
M1	Primary motor cortex
M_H	M-wave at H-reflex
MLS	Muscle length specific normalisation
M_{max}	Maximal compound action potential

MTU	Muscle-tendon unit
MVC	Maximal voluntary contraction
MT	Motor threshold
PMCT	Peripheral motor conduction time
PSCT	Peripheral sensory conduction time
RBE	Repeated bout effect
RMS	Root mean squared
rMT	Resting motor threshold
ROM	Range-of-motion
SD	Standard deviation
SICI	Short-interval intracortical inhibition
SO	Stimulator output
SOL	Soleus
SP	Silent period
TA	Tibialis anterior
TE	Typical error
rTE	Relative typical error
TMS	Transcranial magnetic stimulation
TS	Test stimulus
η_p^2	Partial eta squared

PUBLICATIONS

Peer-reviewed publications arising from this thesis

Škarabot, J., Ansdell, P., Howatson, G., Goodall, S., and Durbaba, R. (2019). Corticospinal responses during passive shortening and lengthening of tibialis anterior and soleus in older compared to younger adults. *Experimental Physiology*, DOI: 10.1113/EP088204.

Škarabot, J., Ansdell, P., Temesi, J., Howatson, G., Goodall, S., and Durbaba, R. (2019). Neurophysiological responses and adaptation following repeated bouts of maximal lengthening contractions in young and older adults. *Journal of Applied Physiology* 127, 1224-1237.

Škarabot, J., Ansdell, P., Brownstein, C.G., Hicks, K.M., Howatson, G., Goodall, S., and Durbaba, R. (2019). Corticospinal excitability of tibialis anterior and soleus differs during passive ankle movement. *Experimental Brain Research* 237, 2239-2254.

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Conference communications

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Škarabot, J., Ansdell, P., Brownstein, C.G., Howatson, G., Goodall, S., and Durbaba, R. Neural modulation during submaximal dorsiflexion of different contraction types in young and older adults. 15-minute oral presentation at: UK Sensory Motor Conference; 21-23 June 2018; Leeds, United Kingdom.

Peer-reviewed publications arising from studies conducted alongside this thesis

Škarabot, J., Mesquita, R.N.O, Brownstein, C.G., and Ansdell, P. (2019). Myths and methodologies: how loud is the story told by the transcranial magnetic stimulation-evoked silent period?. *Experimental Physiology* 104, 635-642.

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Brownstein, C.G., Ansdell, P., **Škarabot, J.**, Howatson, G., Goodall, S., and Thomas, K. (2018). An optimal protocol for measurement of corticospinal excitability, short interval intracortical inhibition and intracortical facilitation in the rectus femoris. *Journal of the Neurological Sciences* 394, 45-56.

Brownstein, C.G., Ansdell, P., **Škarabot, J.**, Kidgell, D., Frazer, A., Howatson, G., Goodall, S., and Thomas, K. (2018). Motor cortical and corticospinal function differs during an isometric squat compared to isometric knee extension. *Experimental Physiology* 103, 1251-1263.

DECLARATION

I declare that the work contained in this thesis has not been submitted for any other award and that it is all my own work. I also confirm that this work fully acknowledges opinions, ideas and contributions from the work of others.

Any ethical clearance for the research presented in this thesis has been approved. Approval has been sought and gained by the Faculty of Health and Life Sciences Ethics committee for each study.

Name: Jakob Škarabot

Signature:

Date: 12th August, 2019

CHAPTER 1: INTRODUCTION

1-1 Introduction

In developed countries, there has been a surge in the proportion of the aging population in the last couple of decades, with this increase expected to continue into the future. For example, in the UK, between 2014 and 2039, 70% of the population growth is estimated to be in the over 60 age group (from 14.9 to 21.9 million people; Goverment Office for Science, 2016). With this projected increase, there is an obvious need to develop strategies to optimise health of the older population.

Even in the absence of acute and chronic pathological conditions, aging is accompanied by many physiological alterations. One of the biological systems experiencing a decline in function and performance in older age is the neuromuscular system. Preserving function and performance of the neuromuscular system is imperative to maintain mobility and independence in older age. Since the introduction of the term sarcopenia ~30 years ago (Rosenberg, 1989), initially characterised as a progressive loss of strength and muscle mass with aging, there has been a rise in research on the age-related reduction in neuromuscular function and performance. Since then, the definition of sarcopenia has been extended to encompass the age-related decrements in the central nervous system (CNS) function, hormonal status, inflammation and nutritional intake (Doherty, 2003). Despite the increase in study of the aging population, the effects of sarcopenia remain underappreciated (McNeil and Rice, 2018).

Aging is accompanied by motor unit (MU) loss and remodelling and alterations of the synaptic inputs from CNS (Hunter et al., 2016b). These changes lead to many functional decrements including the loss of strength and the ability to control muscle force output. The latter is particularly challenging during lengthening contractions (Hughes et al., 1996; Moxley Scarborough et al., 1999). The ability to control muscle

force output during lengthening contractions has been suggested to be impaired in older individuals with a history of falling as compared to age-matched peers without any history of falling (Carville et al., 2007). This suggests that steadiness during lengthening contractions might be important for functional tasks involved with daily living.

The corticospinal tract is the main pathway transmitting motor signals from the primary motor cortex to the muscle. The excitability of the corticospinal tract is constantly modulated during passive and active changes in muscle length. Lengthening contractions purportedly require a unique activation strategy and receive different synaptic input by the CNS compared to isometric and shortening contractions (Duchateau and Enoka, 2016). Whilst this has been repeatedly shown in younger populations, the data on corticospinal output during dynamic contractions in the elderly remains scarce, despite its greater functional relevance (McNeil and Rice, 2018). Given the aforementioned decrements in neuromuscular performance during lengthening contractions in the elderly, elucidating the differences in the synaptic input in comparison with younger individuals during muscle shortening and lengthening is important to elucidate.

Even in the absence of disease, it is crucial to understand the effect of aging on CNS responses and adaptation in order to be able to make a distinction between age-related and disease-related alterations in physiology and develop appropriate interventions aimed to improve neuromuscular function and performance in healthy older and clinical populations. Accordingly, the overarching aim of this thesis was to elucidate neurophysiological responses and adaptation to muscle shortening and lengthening and how these responses and adaptation interact with the aging CNS. Specifically, over the course of three experimental chapters, this thesis examines:

1) Neurophysiological responses to passive shortening and lengthening
2) Neurophysiological responses to submaximal isometric, shortening and lengthening contractions
3) Neurophysiological responses and adaptation to repeated bouts of maximal lengthening contractions

CHAPTER 2: LITERATURE REVIEW

2-1 Introduction

The purpose of this review is to provide an overview of neurophysiological responses and adaptations to muscle shortening and lengthening and their interaction with age-related CNS alterations. Firstly, the review broadly presents the neurophysiological control of muscle length and the generation of voluntary muscle contraction. Secondly, an overview of muscle shortening and lengthening is provided that details neurophysiological responses to passive shortening and lengthening, neurophysiological responses to shortening and lengthening contractions, and responses and adaptations to a bout of maximal lengthening contractions. This section is concluded with presenting the benefits of lengthening contractions. Thirdly, the review presents the age-related alterations within the CNS with a focus on adaptations within the MU and synaptic inputs originating from spinal and supraspinal centres. Lastly, the review focuses on differences in maximal and submaximal neuromuscular performance across different contractions types between young and older adults.

2-2 Definition of terminology

Whilst this thesis is centred around neurophysiological responses, as the title implies, the responses are to be assessed in relation to muscle shortening and lengthening. Thus, neurophysiological responses are considered in the context of muscular actions, making it important to define terminology to avoid confusion. In this thesis, the term *neurophysiological responses* refers to the responses of the central nervous system, discounting potential muscular changes that might influence the size of those responses. For example, when responses to stimulations are assessed, normalisation to maximal compound action potential is performed to discount the influence of changes within the muscle (Carroll et al., 2011). In physiological terms, the term

neurophysiological responses involves the processes in the nervous system above the neuromuscular junction. Conversely, the term *neuromuscular* encompasses both the neural and muscular systems. For example, in the context of aging, alterations are commonly found in the MU, which comprises an alpha motoneuron and the muscle fibres innervated by its axon. Adjustments in the MU are thus influenced by both the synaptic input from the central nervous system, and the muscle fibres innervated by the motoneuron axon. By extension, *neuromuscular* is used as an adjective when describing both the neural and the muscular system in a single context (*neuromuscular system*), when describing interactive processes between the two systems and their functionality (*neuromuscular properties* and *neuromuscular function*), when referring to adaptations in both systems in a given context, e.g. following repeated damaging exercise (*neuromuscular adaptation*), and when referring to behavioural characteristics of muscular actions, that is – the size of the muscular output and its variability (*neuromuscular performance*), as muscular actions are the result of the nervous system output, the inputs from somatosensory receptors and the processes within the skeletal muscle itself (see section 2-4 below).

2-3 Control of muscle length

The control of muscle length is primarily modulated by sensory receptors and is based on the reflexive principle of short-latency connections between the afferent input signal and the motor (efferent) output response (Pierrot-Deseilligny and Burke, 2005). The sensors receiving the afferent signals are many, but the primary somatosensory receptors in human skeletal muscle that relay the information about changes in muscle length are the muscle spindles.

Human skeletal muscles contain thousands of muscle spindles. Muscle spindles lie in parallel with the skeletal muscle (extrafusal) fibres and are characterised by a fusiform shape, the ends of which attach to intramuscular connective tissue (Figure 2-1). The nuclei of muscle spindle (intrafusal) fibres are organised in two distinctive ways, either end-to-end along the central region (nuclear chain fibre) or clustered in a long group into a central bulbous region (nuclear bag fibres), with the latter having two types of fibres, static and dynamic. Intrafusal fibres are enclosed in a capsule and innervated by Ia (primary) afferent and II (secondary) afferent sensory axons, with the former connecting to both nuclear chain and nuclear bag fibres and the latter principally innervating nuclear chain fibres (Figure 2-1). Additionally, muscle spindles receive efferent input from alpha motoneurons (known as beta, innervate extrafusal fibres) and gamma motoneurons (innervate intrafusal fibres at the polar region). The latter is principally responsible for modulating sensitivity of the muscle spindles (Prochazka, 1989) by transmitting an action potential that results in contraction of intrafusal fibre in the polar region of the muscle spindle and a stretch of the central region, which determines the responsiveness of the muscle spindle to the detected length change.

Axons of the Ia and II afferents enter the spinal cord through the dorsal root and make connections to motoneurons monosynaptically or via one or more interneurons. There is a convergence of input from central and peripheral sources onto these interneurons resulting in a variety of motoneuron responses (Hultborn, 2001; Nielsen, 2004). Ia afferents make significant direct monosynaptic excitatory homonymous and heteronymous connections to spinal motoneurons. Furthermore, Ia afferents make a well-known connection to antagonist motoneurons via an interneuron (Tanaka, 1974). This pathway evokes inhibitory postsynaptic potentials in the motoneurons and the interneuron is thus known as the Ia inhibitory interneuron. Group II afferents also

contribute to transmission of the signal originating from the muscle spindles, but their actions are less known (Pierrot-Deseilligny and Burke, 2005). They make monosynaptic connections onto homonymous motoneurons, but are perhaps more known for their oligosynaptic connections eliciting excitatory and inhibitory postsynaptic potentials in the flexor and extensor motoneurons, respectively (Lundberg et al., 1977). Generally, Ia afferents are sensitive to both static and dynamic components of muscle lengthening, whereas II afferents are sensitive to static, but much less to dynamic (Matthews, 1972). Overall, the convergence of inputs from many sources, including other somatosensory receptors, makes the motoneuron outputs variable with changes in muscle length. However, the information originating from muscle spindles and transmitted through group Ia and II sensory axons is the primary regulator of muscle length in the human CNS.

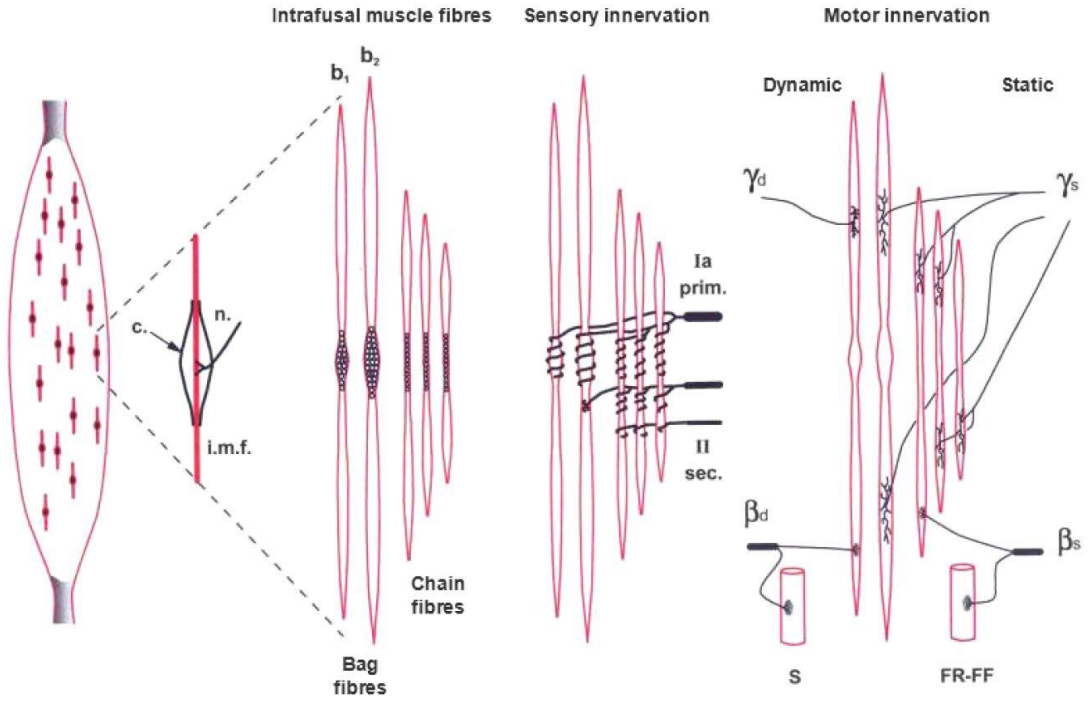

Figure 2-1. The anatomy of muscle spindle. From left to right: parent muscle; capsule (c.) with nerve supply (n.) and intrafusal muscle fibre bundle (i.m.f); two types of organisation based on nuclei organisation; sensory innervation with group Ia and II afferents; motor innervation with gamma and alpha motoneurons, the latter innervating both extrafusal and intrafusal fibres (referred to as beta). From Ellaway et al. (2015).

2-4 Voluntary muscle contraction

Voluntary contractions are a manifestation of the action of the motoneurons innervating the muscles that receive inputs from both descending systems and afferent pathways (Figure 2-2; Enoka, 2008a). The afferent pathways provide both excitatory and inhibitory inputs to spinal motoneurons, with voluntary movement typically being the result of facilitation and suppression of excitatory and inhibitory descending pathways, respectively (Enoka, 2008). Voluntary movements are a consequence of the chain of physiological events that start in the supra-cortical structures. Following the command in the supra-cortical structures, descending drive is initiated from the motor cortex. Cortical neurons originating from the primary motor cortex make monosynaptic connections with spinal motor neurons, forming the corticospinal tract (Enoka, 2008), the primary pathway between the brain and muscle involved in the control of voluntary movement. The action potential from the primary motor cortex (M1) propagates to spinal motoneurons and via efferent axons in the ventral horn of the spinal cord eventually reaches the muscle. Once the action potential reaches the neuromuscular junction, acetylcholine diffuses across the synaptic cleft and attaches to the receptors on the muscle fibre membrane initiating the process known as excitation-contraction coupling. Attachment of acetylcholine to the receptor causes sodium influx into the muscle, generating an action potential that propagates along the sarcolemma, down to transverse tubule into the interior of the muscle fibre. The latter causes a release of calcium from terminal cisternae of the sarcoplasmic reticulum into the sarcomere. Calcium then binds to the protein troponin attached to the actin filament. The latter facilitates the interaction of actin and myosin contractile proteins (the process known as the cross-bridge cycle), such that the myosin-binding site on actin is uncovered allowing for the two filaments to interact, causing muscle

contraction. When electrical stimulus ceases, calcium reuptake into the sarcoplasmic reticulum occurs against a concentration gradient by calcium pumps attached to the sarcoplasmic reticulum. The latter causes muscle relaxation.

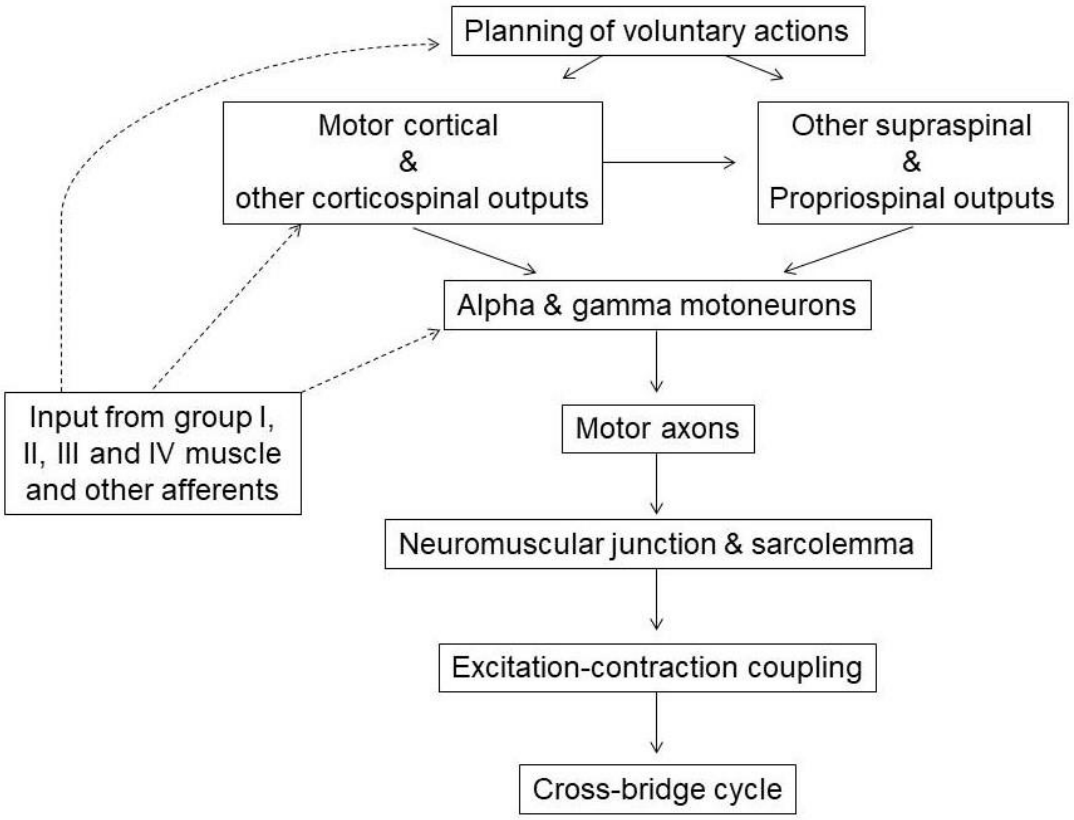

Figure 2-2. The structures involved in voluntary actions. Adapted from Gandevia (2001)**.**

2-5 Monitoring modulation of the nervous system output

2-5.1 Percutaneous nerve stimulation

Peripheral nerve stimulation has been extensively used for investigation of the nervous system in research and clinical settings. Various techniques have been employed ranging from interpolated twitch technique to assess the level of muscle activation (Merton, 1954) to techniques used to assess spinal plasticity, such as V-wave and the Hoffman reflex (H-reflex; Aagaard et al., 2002). The latter will be the focus of this section.

The H-reflex is thought to be an electrical equivalent of the monosynaptic stretch reflex, excluding the effect of gamma motoneurons and muscle spindle discharge (Brooke et al., 1997; Knikou, 2008; Schieppati, 1987). First described by Paul Hoffman (Hoffmann, 1910), it is a tool most commonly implemented in investigations concerning electrophysiological changes occurring at the spinal level (Grospretre and Martin, 2012). The H-reflex can be elicited in most muscles as long as the nerve can be accessed by percutaneous electrical stimulation (Misiaszek, 2003), with flexor carpi radialis and soleus having been most commonly tested (Zehr, 2002).

The H-reflex is evoked by electrical stimulation of a peripheral nerve at submaximal intensities and is recorded using surface electromyography (EMG; Zehr, 2002). Since the Ia afferents are of larger diameter (Li and Bak, 1976), they will be recruited first when increasing the stimulus intensity. Once the threshold for Ia afferent activation has been reached, the action potential travels toward the spinal cord activating the alpha motoneurons which results in contraction of the muscle (H-reflex threshold). Further increases in stimulus intensity will eventually result in direct activation of alpha motoneurons and thus the production of an EMG response called the M-wave (M-wave threshold). Eventually, increase in stimulation results in the antidromic collision with the reflex (past the point of maximal H-reflex), causing phase-out cancellation and complete abolishment of the H-reflex (Aagaard et al., 2002). By incrementally increasing stimulus intensity, an H-M recruitment curve (Figure 2-3) can be obtained with distinct parts: H-reflex threshold, M-wave threshold, maximum H-reflex (H_{max}) and maximum compound action potential (M_{max}). Recruitment of MUs with increases in stimulus intensity is likely to be in an orderly fashion (Henneman, 1957), but the evidence is not conclusive and mostly inferential (Zehr, 2002).

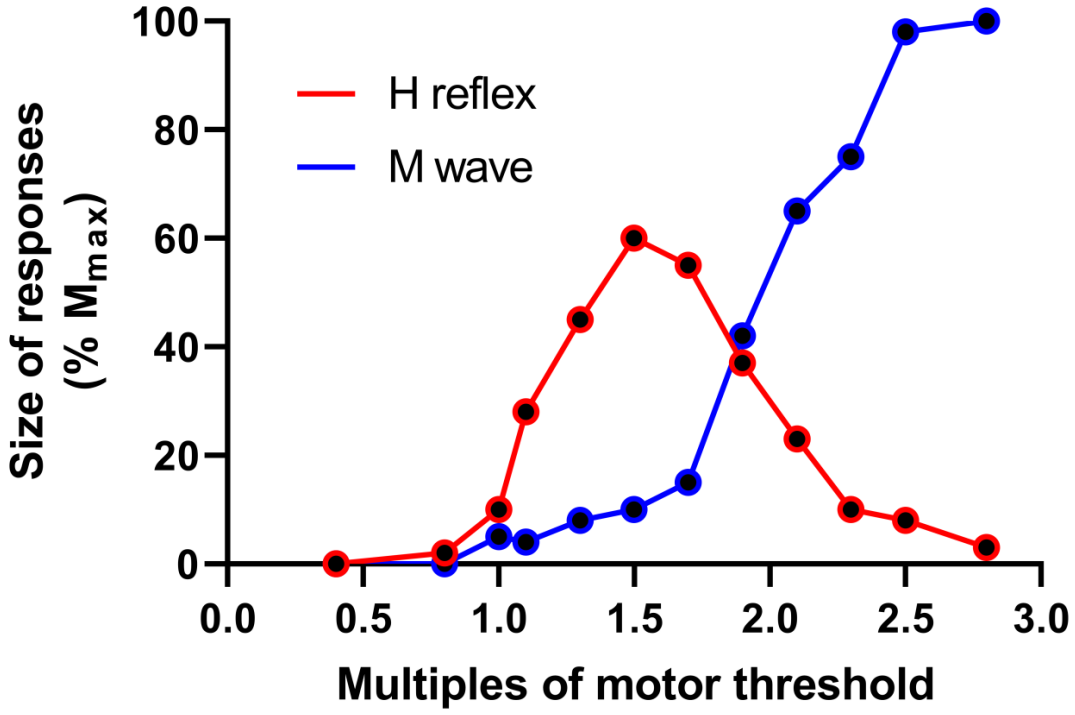

Figure 2-3. H-M recruitment curve. Adapted from Knikou (2008).

It has often been assumed that the H-reflex is an indicator of alpha motoneurons excitability due to a monosynaptic connection between Ia afferents and alpha motoneurons (Zehr, 2002). However, the H-reflex is modulated by the extent of presynaptic inhibition (Brooke et al., 1997; Stein, 1995), and as such, cannot be always interpreted to reflect the excitability of the alpha motoneurons (Zehr, 2002). Presynaptic inhibition can be altered by afferent feedback from other somatosensory receptors (e.g. Golgi tendon organs and cutaneous mechanoreceptors) as well as descending supraspinal commands (Zehr, 2002). As such, it is important that the same set of conditions are applied during an experiment, e.g. standardizing postural orientation and intention of the participant, in order to minimize confounding factors (Zehr, 2002).

In addition to presynaptic inhibition, there are other factors that can influence the size of the H-reflex. Firstly, the H-reflex amplitude has been shown to increase linearly with an increase in motoneuron recruitment (Burke et al., 1989; Schieppati, 1987). However, other reports suggest non-linearity to this relationship, characterized by a bell-shaped relationship relative to the background level of muscle activity, increasing in gain up to 30-40% of MVC, a plateau region between 40-50% MVC, and a decline for high contraction levels (Cathers et al., 2004; Cronin et al., 2008; Heckman, 1994; Matthews, 1986; Mrachacz-Kersting and Sinkjaer, 2003; Toft et al., 1989). Thus, H-reflexes are suggested to be elicited with a background level of muscle activation, ensuring a similar level of motoneuron excitability (Zehr, 2002). Second, it is important to ensure a constant synaptic input received by the alpha motoneurons – this is usually done by evoking the H-reflex at an intensity that also evokes a direct motor response, i.e. M-wave (Zehr, 2002). This also ensures that that the test reflex lies on the ascending limb of the input/output relationship for the motor neuron pool and is subjective to changes with different conditions (Knikou, 2008; Pierrot-Deseilligny and Burke, 2005). Third, when Ia afferents are activated repetitively, it can lead to a reduction in the amount of neurotransmitters and thus neurotransmitter release at the alpha motoneurons (Hultborn et al., 1996), a phenomenon known as post-activation depression. Because of that, successive stimulations should be interspersed by an interval of at least 3-4 s in duration (Pierrot-Deseilligny and Mazevet, 2000). Fourth, for the purposes of reliable comparison between subjects and conditions, it is important to normalise H-reflexes to M_{max}. The latter needs to be evoked in each respective condition and position in order to represent a reliable reference (Zehr, 2002). This is especially important in investigations trying to avoid time-dependent or movement-dependent changes (Zehr, 2002). Lastly, H-reflex size can be affected by

behavioural state and posture of the participant and these need to be kept constant throughout the whole measuring protocol (Zehr, 2002).

2-5.2 Transcranial magnetic stimulation

Attempts to stimulate the brain in animals can be dated back to 19th century (Fritsch and Hitzig, 1870), whilst attempts on humans followed roughly 80 years later (Gualtierotti and Paterson, 1954). However, it was not until 1980, that the primary motor cortex of intact scalp in humans was stimulated for the first time by transcranial electrical stimulation (Merton and Morton, 1980). This type of stimulation is accompanied by a strong sensation of pain and is thus kept from wide clinical use (Terao and Ugawa, 2002). Five years after the first successful use of transcranial electrical stimulation, Barker and colleagues (Barker et al., 1985) demonstrated painless activation of the corticospinal pathway by a magnetic field applied over the M1. This method, called transcranial magnetic stimulation (TMS), had a benefit of being relatively painless and safe (Hallett, 2000; Wassermann et al., 1996). The only reported side effects appear to be muscle tension-type headache and slight discomfort at the site of stimulation (Wassermann, 2000). The availability of TMS has grown steadily over the years and has now been used extensively to study motor control (Avela and Gruber, 2011) and neural adaptations to exercise (Carroll et al., 2011), among others.

TMS is applied through a coil of wire placed over the scalp that produces a time varying magnetic field (Terao and Ugawa, 2002). As a result, the magnetic field penetrates the cranium and in turn depolarizes neuron membranes leading to an excitatory postsynaptic potential (Terao and Ugawa, 2002). TMS does not involve a small and predictable site of stimulation due to divergence of magnetic fields after

leaving their source (Avela and Gruber, 2011). Rather, the amplitude and spatial distribution of activation is determined by the geometry of the stimulating coil in conjunction with the structural organization of the neuronal circuitry of the cortex, which in turn determines the site of stimulation (Avela and Gruber, 2011). A model of the motor homunculus (Figure 2-4) can be used to guide one to the appropriate area to target motor cortex through a coil.

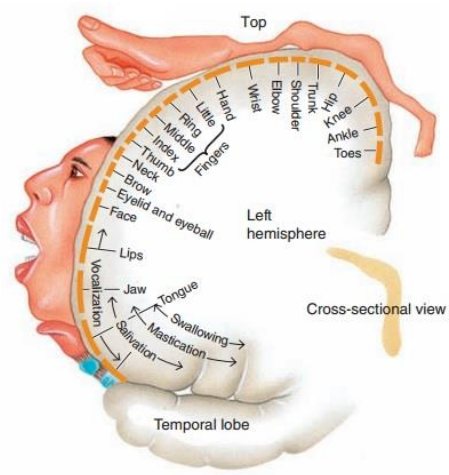

Figure 2-4. Motor homunculus representing motor output from the primary motor cortex to different body parts (Sherwood, 2016)**.**

Different shapes of coils have been used when applying magnetic pulses over M1. There are circular coils with the maximal current just underneath its entire winding (Figure 2-5A and 2-5C). Whilst these coils are generally able to activate neurons up to 2 cm below the scalp surface (Epstein et al., 1990), they are also characterized with the relative uncertainty regarding the exact site of stimulation (Pascual-Leone et al., 2002). A figure-of-eight coil consists of essentially two circular coils side by side on the same plane, with the current of each passing in the opposite direction (Figure 2-5B and 2-5D). This coil produces a more focal field (Ueno et al., 1988) and has the highest current at the intersection of the windings. Lastly, there is a double-cone coil which is composed of two coils connected to each other at an angle of 90-100°.

Similarly to figure-of-eight, a double-cone coil has the highest current at the intersection of the windings, but can induce an electrical field of greater intensities, thus penetrating deeper parts of the brain. Hence, it has been considered the best tool to stimulate the lower limb motor area (Roth et al., 2002) due to their location in the motor homunculus (Figure 2-4).

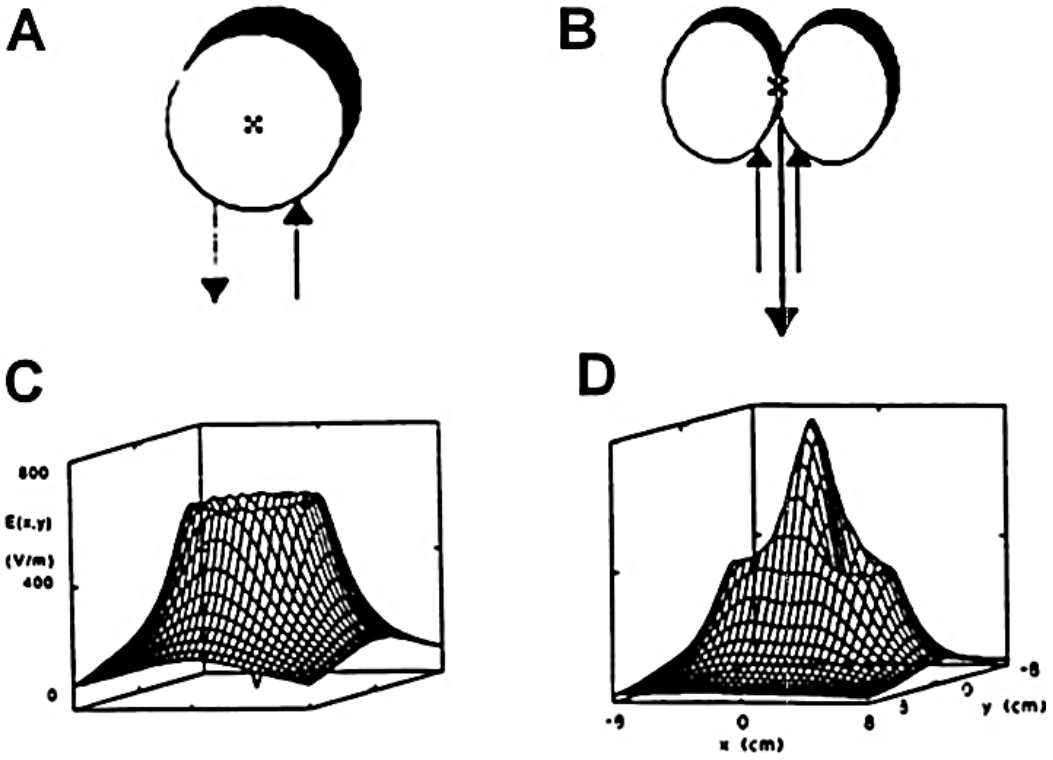

Figure 2-5. Circular (A) and double-cone (B) coil with their resultant electric fields (C and D, respectively). From Hallett (2007).

TMS activates corticospinal cells both directly, i.e. through direct stimulation of the corticospinal axons, and indirectly, i.e. via cortical interneurons. Direct activation is usually achieved via transcranial electric stimulation, although with high enough intensity it is also possible with TMS (Di Lazzaro et al., 1998), and results in the so-called direct (D) waves. Conversely, indirect activation, usually achieved via TMS, results in a series of indirect (I) waves, numbered in order of their latency. D-wave and I-waves have different latencies and so the first I-wave is observed approximately

1.5-2 ms after the D-wave (Di Lazzaro et al., 2004). Whether corticospinal cells are going to be activated directly or indirectly also depends on the orientation of the induced electric field to the cortical network (Di Lazzaro et al., 1998), and thus it is usually recommended that the current runs approximately perpendicular to the central sulcus (Avela and Gruber, 2011).

Application of TMS involves the use of EMG as an indirect indicator of the corticospinal response. When a single TMS pulse is applied to M1 it evokes an EMG response with a brief latency (~15-30 ms depending on the muscle), called the motor evoked potential (MEP; Abbruzzese and Trompetto, 2002). Given that the MEP is recorded via EMG, the signal can be influenced by various peripheral factors and thus, it should be normalised to maximum compound action potential elicited by supramaximal stimulation of the peripheral nerve (Carroll et al., 2011). When MEPs are normalised to M_{max}, their amplitude or area are thought to represent an index of 'corticospinal excitability' (Avela and Gruber, 2011). MEPs are facilitated by voluntary contraction such that with increases in contraction strength there is an increase in size and shortening of MEP latency (Abbruzzese and Trompetto, 2002). However, responses eventually peak at higher contraction strengths followed by a plateau or decline in response size during an MVC (Goodall et al., 2009; Oya et al., 2008). This reduced responsiveness of corticospinal axons at higher contractions strength is muscle-dependent, owing to the relationship between MU recruitment and firing frequency since the probability of an evoked response decreases with increases in firing rate (Bawa and Lemon, 1993; Brouwer et al., 1989; Jones and Bawa, 1999; Matthews, 1999).

Following a MEP elicited in a contracting muscle, there is a pause in the ongoing EMG activity called the silent period (SP). The SP duration varies depending on the muscle

stimulated (Abbruzzese and Trompetto, 2002), increases in duration with increased stimulus intensity (Holmgren et al., 1990; Wilson et al., 1993), but seems to be independent of background muscle activity (Haug et al., 1992; Inghilleri and Berardelli, 1993; Roick et al., 1993). SP is both spinal and cortical in nature, with the former being attributed to the first 50 ms, although recent evidence suggests that the contribution of spinal motoneurons to the generation of SP might last up to 150 ms (Yacyshyn et al., 2016). The SP is thought to be, at least partially, mediated by gamma-aminobutyric acid (GABA) receptors within the primary motor cortex (Werhahn et al., 1999). There are various approaches used to determine SP duration, both visual and mathematical (Damron et al., 2008). If analysis of SP duration includes MEPs (i.e. SP onset is considered the point of stimulation or the onset of MEP) it is sometimes termed 'relative SP', while it is sometimes termed 'absolute SP' if it does not include an MEP (Säisänen et al., 2008). Quantification of SP duration with visual inspection from stimulus onset to the return of continuous EMG activity seems to be the most reliable approach (Damron et al., 2008). SP and MEP are thought to be separate phenomena (Davey et al., 1994; Wassermann et al., 1993) owing to different modulation of the two via activation of intracortical inhibitory circuits (Trompetto et al., 2001), although research is not conclusive, with suggestions having been made about the dependency of SP duration on MEP size (Orth and Rothwell, 2004; Škarabot et al., 2019).

When TMS is applied, stimulus intensity needs to be standardized. This is usually done through determination of a motor threshold (MT). The MT is defined as the lowest TMS output that evokes a response in the target muscle (Rossini and Rossi, 2007). The MT can be determined at rest (resting motor threshold; rMT) and is quantified as a stimulus intensity resulting in a MEP greater than 50 μV in 3 out of 5,

or 5 out of 10 trials (Rossini et al., 2015, 1994). Alternatively, motor threshold can be sought at a low contraction level (active motor threshold; AMT), e.g. 10-20% MVC, and is quantified as a stimulus intensity resulting in a MEP amplitude ≥200 μV in 3 out of 5 trials (Kidgell et al., 2010).

It is important to note that the corticospinal pathway as a whole encompasses not only cortical circuitry, but also the motoneuron pool and any spinal interneuronal connections (Devanne et al., 1997). Thus, whilst a MEP is generally considered an index of corticospinal excitability, it does not allow one to distinguish between the contribution of cortical neurons and spinal motoneurons to the overall response. The H-reflex has been probed in an attempt to make such a distinction (Nielsen et al., 1999). However, there are a few issues with this approach that need to be kept in mind when trying to draw conclusions. As mentioned above, H-reflexes can be influenced by presynaptic effects, whilst TMS is thought to be devoid of presynaptic influence (Jackson et al., 2006; Nielsen and Petersen, 1994). Thus, they may activate different interneuronal circuits (Carroll et al., 2011). Furthermore, monosynaptic components in both Ia afferent and corticospinal pathways have different sensitivity to changes in motoneuronal excitability (Hortobágyi et al., 2003; Hultborn and Nielsen, 1995). Lastly, MEP size may be influenced by changes in transmitter release from corticospinal cells onto spinal motoneurons (Gandevia et al., 1999). Therefore, explicit conclusion as to the site of change (cortical vs. spinal) cannot be delineated by comparing MEPs with H-reflexes (Avela and Gruber, 2011). However, various experiments have employed this strategy and made inferences about the contribution of cortical neurons and spinal motoneurons to the overall response, but mostly when a diverging effect had been expected (Duclay et al., 2014, 2011, Lundbye-Jensen and Nielsen, 2008a, 2008b; Solopova et al., 2003; Taube et al., 2008).

TMS can also be applied with two consecutive pulses interspersed by a short interstimulus interval (ISI). The first pulse is usually referred to as the conditioning stimulus (CS) and is followed by a test stimulus (TS). The size of the response to CS is normalised to the response to TS and gives an indication of intracortical inhibition and facilitation (Rossini et al., 2015). When a subthreshold CS is followed by a suprathreshold TS at an ISI of 1-6 ms, it results in a supressed response to paired-stimulation relative to the response to a single pulse (Kujirai et al., 1993). This paired-pulse paradigm is termed short-interval intracortical inhibition (SICI) and is thought to be mediated by the activity of $GABA_A$ receptors. Similarly, the paradigm of intracortical facilitation (ICF) involves applying a subthreshold CS followed by a suprathreshold TS, but with a longer ISI (6-30 ms), resulting in a facilitated response (Kujirai et al., 1993). This has been postulated to represent the activity of glutamatergic circuits in M1 (Ziemann et al., 2015), although subcortical contributors have been suggested as well (Wiegel et al., 2018). Whilst SICI and ICF are generally accepted measures of the activity of interneuronal circuitry, the results need to be interpreted within the context of the configuration employed in studies and other potential confounding factors, e.g. background EMG activity (Rossini et al., 2015).

2-6 Muscle shortening and lengthening

If a force is produced at a fixed joint angle, the action is called isometric, since no change in whole muscle length and/or movement about related joint occurs and hence no work is performed. This is the case, for example, when the torques of the muscle and the load are equal (Enoka, 1996), or when contraction is performed against a rigid resistance. However, with articular joint rotation, changes in the length of the muscles that cross that joint usually occur. A muscle can shorten and lengthened passively, e.g.

through an external facilitator such as an isokinetic dynamometer, or actively, i.e. during a muscle contraction. In the case of the latter, actions are called anisometric. If the load torque is smaller than the muscle's capacity, the load is lifted and so the muscle performs a shortening (concentric) contraction. Conversely, if the load torque is greater than that of a muscle, or the muscle is resisting the stretch (e.g. controlled lowering), the muscle performs a lengthening (eccentric) contraction (Enoka, 1996). The force a muscle can produce during lengthening contractions might be greater than during shortening and isometric contraction and is largely unaffected by contraction velocity (Figure 2-6; Enoka, 1996).

During movements, it is common for lengthening and shortening contractions to be combined into a sequence known as the stretch-shortening cycle, which due to greater mechanical efficiency and attenuation of impact forces is a beneficial strategy for increased performance (Komi, 2003). However, lengthening contractions are not only used as part of the stretch-shortening cycle in everyday activity, but also during controlled lowering of a load (Enoka, 1996). Evidence suggest that the CNS operates differently during muscle lengthening when compared to muscle shortening, be it in a quiescent or contracting muscle, and this will be presented in the subsequent section. A bout of lengthening contractions might be accompanied by delayed onset muscle soreness. However, after repeated exposure, the muscle manages to protect against it, and soreness is minimal to non-existent, the adaptive phenomenon known as 'repeated-bout effect' (Hyldahl et al., 2017). There are neural mediators that are involved in this process, which will be outlined in this section. Lengthening contraction exercises can also be considered beneficial in some cases, which will also be discussed below.

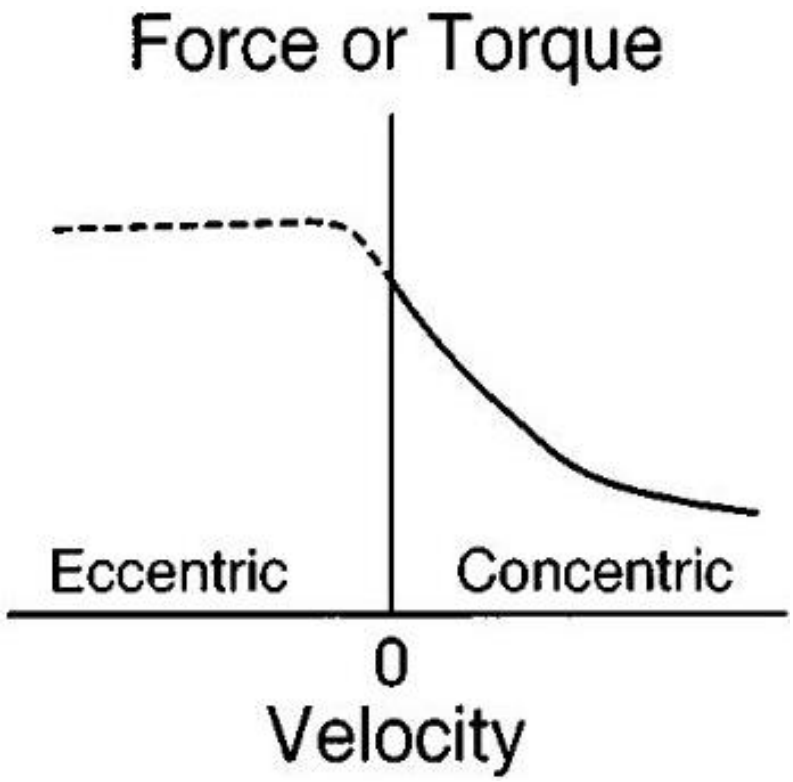

Figure 2-6. In vivo torque-velocity relationship during shortening (concentric) and lengthening (eccentric) contractions. Adapted from Enoka (1996)**.**

2-6.1 Neural control of muscle shortening and lengthening

Neural control of passive shortening and lengthening

A passive rotation of an articular joint and the corresponding changes in muscle length result in modulation of neurophysiological responses. At the somatosensory receptor level, the extent of muscle deformation with joint rotation influences the sensitivity of muscle spindles (Herbert and Gandevia, 2018). When the muscle is lengthened, the firing of the muscle spindle increases proportionally to the magnitude of the stretch, but remains low during shortening of a muscle (Matthews, 2011). Subsequently, the muscle spindle feedback can influence the activity in the motor pathway via monosynaptic connections to spinal motoneurons, input to the primary motor cortex or via premotor areas onto the spinal motoneurons (Lewis et al., 2001).

Indeed, corticospinal responses, as assessed by the amplitude or area of MEPs, are modulated with passive muscle length changes. Corticospinal excitability has been shown to be augmented and depressed during passive cyclical shortening and lengthening of the wrist flexors, respectively (Figure 2-7; Lewis *et al.*, 2001; Lewis & Byblow, 2002; Coxon *et al.*, 2005). These studies favour the mechanism of modulation to be related to inputs from muscle spindle activity to the primary motor cortex or via premotor areas onto the spinal motoneurons (Lewis et al., 2001). When the muscle is lengthened, the increased feedback arising from muscle spindle receptors might supress the corticospinal output (Lewis et al., 2001). Conversely, this suppression is disinhibited during passive shortening when muscle spindle output is reduced. Intracortical inhibition also has been shown to be reduced during cyclical passive lengthening of wrist flexors (Lewis et al., 2001). However, this reduction was only evident in the initial phases of wrist flexion; i.e. the transition from extension to flexion, and might thus be related to a sudden change in muscle length and the corresponding initial burst in muscle spindle firing (Matthews, 2011). Despite the aforementioned findings, it is still largely unclear at what level of the corticospinal pathway the modulation of the CNS output occurs during passive shortening and lengthening of the muscle. Also, it remains difficult to extrapolate the results from cyclical passive motion to discrete shortening and lengthening of the muscle. In the case of the latter, Ia afferent input onto the spinal motoneurons has been shown to be reduced during passive lengthening and augmented during passive shortening of the plantar flexor muscles (Pinniger et al., 2001), with findings attributed to presynaptic inhibition and post-activation depression of Ia afferents (Hultborn et al., 1996).

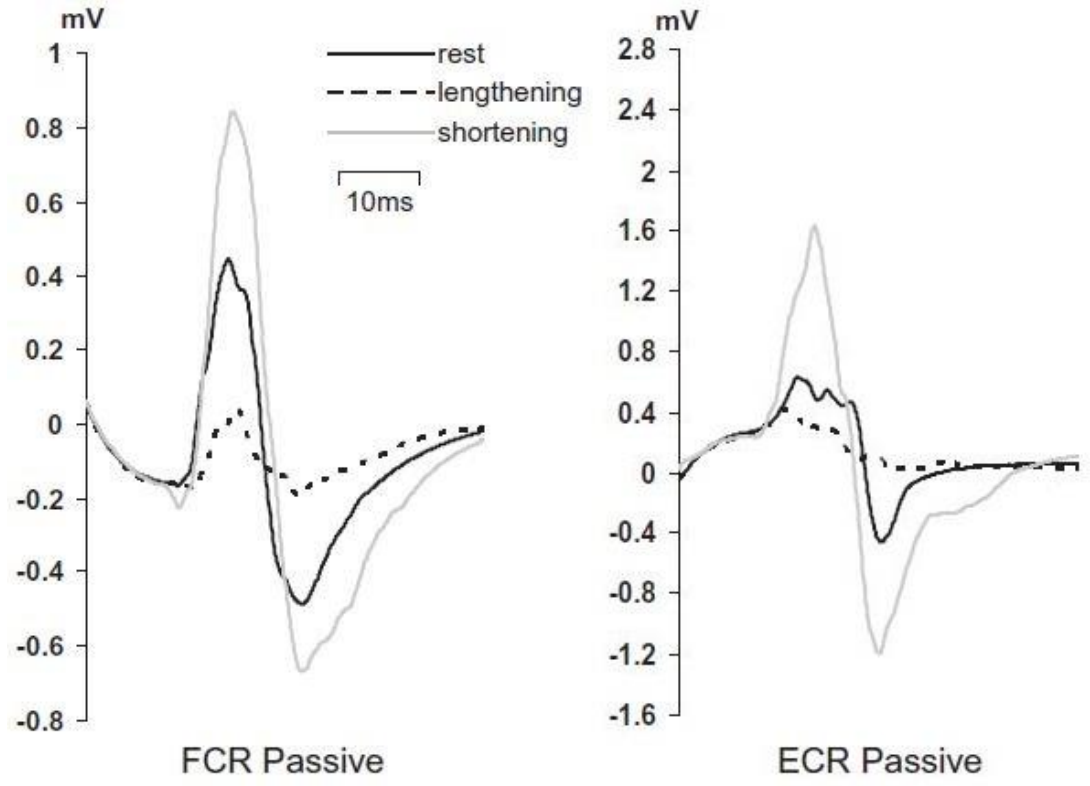

Figure 2-7. Corticospinal excitability during passive shortening and lengthening relative to rest in the wrist controlling muscles (Chye et al., 2010)**.**

The abovementioned findings are difficult to surmise however, due to differences in the study design (cyclical vs. distinct passive movement of the joint) and scarcity of the work on the lower limb relative to upper limb muscles. Whilst the functional significance of such findings is limited, investigating passive, rather than active movement allows one to discern whether the modulation of responses in the corticospinal pathway is due to a change in proprioceptive feedback related to alterations in muscle length, and is not confounded by potential differences in neural drive that can influence neurophysiological responses during isotonic movements (Abbruzzese et al., 1994; Morita et al., 2000) as will be discussed in the next section.

Neural control of shortening and lengthening contractions

The activation signal originating from the CNS purportedly differs during lengthening compared to shortening/isometric contractions. The extent to which the differences are apparent will principally depend on the task, with load resistance causing minimal activation differences compared to load displacement (Duchateau and Enoka, 2008). Modulation of the CNS activation signal is regulated by the number of activated MUs and the rate at which they discharge action potentials. For example, when an isometric task involves matching the intended force trajectory, the activation strategy regulated by the MUs accommodates the rise and decay of muscle force (Kimura et al., 2003; Nardone and Schieppati, 1988; Semmler et al., 2002). This is further complicated during movement as evidenced by the gain of the stretch reflex (Doemges and Rack, 1992) and augmented feedback from muscle spindles (Burke et al., 1978). Despite that, the stretch reflex amplitude remains largely depressed during movement, especially during lengthening contractions, suggesting central control of peripheral feedback information (Bawa and Sinkjaer, 1999; Nakazawa et al., 1998, 1997).

Whilst initial studies have proposed that a differing activation signal during lengthening contractions might stem from a tension-regulating inhibitory mechanism (Amiridis et al., 1996; Del Valle and Thomas, 2005; Seger and Thorstensson, 2000; Westing et al., 1991), the subsequent studies have dismissed it (Duclay et al., 2014; Grabiner and Owings, 2002; Pinniger et al., 2000). Given that lengthening contractions are accompanied by lower discharge rate of MUs (Del Valle and Thomas, 2005; Kallio et al., 2010; Laidlaw et al., 2000; Pasquet et al., 2006), it has been suggested that the signal controlling anisometric contractions originates from supraspinal or spinal elements of CNS (Duchateau and Enoka, 2016). The evidence from TMS studies shows a reduced size of MEPs (Figure 2-8A; Duclay et al., 2011,

2014; Gruber et al., 2009; Abbruzzese et al., 1994), and a lower plateau and maximum slope in the TMS input-output curve (Sekiguchi et al., 2007, 2003b, 2001) during lengthening compared to isometric/shortening contractions, suggesting reduced corticospinal excitability. Furthermore, TMS-induced SP is reduced during lengthening contractions, at least in soleus (Duclay et al., 2011; Valadão et al., 2018). Similarly, H-reflexes (Figure 2-8B; Abbruzzese et al., 1994; Duclay et al., 2011, 2014; Duclay and Martin, 2005; Nordlund et al., 2002; Romanò and Schieppati, 1987; Valadão et al., 2018) and motoneuron excitability as assessed by cervicomedullary evoked potentials (CMEPs; Gruber et al., 2009) have been found to be reduced during lengthening contractions.

Given the aforementioned observations in response to several different stimulation techniques, a few different mechanisms have been proposed for neural modulation of lengthening contractions. As the size of an MEP is influenced by inputs from both cortical neurons and spinal motoneurons, Gruber and colleagues (2009) compared MEPs and CMEPs during lengthening and isometric contractions and observed a greater reduction in the size of CMEPs during lengthening contractions. This observation was suggestive of compensation for spinal inhibition by increases in descending drive (Gruber et al., 2009), and is consistent with findings of greater movement-related cortical potentials obtained via electroencephalography recordings during lengthening contractions (Fang et al., 2004, 2001). Evidence of increased cortical output during lengthening contractions is further supported by reduced SICI and ICF in the ipsilateral motor cortex (Howatson et al., 2011). Similarly, Opie and Semmler recently observed reduced SICI, but an increase in long-interval intracortical inhibition, suggesting differential modulation of intracortical connectivity (related to $GABA_A$ vs. $GABA_B$ neurotransmitters) during lengthening contractions (Opie and

Semmler, 2016). In this light, it is also worth noting that lengthening contractions are accompanied by reduced duration of the TMS-induced SP. The SP has generally been accepted as an index of intracortical inhibition, however, this interpretation is debatable (Škarabot et al., 2019).

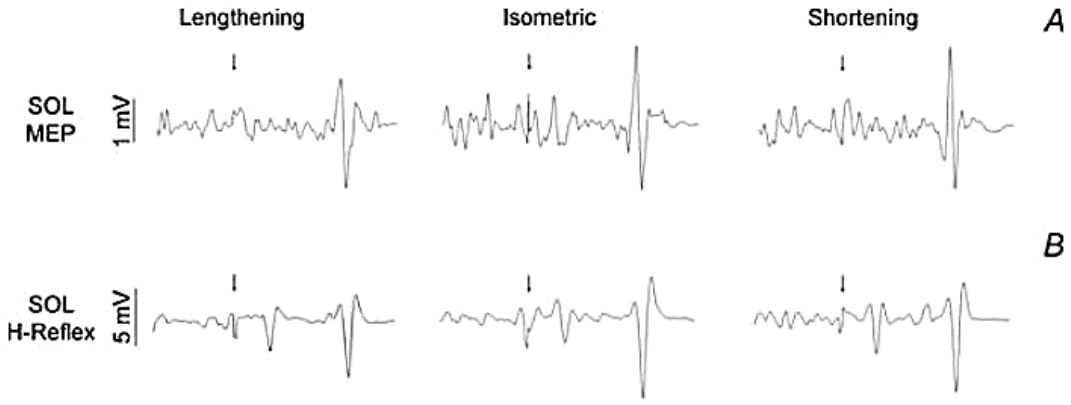

Figure 2-8. Motor evoked potentials (A) and H-reflex (B) behaviour during lengthening, isometric and shortening contractions. Downward arrow denotes the point of stimulus (Duclay et al., 2011).

In an attempt to distinguish between the contribution of cortical neurons and spinal motoneurons, comparison between H-reflexes that test the Ia synaptic input and responsiveness of motoneurons, and MEPs has been made (Duclay et al., 2014). As a greater degree of reduction in the size of H-reflexes was observed compared to MEPs during lengthening contractions, inferences have been made as to the origin of reduction being at the spinal, rather than the corticospinal pathway. However, H-reflexes, unlike CMEPs (Nielsen and Petersen, 1994), are influenced by presynaptic mediators, which is why the reduced H-reflexes during lengthening contractions have been most often attributed to alterations in presynaptic inhibition (Abbruzzese et al., 1994; Duclay et al., 2014, 2011; Duclay and Martin, 2005; Romanò and Schieppati, 1987). The increase in presynaptic inhibition via primary afferent depolarization seems a more likely mechanism (Rudomin and Schmidt, 1999) than homosynaptic postactivation depression (Hultborn et al., 1987), due to a greater influence of the latter

during rest than activity (Petersen et al., 2007), and evidence of descending pathways controlling spinal inhibition during lengthening contractions supporting the former (Grosprêtre et al., 2014). Based on indirect evidence (Duclay et al., 2011), postsynaptic mechanisms may also play a role, the most likely being recurrent (Renshaw cell) inhibition (Duchateau and Enoka, 2016) due to its potential role in reduced MU discharge during lengthening contractions (Del Valle and Thomas, 2005; Laidlaw et al., 2000; Pasquet et al., 2006; Søgaard et al., 1996; Stotz and Bawa, 2001). By comparing modulations of reference, conditioning and test H-reflexes during different actions types, Barrue-Belou and colleagues showed a reduction in the test H-reflex during lengthening contractions, whilst reference and conditioning H-reflexes remained unchanged across contraction types. Their finding further supports the notion of increased recurrent inhibition during lengthening contractions (Barrué-Belou et al., 2018). It is also worth noting that a recent study suggested that the rate of sensory information input does not play a major role in the neural control of lengthening contractions, with responsiveness of corticospinal pathway being similar with different velocities of fascicle lengthening (Valadão et al., 2018). However, the study was limited by the low number of different velocities investigated as well as disagreeing with the findings that showed differing corticospinal responses during lengthening contractions depending on the muscle length at the point of stimulation (Doguet et al., 2017).

Collectively, the abovementioned evidence suggests that both supraspinal and spinal mechanisms are involved in the neural control of lengthening contractions, with the inhibition occurring at the spinal level, but can be modulated by descending pathways from supraspinal centres (Duchateau and Enoka, 2016). This proposed neural control might fit a feedforward (Valadão et al., 2018) or feedback (Doguet et al., 2017) control

model. Despite seemingly overwhelming evidence for the notion of unique control of lengthening contractions, it should be noted that comparison between studies is difficult due to different loading conditions (isokinetic vs. inertial), contraction intensity (maximal vs. submaximal) and methodological constraints (see next section).

Methodological consideration for interpretation of neural control of lengthening contractions

A muscle consists of the contractile elements arranged in-series and connective tissue attachments to the skeleton. Thus, changes in whole muscle length might not necessarily parallel changes in the length of the contractile elements (Ishikawa et al., 2005; Kawakami and Fukunaga, 2006). In particular, this might be the case at the beginning of a lengthening contraction, whereby muscle fascicles perform a shortening action to take up tendon compliance before they start lengthening, regardless of whether the entire muscle-tendon unit (MTU) is being lengthened via joint rotation (Hahn, 2018). Another confounding factor that limits the interpretation of neural behaviour of shortening and lengthening actions is the muscle length at which the assessment is performed. Typically, comparison between different action types is performed at the same joint angle and the presumption is made that the assessment is performed at the same muscle length. However, given the compliance of the MTU, this merely confirms comparison is made between the same MTU length, and not identical lengths of the contractile elements (Hahn, 2018). Thus, rigorous control of active elements of the muscle is needed in studies investigating neural responses during muscle shortening and lengthening.

Another important consideration is that with a lengthening contraction, the force-production capacity of a muscle or muscle fibre can be greater as compared to

shortening or isometric contractions. For example, data from *in situ* muscle fibres or whole animal muscles shows 50-80% greater force production during lengthening compared to isometric contractions (Edman, 1988; Morgan et al., 2000). However, in humans this enhanced force production has failed to be detected (Amiridis et al., 1996; Babault et al., 2001; Beltman et al., 2004; Colson et al., 2009), or has been observed to be smaller compared to animal data and *in situ* muscles, i.e. <40% (Aagaard et al., 2000; Duclay et al., 2011; Kellis and Baltzopoulos, 1998; Linnamo et al., 2006; Reeves et al., 2009). This seems to be somewhat dependent on the muscle group, with dorsiflexors consistently showing greater torque-producing capacity during lengthening contractions (Klass et al., 2007; Pasquet et al., 2000; Reeves and Narici, 2003), and age, with aging adults more likely to exhibit force enhancement (Roig et al., 2010). Therefore, it has been suggested that caution should be taken when interpreting the unique activation signal purported to modulate lengthening contractions (Hahn, 2018). For example, some ambiguity exists in the literature showing that a reduction in MEP and CMEP size is absent when torque enhancement is observed (Hahn et al., 2012). The notion of unique activation strategy during lengthening contractions may have stemmed from observations that the level of voluntary activation as assessed by interpolated twitch technique is usually lower during lengthening contractions (Amiridis et al., 1996; Beltman et al., 2004; Westing et al., 1991), which has been suggested to be mediated by a tension-regulating mechanism (Amiridis et al., 1996; Del Valle and Thomas, 2005; Gruber et al., 2009; Seger and Thorstensson, 2000; Westing et al., 1991). Specifically, this hypothesis suggests the involvement of Golgi tendon organ and its inhibitory action on motoneurons in order to limit the production of force (Duchateau and Enoka, 2016). However, this seems unlikely (Duchateau and Baudry, 2014; Duchateau and Enoka,

2016) given that normalised force-velocity relationships are similar during maximal and submaximal lengthening contractions (Pinniger et al., 2000) and a depression in EMG activity might be evident in the maximal isometric contraction preceding a lengthening contractions (Grabiner and Owings, 2002). Also, this hypothesis does not seem to take into account that in certain conditions (e.g. when dorsiflexors are studied or elderly individuals) the force enhancement during lengthening contractions is present. Furthermore, following training (Aagaard et al., 2000; Colson et al., 2009) and in trained individuals (Amiridis et al., 1996), where force enhancement is exhibited, decreased voluntary activation during lengthening contractions is absent. This supports the need for a thorough familiarisation procedure that can result in the realisation of force enhancement in as few as 1-4 sessions (Hahn, 2018).

The associated EMG activity has been shown to be lesser during lengthening when compared to shortening contractions at a given absolute load (Bigland & Lippold, 1954; Enoka, 1996; Duchateau & Enoka, 2016). However, there are some methodological issues that confound this simplistic view (Duchateau and Enoka, 2016), particularly as it relates to the evidence of greater force-producing capacity of muscle fibres during lengthening contractions. Specifically, greater MU activity is needed during shortening contractions to achieve the same absolute force than during lengthening contractions (Duchateau and Enoka, 2016). For example, if the same absolute load is used during both lengthening and shortening contractions, it is likely to be more submaximal for the former than the latter and hence it will result in less MU activity. It has been suggested that the differing MU activity between shortening and lengthening contractions might stem from preferential recruitment of high-threshold MUs during lengthening contractions (Nardone et al., 1989) and alterations in the MU recruitment order. However, this notion was not supported by subsequent

studies showing lack of differences in MU recruitment between contraction types (Bawa and Jones, 1999; Laidlaw et al., 2000; Pasquet et al., 2006; Stotz and Bawa, 2001). It is more likely that recruitment/derecruitment of certain MUs during lengthening contractions are due to variations in muscle length and resulting small stretches, or slight oscillations in joint position in the transition between shortening and lengthening contraction (Bawa and Jones, 1999).

Furthermore, the rate of change of muscle length must be similar between contraction types to discount the influence of contraction velocity on afferent feedback and thus neural activation of the muscle (Duchateau and Enoka, 2011). Most experiments use isokinetic dynamometry to assess differences between contraction types as it allows for rigorous control of angular velocity and torque output (Duchateau and Enoka, 2016). Thus, given the potential for aforementioned differences in MU activity, it is important that the torque level is proportional to contraction type specific maximal voluntary contraction (MVC) for appropriate comparison between shortening and lengthening submaximal contractions.

2-6.2 Response and adaptation to a bout of lengthening contractions

Performing a bout of unaccustomed exercise, particularly that involving (maximal) lengthening contractions, results in a myriad of physiological changes as a consequence of muscle damage that are most often accompanied by sensations of muscle soreness. These physiological mediations can be roughly categorised as symptomatic, systematic and histological (Hyldahl et al., 2017). The symptomatic consequences of unaccustomed, damaging exercise include a prolonged reduction in force-generating capacity of a muscle, delayed-onset muscle soreness (DOMS), and the accompanying increase in muscle stiffness and swelling (Hyldahl et al., 2017). The

systemic responses include increases in circulating levels of muscle proteins such as creatine kinase (CK) and myoglobin (McHugh, 2003). The histological alterations include disruptions within the muscle fibres themselves at the level of myofilaments and the surrounding structures in the extracellular matrix, i.e. endomysium, perimysium and epimysium (Hyldahl and Hubal, 2014). The time course is not necessarily the same for all variables. For example, force-generating capacity of the muscle is reduced to the greatest extent one day post unaccustomed lengthening exercise with complete restoration in 7-14 days (Hyldahl et al., 2017). Conversely, muscle soreness might be present one day post, but peaks 2-3 days post lengthening exercise, whilst peak swelling is more likely to occur 4-7 days post (Hyldahl et al., 2017). Blood CK levels usually increase 2 days post with the peak occurring 4-5 days post (Hyldahl et al., 2017). Changes to the extracellular matrix are less clear, largely due to the difficulty in measuring them, but they are likely to be more pronounced in the days after exercise than immediately post (Hyldahl et al., 2017). The magnitude of muscle soreness and physiological changes in response to unaccustomed lengthening exercises will depend on many factors and will usually be greater with greater intensity, higher velocity, a greater number of contractions, longer muscle length, in the upper compared to lower limb muscle groups and less exposure to lengthening contractions in daily activity. To a lesser extent muscle damage might also be dependent on age, with prepubescent children being less susceptible to damage, and biological sex, but the data on the latter is equivocal (Hyldahl et al., 2017).

If the exercise involving lengthening contractions resulting in muscle damage is repeated, a protective adaptive response occurs leading to an attenuation of muscle damage and soreness (Byrnes et al., 1985), and the associated physiological changes (e.g. smaller reduction in MVC; Newham et al., 1987). This adaptive response is

known as the repeated bout effect (RBE), a term first coined by Nosaka and Clarkson (1995), which may also extend to the contralateral homonymous muscle group, a phenomenon known as the contralateral RBE (Howatson et al., 2007). The phenomenon of RBE is mediated by alterations to mechanical properties of MTU, structural remodelling of extracellular matrix, biochemical signalling pathways modifying the inflammatory response and a host of neural adaptations operating in concert (Hyldahl et al., 2017). Due to the overarching theme of this thesis, the focus will be placed on neural mechanisms in response and adaptation to a bout of maximal lengthening contractions that remain understudied.

Neural responses and adaptation to a bout of maximal lengthening contractions

There are many lines of evidence that suggest the involvement of the CNS in response and adaptation to a bout of maximal lengthening contractions. Following lengthening exercise, there is a reduction in voluntary activation as assessed using motor nerve stimulation (Goodall et al., 2017; Prasartwuth et al., 2005) and TMS (Goodall et al. 2017), suggesting reduced capacity of the CNS to recruit available MUs in the motoneuron pool. If the same exercise is repeated, the reduction in voluntary activation is less pronounced.

A number of studies have shown that adjustments in the motoneuron pool are greatly involved in the response and adaptation to maximal lengthening exercise. Firstly, the EMG activity has been found to increase during lower force levels (≤50% MVC) post damaging exercise (Carson et al., 2002; Leger and Milner, 2001; Meszaros et al., 2010; Prasartwuth et al., 2005; Semmler et al., 2007; Weerakkody et al., 2003), despite the reduction in MVC (Figure 2-9).

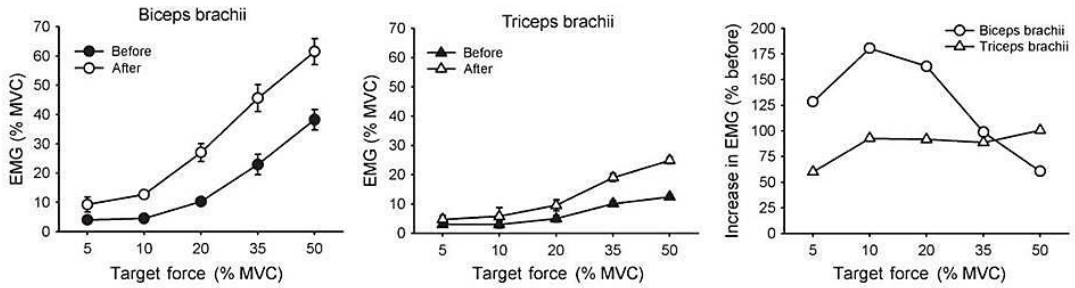

Figure 2-9. EMG activity at the same relative force output pre and post damaging exercise (Semmler, 2014)**.**

Second, there is an increase in the activation of the antagonist muscle post exercise (Leger and Milner, 2001; Semmler et al., 2007), which has been suggested to contribute to joint stiffness and improved neuromuscular performance in a damaged muscle (Semmler, 2014). This increased in antagonist activation might also contribute to increased EMG activity of the agonist, although the dissimilar increase pattern post damaging exercise suggests multifactorial contribution (Semmler, 2014). Third, following a bout of maximal lengthening contractions there is an increase in MU synchornisation, and coherence between pairs of MUs in the low frequency range (0-10 Hz; Dartnall *et al.*, 2008). Fourth, a reduction in recruitment threshold has been observed following damaging exercise, suggesting greater MU activity at the same relative force level post exercise (Dartnall et al., 2009). Lastly, damaging exercise has been associated with decreased MU conduction velocity (Hedayatpour et al., 2009; Piitulainen et al., 2012, 2011), which combined with increased MU activity, likely contributes to increased EMG activity at the same force level post exercise. Whilst a significant amount of data is available to suggest MU plasticity in response to damaging exercise involving maximal lengthening contraction, experiments on the adaptation process are lacking. Dartnall and colleagues provide one of the few pieces of direct evidence that MU adjustments occur in the adaptive process from damaging exercise. Following the second bout of damaging exercise they found no change in

recruitment thresholds relative to an increase after the first bout, and a return of increased MU synchornisation levels post the second bout to pre-exercise levels (Dartnall et al., 2011). This return to normal level of syncronisation suggests a decrease or increase of common or independent inputs to the motoneuron pool, respectively (Dartnall et al., 2011).

Because the MUs represent the common synaptic input, it is likely that the response and adaptation at the level of the MU is the result of modulation of synaptic inputs originating from other sources. Furthermore, the existence of contralateral RBE would suggest that the processes above the level of the motoneuron pool are involved in the adaptive process in response to maximal lengthening exercise. A bout of maximal lengthening exercise has been shown to reduce the degree of intracortical inhibition and has been related to a short-term change in afferent feedback stemming from the release of biochemical substrates and inflammation (Pitman and Semmler, 2012). However, it remains unknown whether the response would differ if the bout of exercise was repeated, when disruptions in biochemistry are expected to be smaller. Additionally, DOMS in response to a bout of maximal lengthening contractions has been shown to increase somatosensory cortical excitability and reduce the hotspot area required to elicit an MEP (De Martino et al., 2018). These data provide strong evidence of an acute neuroplastic response to a bout of maximal lengthening contractions that is likely related to DOMS (De Martino et al., 2018).

Overall, CNS does seem to play a role in the response and adaptation to maximal lengthening exercise. However, despite the evidence from studies examining MU activity and mechanically-induced variables, no study has systematically examined the contribution of both supraspinal (i.e. the cortical activity and modulation of

intracortical networks) and spinal components in this adaptive process (Hyldahl et al., 2017).

2-6.3 The benefits of lengthening contractions

Lengthening contractions may be beneficial for a number of reasons. Firstly, the higher forces produced during lengthening contractions, which provide greater overload during training, may present an effective strategy to prevent musculoskeletal injuries (LaStayo et al., 2003c). Second, given the lower metabolic cost of lengthening contractions (Abbott et al., 1952; Abbott and Bigland, 1953), training using this contraction type may be better suited for energetically impaired populations (LaStayo et al., 2003c). Such a population are the elderly, who are at a greater risk for musculoskeletal injuries than younger individuals due to sarcopenia. Moreover, there is a great amount of evidence to suggest that training involving lengthening contractions may be superior to traditional training strategies for increasing muscle strength and mass in the elderly as well as their functional ability (LaStayo et al., 2003b; Reeves et al., 2009). Third, lengthening contractions may be suitable for prevention of muscle-tendon injuries, particularly strains, potentially due to increase in tendon stiffness and improved ability to absorb energy at the musculotendinous junction as a result of training involving lengthening contractions (LaStayo et al., 2003b; Reich et al., 2000; Roig Pull and Ranson, 2007). However, there are opposing views to this approach which suggest having a more compliant muscular system may be better at mitigating said injuries (Brockett et al., 2001; Proske and Morgan, 2001). Fourth, since the magnitude of increases in bone mass and mineral density is dependent on the magnitude of force, lengthening contractions are also a viable strategy for preventing osteopenia (Braith et al., 1996; Chambers et al., 1993; Hawkins

et al., 1999; Lanyon, 1987; Vincent and Braith, 2002). Fifth, lengthening contractions are relied upon when descending the stairs (Andriacchi et al., 1980; Lindstedt et al., 2001; McFadyen and Winter, 1988), an activity with the greatest risk of falls in the elderly (Jacobs, 2016; Startzell et al., 2000), linked to impaired ability to perform graded lengthening contractions (Bean et al., 2002; Hughes et al., 1996; Moxley Scarborough et al., 1999) despite greater preservation of lengthening contraction strength (Roig et al., 2010). Steadiness seems to be especially reduced during lengthening contractions in older people with a history of falling when compared to age-matched peers without any history of falling (Carville et al., 2007). Thus, improving strength during lengthening contractions could be of functional significance for older individuals (LaStayo et al., 2003b). Sixth, lengthening contractions are a viable strategy for rehabilitation of musculoskeletal impairments, such as tendinopathies (Alfredson et al., 1998b, 1998a; Frizziero et al., 2014; Jensen and Di Fabio, 1989; Stanish et al., 1986, 1985). Lastly, because lengthening contractions have the ability to overload the muscle to a greater extent, they may result in greater enhancement of muscle mass, strength and power (Brandenburg and Docherty, 2002; Hortobágyi et al., 2001a, 1996b, 1996a; Komi and Buskirk, 1972; Lastayo et al., 1999; LaStayo et al., 2000; LaStayo et al., 2003b; Lindstedt et al., 2001).

2-7 Alterations in central nervous system with aging

It is well recognised that healthy aging is characterised by many neuromuscular performance decrements associated with both muscular and neural alterations. The most apparent is the loss of muscle mass and strength, induced by loss of skeletal muscle fibres, apoptosis of motoneurons and incomplete re-innervation of remaining muscle fibres, resulting in fewer, but larger MUs (Doherty, 2003). In light of the topic

of this thesis, the focus of this section will be placed on age-related alterations in the CNS, encompassing alterations at the level of the MU, i.e. the alpha motoneuron and the muscle fibres it innervates, and the synaptic inputs from the CNS. Whilst the definitions vary across literature, in this thesis adults will be considered aging if they are older than 60 years of age (Hunter et al., 2016b).

2-7.1 Alterations at the level of motor unit

Healthy aging is accompanied by a progressive loss of MUs (McNeil et al., 2005), which might be a precursor for many neuromuscular adaptations in older age (McNeil and Rice, 2018). Due to the adaptive nature of the neuromuscular system, following the loss of MUs the process of collateral reinnervation occurs, with nearby surviving motor axons sprouting to reinnervate the denervated muscle fibres. This results in larger MUs, with surviving motoneurons now innervating a greater number of muscle fibres. However, reinnervation leads to greater oxidative stress to motoneurons, which could lead to their death, as well as instability of the neuromuscular junction (Deschenes, 2011; Hepple and Rice, 2016). The process of sprouting appears to be finite with individual motoneurons being limited in the number of muscle fibres they can sustain (Rafuse et al., 1992). The latter might explain the greater success rate of collateral sprouting in older compared to very old adults (Gilmore et al., 2018; Piasecki et al., 2018). Overall however, studies indicate that reinnervation leads to a reduction in the estimated number of MUs (Gooch et al., 2014) in older population (Gilmore et al., 2017; McNeil et al., 2005; Piasecki et al., 2018; Power et al., 2016a). The rate of reduction in the estimated number of MUs is ~1% per year between the third and sixth decades, but thereafter accelerates to ~2% a year (Campbell et al., 1973; McNeil et al., 2005; Tomlinson and Irving, 1977).

Despite the process of reinnervation, its finite nature might eventually lead to the death of motoneurons, which has been suggested to preferentially occur in motoneurons innervating fast-twitch muscle fibres (Kanda and Hashizume, 1989). The latter might potentially explain the reduction in MU discharge rates and the slowing of contractile properties. Indeed, discharge rates of MUs during moderate and high intensity contractions has consistently been shown to be lower in older compared to younger adults (Dalton et al., 2010; Kirk et al., 2018; Klass et al., 2008).

2-7.2 The influence of synaptic inputs from spinal and supraspinal centres

Whilst the actions of the muscles are dependent on the output from motoneurons, the activity of the latter is dependent on synaptic inputs that they receive. Thus, the age-related changes at the level of the MU are determined by the gain of the neuromuscular system, i.e. the ratio of the output to the input (Hunter et al., 2016b). The synaptic inputs to the motoneurons originate from numerous sites in the CNS. This section will focus on the reflex responses that alter motoneuron excitability through the afferent pathway as well as inputs originating from the supraspinal centres emphasising those related to the primary motor cortex.

Reflex inputs to motoneurons

Aging has been associated with a concomitant reduction in responses from the afferent sensory receptors to spinal motor neurons. The H-reflex has been shown to be lower in amplitude in older adults when compared to young (Figure 2-10), and has often been related to increased presynaptic inhibition (Kallio et al., 2010; Morita et al., 1995; Sabbahi and Sedgwick, 1982; Scaglioni et al., 2002). There is also a reduction in peripheral nerve conduction velocity with advancing age (Dorfman and Bosley, 1979;

Rivner et al., 2001), possibly stemming from the loss of large-diameter axons (Kawamura et al., 1977a), smaller density of both myelinated and unmyelinated neurons as well as reduced internodal length (Doherty and Brown, 1993; Jacobs and Love, 1985; Metter et al., 1998; Scaglioni et al., 2002), resulting in a general slowing of responses. Based on the prolonged latencies of the H-reflex, but no change in the latency of direct motor response with advancing age, it has been suggested that aging might affect sensory afferent axons to a greater extent than efferent motor axons (Scaglioni et al., 2002). However, further investigation is warranted, employing more robust measures, such as calculation of conduction times (Udupa and Chen, 2013), to fully elucidate the age-related adaptations in the specific type of axons.

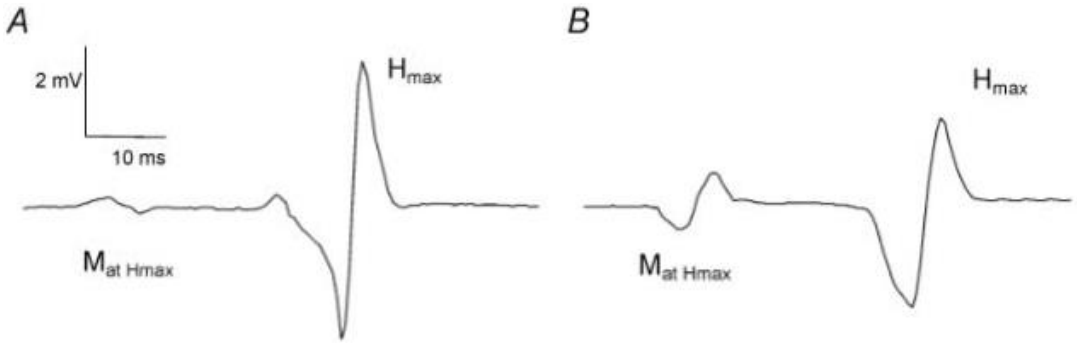

Figure 2-10. H-reflex in young (A) compared to older (B) adults (Scaglioni et al., 2003)**.**

Cortical inputs to motoneurons

Unlike the loss of spinal motoneurons, the number of cortical neurons appears to be unchanged in older populations (Salat et al., 2004). However, aging is accompanied by reductions in the size of cortical neuronal cell bodies (Ward, 2006a), and reduced white (Marner et al., 2003) and grey (McGinnis et al., 2011; Salat et al., 2004) matter volume, which have been associated with decreases in neuromuscular performance (Kennedy and Raz, 2005). Furthermore, instability of neuromuscular transmission has been observed in aging adults (Power et al., 2016a). With regards to the latter, MEP latencies have been shown to be longer in older individuals, which might stem from

age-related alterations in temporal characteristics of interneuronal (I-wave) circuitry (Opie et al., 2018).

Aging has been associated with decreased interhemispheric inhibition (Mattay et al., 2002; Ward, 2006a), shorter TMS-induced SPs (Oliviero et al., 2006; Sale and Semmler, 2005) and reduced intracortical inhibition (Heise et al., 2013; Marneweck et al., 2011; Opie and Semmler, 2016). This decreased motor cortical inhibition could result in inappropriate activation of MUs leading to unnecessarily greater muscle activation and reduced motor control (Johnson et al., 2017; Seidler et al., 2010), particularly during fine motor tasks (Shinohara et al., 2003). However, the findings pertaining to the indices of corticospinal excitability, such as the size of MEPs and the slope of input-output relationship of TMS have been equivocal (Bhandari et al., 2016), with studies showing similar (Hassanlouei et al., 2017; Smith et al., 2011b; Stevens-Lapsley et al., 2013) or reduced (Oliviero et al., 2006; Pitcher et al., 2003; Sale and Semmler, 2005) corticospinal excitability in older adults compared to young. Similarly, results from paired-pulse stimulation measures, such as intracortical inhibition and facilitation, have been mixed (Bhandari et al., 2016). Furthermore, SP duration has been found to be unchanged (Hunter et al., 2008; Rozand et al., 2017; Stevens-Lapsley et al., 2013) or shorter (Oliviero et al., 2006; Sale and Semmler, 2005) in older adults. These discrepancies could be multifactorial (Hassanlouei et al., 2017), ranging from biological sex, contractile state (relaxed vs. active) and the muscle investigated (upper vs. lower limb). Importantly, the findings were obtained only during isometric contractions. As highlighted recently, there is a need to assess responses during dynamic movements as they provide greater functional information about neuromuscular output (McNeil and Rice, 2018).

Interestingly, the age-related changes in intracortical inhibition, which reflect the state of neurotransmitters in the brain (McGinley et al., 2010; Opie and Semmler, 2016) have been linked to the capacity for neuroplasticity (Cash et al., 2016). As such, the latter might be reduced in older adults (McNeil and Rice, 2018; Opie et al., 2018). Investigation of supraspinal responses in older adults is thus not only important in elucidating the age-related changes in corticospinal input that can alter synaptic input at the motoneuronal level, but also to elucidate the potential mechanisms of the neuroplastic response to different types of exercise, which is crucial to inform clinical practice.

2-8 Neuromuscular performance during shortening and lengthening contractions in healthy aging adults

As a result of neuromuscular alterations described in the preceding section, aging adults exhibit reduction in neuromuscular performance during both maximal and submaximal contractions. Within the population of older adults however, there might be differences depending on age, with very old adults (>80 years old; Hunter et al., 2016) usually exhibiting significantly greater decrements in neuromuscular performance compared to old adults (60-80 years old). However, even when matched for age and sex, great heterogeneity of responses is typically observed in older compared to young adults (Degens and Korhonen, 2012; Rantanen et al., 1998; Vanden Noven et al., 2014). Furthermore, older adults exhibit greater trial-to-trial variability (Degens and Korhonen, 2012; Rantanen et al., 1998; Vanden Noven et al., 2014). The origin of this greater heterogeneity has been ascribed to age-related changes in the CNS (Hunter et al., 2016b) and needs to be taken into consideration when investigating neuromuscular performance. The purpose of this section is to

provide an overview of the age-related decrements and variability in neuromuscular performance measures that are pertinent to this thesis.

2-8.1 Neuromuscular performance during maximal contractions

Isometric strength

Aging is usually accompanied by a reduction in isometric strength (Doherty, 2003; Grabiner and Enoka, 1995; McNeil et al., 2005; Piasecki et al., 2018), which parallels the age-related reduction in muscle mass (Frontera et al., 2000; Metter et al., 1999). Furthermore, aging adults exhibit lower specific force, i.e. force per unit of cross-sectional area, related to the greater proportion of intramuscular fat and connective tissue (Goodpaster et al., 2008; Schaap et al., 2013). Cross-sectional studies show that the average age-related loss in isometric strength is ~10% per decade from ~50 years of age onwards, with acceleration of this decline in very old adults (Hunter et al., 2000; Lindle et al., 1997). However, longitudinal studies suggest that this decline might be underestimated (Frontera et al., 2000; Metter et al., 1999). The degree of age-related strength loss might also vary across different muscle groups (Doherty, 2003), with bigger muscle groups such as knee extensors often exhibiting a greater decline in strength, compared to hand, arm or lower leg muscles (Frontera et al., 2000; Hunter et al., 2000; Janssen et al., 2000; Klass et al., 2011; Kwon et al., 2011). This could be explained by variability in the rate of sarcopenia (Gilmore et al., 2017; Morat et al., 2016; Piasecki et al., 2018) and the associated failure to expand the MU size to compensate for a reduction in the number of MUs (Piasecki et al., 2018), which might be related to the variability of use across muscles in activities of daily living. Nevertheless, even when older adults might not present a deficit in the generation of maximal force, underlying neural changes associated with aging could still be present

(McNeil et al., 2005). The rate of strength loss could also differ between sexes, with aging males possibly exhibiting a greater decline in isometric strength compared to age-matched females (Ditroilo et al., 2010; Wu et al., 2016).

Strength during dynamic contractions

Similar to maximal isometric contractions, aging adults exhibit a reduction in strength during shortening contractions compared to the young, with the age difference being augmented by increased velocity of the contraction (Raj et al., 2010). The latter might be related to reduced maximal shortening velocity of fast-twitch fibres (Krivickas et al., 2001; Larsson et al., 1997), altered muscle architecture (Thom et al., 2007) and possibly lower maximal MU firing rates (Klass et al., 2008). Converesely, strength during lengthening contractions is better maintained in older age (Roig et al., 2010), as evidenced by greater lengthening to isometric strength ratio irrespective of contraction velocity (Figure 2-11; Power *et al.*, 2015). This has largely been attributed to muscle properties such as slower cross-bridge kinetics and greater stiffness of the muscle-tendon unit (Klass et al., 2005; Larsson et al., 1997; Power et al., 2016b, 2015).

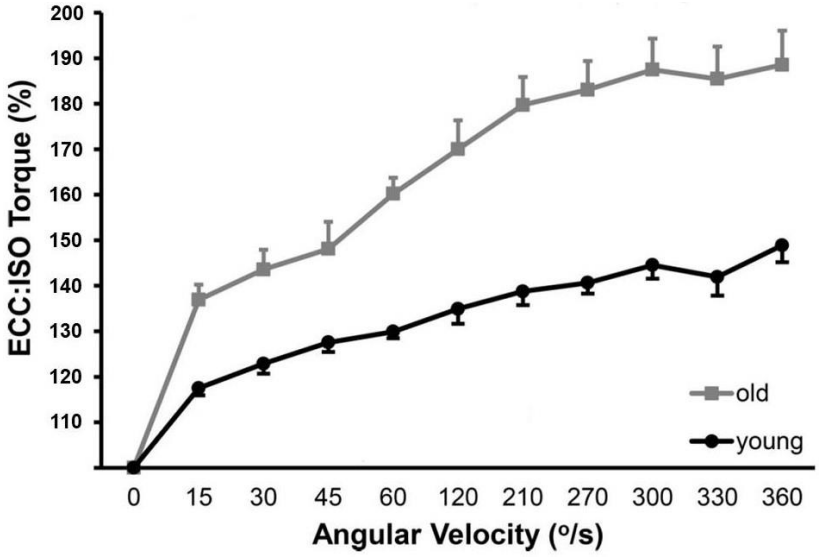

Figure 2-11. Age differences in the ratio of peak torque during lengthening and isometric dorsiflexion at different angular velocities (Modified from Power *et al.*, 2016).

2-8.2 Neuromuscular performance during submaximal tasks

Whilst most studies assess the effect of aging on neuromuscular performance via some type of maximal performance measure (e.g. maximal strength, power etc.), submaximal performance is considered less often (Bellew, 2002). However, the latter may be of greater functional significance for the aging population, given the functional issues (e.g. loss of balance) are more likely related to attenuated control of muscular force than its maximal capacity (Shepard et al., 1993).

Variability of torque/force output

There is a plethora of evidence suggesting that older individuals exhibit greater difficulty in controlling the force output of the muscle during submaximal contractions (Enoka et al., 2003). Control of force/torque is quantified by variability of force/torque output about a mean value as standard deviation or coefficient of variation (Enoka *et al.*, 2003). Generally, the differences in control of muscle force between young and older adults are influenced by the contraction intensity, muscle group, contraction type, and physical activity of the individual (Enoka et al., 2003). The absolute force/torque variability increases with increases in contraction strength regardless of age, due to an increase in MU activity with increased magnitude of contraction (Burnett et al., 2000; Galganski et al., 1993; Keen et al., 1994; Laidlaw et al., 2000; Tracy and Enoka, 2002). However, the variability appears to be a bigger challenge for the elderly particularly during low (≤10% MVC) or higher (>50% MVC) intensity isometric contractions (Graves *et al.*, 2000; Tracy & Enoka, 2002). Whilst the control of muscle force is generally compromised in older adults compared to the young, this finding is not entirely consistent across muscle groups, with some investigations in elbow flexor (Graves et al., 2000) and knee extensor (Tracy and Enoka, 2002) muscles

not showing a difference between young and older individuals. However, older individuals exhibit greater variability across trials when compared to their young counterparts (Christou et al., 2001; Christou and Carlton, 2001), likely due to impaired ability to reproduce timing characteristics of the contraction (Christou et al., 2002). Interestingly, there seems to be a lack of relationship between maximal strength and torque variability during submaximal contractions in older adults (Burnett et al., 2000).

Usually, older adults have a greater difficulty controlling the force/torque output during dynamic contractions, with the difficulty being greater at faster contraction velocities (Burnett *et al.*, 2000; Graves *et al.*, 2000; Laidlaw *et al.*, 2000, 2002; Christou *et al.*, 2003). Interestingly, variability in submaximal force production seems to be especially increased during lengthening contractions in older people with a history of falling when compared to age-matched peers without any history of falling (Carville et al., 2007). Force accuracy is also impaired with aging and is usually due to overshooting the target force, hence making the movement uneconomical (Hortobágyi et al., 2001b). This difference suggests poorer ability of older adults to exert a force with precision during isometric contractions and greater variability of trajectory during movement (Tracy and Enoka, 2002), possibly leading to greater incidence of falls (Bellew, 2002). As evidenced by many investigations suggesting that either strength or skill (Kornatz et al., 2005) training improve force steadiness, it is clear that muscle force control is dependent on physical activity status of an individual.

The mechanisms underpinning impaired neuromuscular performance during submaximal tasks

The greater difficulty of controlling muscle force in aging adults is mainly related to age-related alterations in the CNS. The loss of skeletal muscle fibres, apoptosis of motoneurons and incomplete re-innervation of remaining muscle fibres (Doherty, 2003), resulting in fewer, but larger MUs producing larger and more variable MU action potentials might be responsible for poorer control of muscle force in older individuals (Galganski et al., 1993). Equally, greater variability of MU discharge rates and common synaptic input can explain larger variability of force output (Castronovo et al., 2018). The activity in supraspinal centres, such as cortical and subcortical areas as well as frontal lobes (putamen, insula and contralateral superior frontal gyrus; Yoon *et al.*, 2014).

There are also other adaptations that may be relevant for force steadiness such as age-related changes in transmission efficacy via corticospinal and reflex pathways to motor neurons (Laidlaw et al., 2000). These include a loss of spinal motor neurons and increases in absolute latencies of sensory and motor evoked potentials, particularly in large-diameter, fast-conducting motor fibres in the pyramidal tract (Evans and Starr, 1994). Consequently, transmission efficacy along the corticospinal tract is altered as evidenced by reductions in the amplitudes of motor evoked (Eisen et al., 1991) and compound excitatory postsynaptic potentials (Eisen et al., 1996). Furthermore, aging is associated with impairments in the sensory system, such as joint-position sense, touch, kinesthesis and proprioception (Cole, 1991; Desrosiers et al., 1996; Henningsen et al., 1995; Sathian et al., 1997; Skinner et al., 1984), which would influence afferent feedback mechanisms associated with force control (Hortobágyi et al., 2001b).

2-9 General summary

Aging is accompanied by MU loss and remodelling along with alterations of the synaptic inputs from the CNS, resulting in decrements in neuromuscular performance. These include a reduction in strength during isometric and shortening contractions. Furthermore, aging adults exhibit increased variability of the motor output, particularly during shortening and lengthening contractions. Changing muscle length modulates responses in the corticospinal tract. During passive shortening corticospinal excitability is augmented, and decreased during passive lengthening, with this behaviour likely being related to the modulation of afferent feedback originating from muscle spindles. However, it remains unclear at what level of the corticospinal pathway this modulation occurs, or whether this modulation extends to the muscles of the lower limb. Similarly, discrete shortening and lengthening contractions modulate corticospinal output in younger populations, but this interpretation might be constrained by methodological approaches (Hahn, 2018). Studies have only assessed age-related differences in corticospinal output during isometric contractions, but neglected investigation of dynamic contractions that provide greater functional information about the neuromuscular output (McNeil and Rice, 2018). Repeated lengthening contractions might result in DOMS and disruption of neuromuscular function. However, if the same bout is repeated, the muscle adapts to protect it from further damage. Despite the preservation of eccentric strength, older adults typically exhibit a smaller expression of RBE, which might be related to reduced adaptability of their altered central nervous system (McNeil and Rice, 2018; Opie et al., 2018). Since lengthening contractions are of great importance for the activities of daily life in the elderly, and because of their potential to produce higher muscle forces at lower metabolic cost and could thus be more suited for the elderly, it is imperative we

understand the central nervous system responses and adaptation to lengthening contractions.

2-10 Study aims

The overarching aim of this thesis was to elucidate neurophysiological responses and adaptation to muscle shortening and lengthening and their interaction with the aging CNS. The central hypothesis was that both the sensory and motor components of the CNS will be altered in aging adults, leading to impaired sensorimotor integration of muscle length related afferent feedback and reduced corticospinal responses during dynamic contractions, which will in turn impair the ability of older adults to adapt to novel stimuli involving lengthening contractions. To ensure robust study design, Study 1 and Study 2 are also supplemented with methodological experiments designed to investigate some of the methodological issues outlined in the review of the literature. The specific aims of each experiment are outlined below.

Study 1: Neurophysiological responses to passive shortening and lengthening

Part 1: To validate a novel method of subcortical stimulation of corticospinal axons.

Part 2: To elucidate at what level of the neural axis does the modulation of corticospinal responses during passive shortening and lengthening of lower limb muscles occur, and whether altering muscle sensory feedback results in differing modulation of corticospinal output between young and older adults.

Study 2: Neurophysiological responses to submaximal isometric, shortening and lengthening contractions

Part 1: To find the optimal normalising procedure for estimates of relative torque output during submaximal dynamic contractions.

Part 2: To establish whether the behaviour of the contractile elements of tibialis anterior during submaximal shortening and lengthening contractions is distinct.

Part 3: To assess the interplay between neuromuscular performance, the associated variability and differences in corticospinal and spinal responses during submaximal isometric, shortening and lengthening contractions between young and older adults.

Study 3: Neurophysiological responses and adaptation to a bout of maximal lengthening contractions in young and older adults

To systematically assess the contribution of spinal and supraspinal (cortical and intracortical) properties in response and adaptation to repeated bouts of maximal lengthening contractions and compare it with the adaptive response of older adults.

CHAPTER 3: GENERAL METHODS

3-1 Introduction

This chapter details the general methods applied to studies within the thesis. Specific methods used in individual studies are outlined in the corresponding experimental chapters.

3-2 Pre-testing procedures

3-2.1 Ethical approval

Prior to each study, ethical approval was obtained from Northumbria University Ethics Committee in accordance with Declaration of Helsinki.

3-2.2 Participants

Young (age: 18-35 years) and older (age: ≥60) individuals were recruited. Participants read an information sheet (Appendix 1) and those eligible for the study provided written informed consent prior to any element of the study proceeding (Appendix 2). The lower age limit of 60 years for the older group of adults was chosen based on the reported age-related changes in the neuromuscular system affecting neuromuscular function and performance (Hunter et al., 2016b). To reduce the potential influence of female sex hormones on corticospinal responses (Smith et al., 2002), all premenopausal females were tested in the early follicular phase of the menstrual cycle where quantities of both oestrogen and progesterone are likely to be low (Elliott et al., 2003), or whilst taking oral contraceptives (Ansdell et al., 2019). Prior to testing, participants responded to the questionnaire for contraindications for TMS (Rossi et al., 2009) and a health screening questionnaire to ascertain contraindications to participation (Appendix 3). An important consideration for recruitment of participants

was the level of physical activity performed due to the disparate age-related alterations in corticospinal responses that have been observed in relation to physical activity (Hassanlouei et al., 2017). The included participants were considered recreationally-active, i.e. meeting the recommended physical guidelines of the World Health Organisation (World Health Organisation, 2010). Overall, participants reported 350 ± 235 min of moderate to high intensity physical activity per week on average, with no differences between young (360 ± 130 min/week) and older (330 ± 320 mins/week; p = 0.767). The exclusion criteria for both groups of participants included any neurological or neuromuscular disorders, musculoskeletal injury that may attenuate the ability to produce torque, taking any medications known to affect the nervous system, and having pacemakers and intracranial plates. Participants were instructed to refrain from alcohol and strenuous exercise the day prior to testing and caffeine on the day of testing (Gandevia and Taylor, 2006).

3-3 Testing procedures and instrumentation

3-3.1 The choice of muscle under investigation

Dorsiflexors were chosen as an experimental model due to their unique behaviour during lengthening contractions. Whilst not all human muscles may exhibit greater torque-producing capacity during lengthening contractions (for review see Duchateau and Enoka, 2016), dorsiflexors have consistently demonstrated significantly greater maximal lengthening torque compared to shortening and/or isomeric (Pasquet *et al.*, 2000; Reeves & Narici, 2003; Klass *et al.*, 2007; Duchateau & Enoka, 2016). Furthermore, from a neurophysiological perspective, TA, a primary agonist muscle during dorsiflexion, has been shown to have a comparatively high corticospinal drive during human locomotion (Schubert et al., 1997) owing to the need for accuracy of

toe clearance (Capaday et al., 1999) and its role in the control of foot drop during heel strike and foot lift during the swing phase (Byrne et al., 2007). Lastly, poor function of TA has been linked to greater incidence of falls in older adults (Kemoun et al., 2002; Whipple et al., 1987), thus suggesting the need for study the neurophysiological function of this muscle. The within- and between-day repeatability of corticospinal measures in TA has been established previously in our laboratory (Tallent et al., 2012).

3-3.2 Dynamometry and torque recordings

Participants were sat on an isokinetic dynamometer (Cybex Norm, Computer Sports Medicine Inc., Stoughton, MA, USA). All testing was performed on the dominant limb as per the lateral preference inventory (Coren, 1993; see Appendix 4). Hip angle was positioned at 60° of flexion with the knee and the ankle at 90°. The foot was strapped securely to a metal plate attached to lever arm of the motor with a velcro strap at the level of the talus and phalange bones, with particular attention made to minimise extraneous toe movements. The distal part of the thigh was strapped down with velcro to minimise abduction, adduction and flexion of the hip. Participants were instructed to focus solely on dorsiflexion and activation of TA. Visual feedback of the target torques was provided with the monitor placed approximately 1.5 m from the participant with the *y*-axis scale of visual display kept consistent for all contraction levels for all participants (−10 to 110% of participant's MVC). For shortening and lengthening, range-of-motion (ROM) was from 10° of dorsiflexion to 10° of plantar flexion (total ROM of 20°, anatomical zero was taken when the ankle was at 90°). Velocity of movement for lengthening and shortening was set at $5°\cdot s^{-1}$, giving a total contraction time of 4 s. Before the start, participants were passively moved into the starting position (10° of plantar flexion or 10° of dorsiflexion relative to anatomical

zero for shortening and lengthening, respectively) to minimize thixotropic effects (Proske et al., 1993). In the case of contractions, the dynamometer was programmed to move the ankle once the participant had reached the desired torque level in the starting position. Isometric contractions were made with the ankle joint at anatomical zero and participants were instructed to increase the torque to an appropriate level and then maintain it for 4 s, thereby equating the contraction time to across contraction types. Stimuli were delivered at the same joint angle (anatomical zero) across all conditions via the graphical editor function in Spike2 (v8, CED, UK). Torque was recorded directly from isokinetic dynamometer as a raw analogue signal (mV), converted to torque (N·m) via a two-point calibration (Spike2, v8, Cambridge Electronic Design [CED], UK) and analysed offline. The experimental setup is depicted in Figure 3-1.

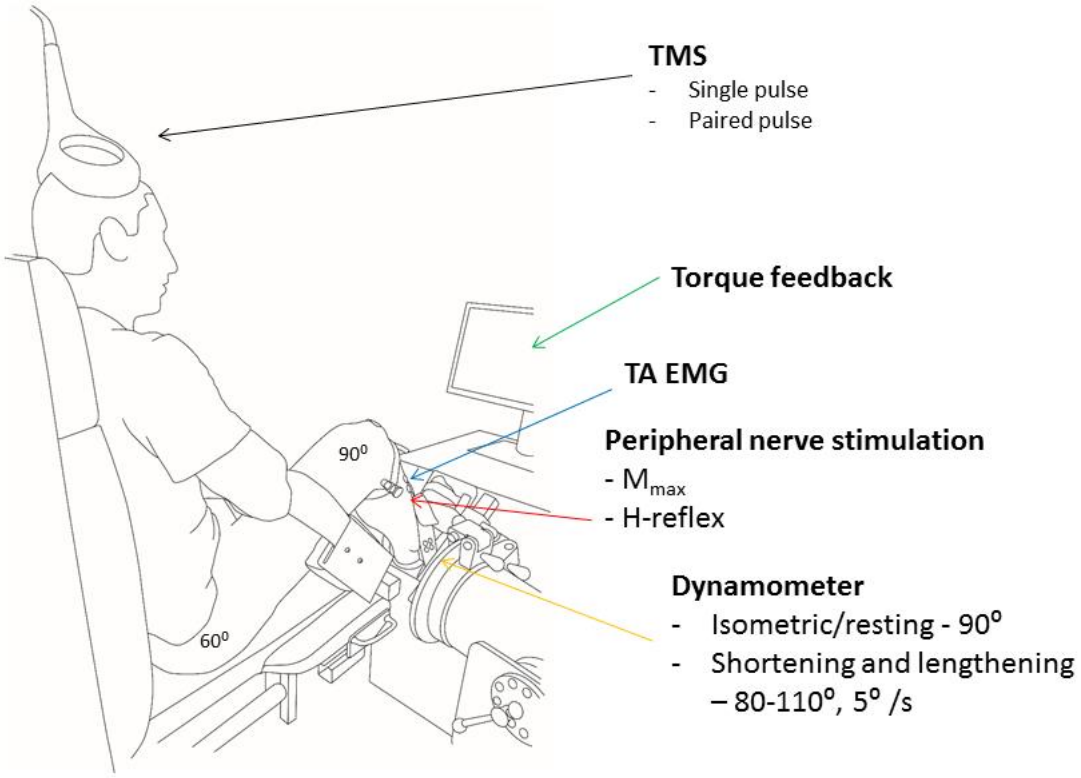

Figure 3-1. Experimental setup.

3-3.3 Electromyography

Electromyographic (EMG) activity was recorded with a bipolar electrode arrangement (8 mm diameter, 20 mm inter-electrode distance; Kendall 1041PTS, Tyco Healthcare Group, USA) over the muscle belly of TA according to Surface ElectroMyoGraphy for the Non-Invasive Assessment of Muscles recommendations (Hermens et al., 2000). The electrodes were placed at one-third of the length between the tip of the fibula and the tip of the medial malleolus, with the reference electrode placed over the medial malleolus. Prior to placement of electrodes, the recording site was shaved, abraded with preparation gel and wiped clean with an alcohol swab to ensure appropriate impedance (<2 kΩ). The EMG signal was amplified (×1000), band pass filtered (20-2000 Hz; Neurolog System, Digitimer Ltd, UK), digitised (5 kHz; CED 1401, CED, UK), acquired and analysed offline (Spike2, v8, CED, UK).

3-3.4 Percutaneous nerve stimulation

Responses in TA to percutaneous stimulation were elicited by delivering an electrical stimulus (1 ms pulse duration; Digitimer DS7AH, Hertfordshire, UK) over the peroneal nerve, below the head of the fibula (40 mm cathode/anode arrangement; Digitimer, Hertfordshire, UK). Upon localization of the optimal site, it was marked with a permanent marker and the stimulating electrode was strapped to the participant's leg (Figure 3-1). The stimulation intensity was increased by 0.3 mA from H-reflex threshold every 3 pulses until the maximum M-wave (M_{max}) was reached. Stimulation current was then further increased by 30% to ensure supramaximal stimulation. M_{max} was elicited at rest (Chapter 4) or in the isometric state at different contraction levels since it has been shown to be dependent on position (Gerilovsky et al., 1989) and contraction intensity (Lee and Carroll, 2005), but not the change in

length, i.e. shortening versus lengthening (Nordlund et al., 2002; Pinniger et al., 2001). Similarly, H/M recruitment curve were obtained at rest (Chapter 4) or during isometric contraction of different intensities since only the amplitude of the H-reflex, but not the slope of the H/M recruitment curve differs between shortening and lengthening (Pinniger et al., 2001). The H-reflex was evoked with a small M-wave (15-20% M_{max}) ensuring the H-reflex lied on the ascending limb of the H/M recruitment curve and was thus sensitive to changes during ankle movement (Pierrot-Deseilligny and Burke, 2005). During construction of H/M recruitment curves, stimuli were separated by 5-10 s to avoid the influence of post-activation depression (Hultborn et al., 1996). During contractions, stimuli were separated by at least 30 s to ensure H-reflex recovery (Howatson et al., 2011).

3-3.5 Transcranial magnetic stimulation

Single and paired-pulse TMS were delivered using one or two Magstim 200^2 magnetic stimulators, respectively (Magstim Co., Ltd., Whitland, UK; maximal output of ~1.4 T) via a concave double-cone coil (110 mm diameter). The coil was positioned over the primary motor cortex contralateral to the target TA muscle, orientated to induce cortical currents in the posterior-to-anterior direction (Figure 3-1). Initially, the centre of the coil was placed 1 cm lateral and posterior to the vertex (Devanne et al., 1997), after which it was moved in small steps around the optimal until the position capable of evoking the biggest potential in TA (hotspot) was found. Once identified, the back of the coil was marked directly on the scalp with a permanent marker to ensure consistent placement of the coil across trials. Thresholds (rMT or AMT) were investigated with the ankle positioned at anatomical zero and determined as the intensity that elicited a MEP with an amplitude of 50 μV (Rossini et al., 1994) or 200

μV (Kidgell et al., 2010) in 3 out of 5 trials, respectively. During single pulse TMS, the intensity of stimulation was set to 1.2 × MT as it lies on the middle portion of the ascending part of the stimulus-response curve (Han et al., 2001) and is thus sensitive to changes in corticospinal excitability. During the paradigms of SICI and ICF, CSs of 0.7 and 0.6 × MT were delivered at ISIs of 2 and 10 ms, respectively, whereas TSs were always delivered at 1.2 × MT (Brownstein et al., 2018). For paired-pulse paradigms, CS and TS pulses were delivered in alternating fashion.

3-3.6 Ultrasonography

Continuous longitudinal images of TA during shortening and lengthening were recorded using a real-time ultrasound apparatus (AU5 Harmonic, Esatoe Biomedica, Genoa, Italy). After identification and marking of the proximal and distal insertion of the muscle, a B-mode linear array probe (7.5 MHz, 55 mm width) was held with a constant light pressure perpendicular to the dermal surface along the midsagittal plane of a muscle. A hypo-allergenic ultrasound gel (Parker, Park Laboratories Inc., Fairfield) was used to enhance coupling between the skin and the probe. The probe was positioned between the fibular head and medial malleolus (Bland et al., 2011) at the site corresponding to the thickest portion of the muscle as identified by the ultrasound (Reeves and Narici, 2003). An echo-absorptive marker was placed between the skin and the probe to ensure the probe did not move during the recording. The images were recorded in real time and sampled at 25 Hz (AVer Media Capture Studio, AVer Media Technologies, New Taipei City, Taiwan). An externally generated square wave pulse was used to synchronise the ultrasound images with the dynamometer position acquisition system. Frame-capture software (Adobe Premier Elements,

version 15) was used to acquire ultrasound images for offline analysis (ImageJ 1.45, National Institutes of Health, USA).

3-4 Data analysis

3-4.1 Torque

The greatest instantaneous torque value was recorded as the MVC. Torque variability during submaximal contractions was assessed as coefficient of variation (CV_{torque} = standard deviation (SD) of torque /mean torque*100).

3-4.2 Electromyography

EMG activity was quantified as root mean square (RMS) in the 100-ms window prior to stimulus. RMS EMG was normalised to M_{max} obtained during the same contraction intensity (RMS/M_{max}) in order to remove the confounding effect of electrode location and body fat (Lanza et al., 2018), and account for changes at the skin-electrode interface and differences in propagation along the sarcolemma (Neyroud et al., 2015).

3-4.3 Evoked responses

Peak-to-peak amplitudes of MEPs were calculated between the initial deflection of the EMG from baseline to the second crossing of the 0 axis (Figure 3-2A) and were normalized to the peak-to-peak amplitude of M_{max} obtained during the same contraction intensity (MEP/M_{max}). Peak-to-peak amplitudes of responses to CS and TS were calculated and CS MEP amplitudes were expressed relative to TS MEP amplitudes to quantify SICI and ICF. SP was calculated as the time interval between the stimulus artefact and the return of pre-stimulus EMG (Damron et al., 2008; Figure

3-2A). Peak-to-peak amplitudes of M_{max} and H-reflex were calculated and were subsequently normalized to peak-to-peak amplitude of M_{max} obtained during the same contraction intensity (H/M_{max}). Additionally, peak-to-peak amplitude of the M-wave evoked with an H-reflex (M_H) was calculated and normalized to peak-to-peak amplitude of M_{max} obtained during the same contraction intensity (M_H/M_{max}) to ensure the same proportion of MUs were activated across all trials (Duclay and Martin, 2005; Figure 3-2B).

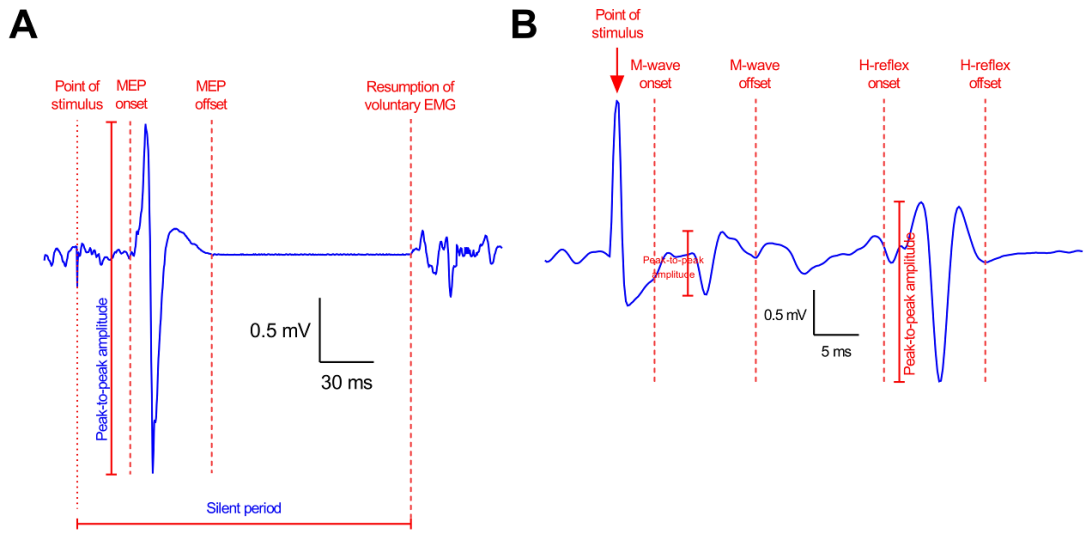

Figure 3-2. Analysis of evoked potentials.

3-4.4 Fascicle length

Using digitising software (ImageJ 1.45, National Institutes of Health, USA) fascicle length was measured from central to the superficial aponeurosis for TA (Reeves & Narici, 2003; Figure 3-3). The fascicle was only measured if it remained visible across the entire ultrasound image. Where the fascicle extended beyond the ultrasound image, linear continuation of the fascicle and aponeurosis was assumed (ICC = 0.853, Ando *et al*. 2014; 2.4% error rate, Reeves and Narici 2003). To reduce error associated with estimation of fascicle length, an average of three fascicles across the image was taken (Guilhem et al., 2011).

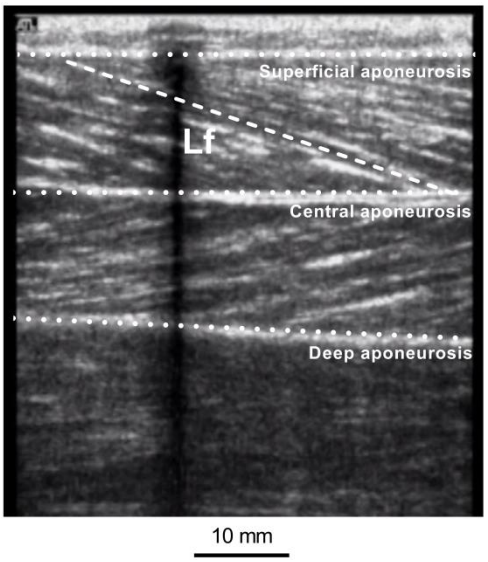

Figure 3-3. Example of an ultrasound sagittal plane scan of tibialis anterior. The image was taken at anatomical zero and show the fascicle length (L_f) measured from the visible insertion of the fibre between the central and the superficial aponeurosis in tibialis anterior. The shadow in the image represents the echo-absorptive marker used to ensure no movement between the skin and the probe occurred throughout ankle motion.

CHAPTER 4: NEUROPHYSIOLOGICAL RESPONSES TO PASSIVE SHORTENING AND LENGTHENING

PART 1: ELECTRICAL STIMULATION OF CORTICOSPINAL AXONS AT THE LEVEL OF THE LUMBAR SPINAL SEGMENTS

Publication arising from this part of the chapter:

Škarabot, J., Ansdell, P., Brownstein, C.G., Thomas, K., Howatson, G., Goodall, S., and Durbaba, R. (2019). Electrical stimulation of human corticospinal axons at the level of the lumbar spinal segments. *European Journal of Neuroscience* 49, 1254-1267.

4-1 Introduction

Any change in MEP size may be due to changes in the excitation or inhibition of cortical neurons or spinal motoneurons (Rossini et al., 2015). To discern the contribution of spinal motoneurons to the overall response, stimulation of Ia afferents has been used previously (H-reflex; Nielsen et al. 1999). However, the H-reflex is known to be sensitive to the influence of presynaptic mechanisms (Zehr, 2002). An alternative methodological approach for assessment of motoneuron pool excitability is electrical stimulation of descending axons at a subcortical level (Ugawa et al., 1991), which, unlike the H-reflex, is thought to be devoid of presynaptic influences (McNeil et al., 2013; Nielsen and Petersen, 1994). For this reason, it is considered a more direct, and arguably more appropriate method for assessment of spinal motoneuron contribution to the overall corticospinal response. This is reinforced by the fact that the action potentials descending from the motor cortex are attenuated by antidromic collision of those originating from spinal stimulation, indicating activation of some of the same corticospinal axons (Maertens de Noordhout et al., 1992; Martin et al., 2008; Taylor et al., 2002; Ugawa et al., 1991). Moreover, the response to electrical stimulation of the corticospinal axons at a subcortical level with increased contraction strength is similar to that of TMS (Maertens de Noordhout et al., 1992; Weavil et al., 2015) and exhibits no change in onset latency (Martin et al., 2008; Petersen et al., 2002), suggesting a large monosynaptic component (Petersen et al., 2002). Stimulation at subcortical levels has been successfully applied to investigate the contribution of spinal motoneurons to the overall response in upper limbs, but less often in lower limbs (for review see Taylor and Gandevia, 2004).

Measurement of the status of spinal motoneurons that control lower limb function is complicated by methodological challenges. For example, when stimulation is applied

between the mastoids, it is difficult to evoke responses in leg muscles at rest (Ugawa et al., 1995). The stimulation over the thoracic spinous processes has been shown to evoke a corticospinal response in quiescent lower limb muscles, but not in all muscles and participants (Martin et al., 2008). Furthermore, even if responses are evoked, they tend to be small ($\leq$ 10% M_{max}; Martin et al. 2008). Notably, stimulation of the descending tracts at the mastoid or thoracic level also stimulates motoneurons associated with control of upper limb and trunk musculature (Nathan and Smith, 1982; Nathan et al, 1996), therefore the current applied is likely shared between the motoneuron pool of multiple muscle groups (Kendall et al., 2005). Excitable tissues such as muscle and upper limb nerve roots likely also become depolarised by the large current applied (Taylor, 2006), resulting in contraction of back, neck, shoulder and arm muscles (Martin et al., 2008). Thus, an alternative paradigm that mitigates the aforementioned technical challenges would be advantageous for investigation of corticospinal behaviour in the lower limb muscles.

A potential solution to the methodological challenge of subcortical stimulation of the corticospinal axons when lower limb muscles are targeted could be stimulation of the lower spinal column. At the lumbar level, the descending tracts contain a greater relative density of motoneurons projecting to lower limb muscles (Sayenko et al., 2015). Stimulation applied closer to these projections will likely result in a higher current density in the lower limb motoneurons when compared to mastoid or thoracic stimulation. Indeed, Kuck *et al*. (2017) and Fernandes *et al*. (2018) have shown, via modelling techniques, that when the cathode and anode are placed over the lumbar and thoracic spinous processes, respectively, the highest density of electrical field is concentrated around the spinal cord segments associated with lower limb projections. Furthermore, these modelling studies also indicated that electric field magnitude is

likely to be higher in the lateral spinal cord white matter where the lateral corticospinal tract is located. Thus, when targeting the lower limb muscles, stimuli delivered lower on the spinal tract might provide an alternative methodological paradigm to activate the descending corticospinal axons.

The aim of this experiment was to explore whether a single electrical stimulus over the first lumbar spinous process (LS) activates descending corticospinal axons innervating TA by pairing LS with TMS of the motor cortex at appropriately timed ISIs. It was hypothesised that when the stimuli are paired at intervals shorter than the difference in latencies of each stimulus alone there will be an occlusion of the response to paired stimulation relative to the response to TMS alone. This technique has been employed previously with cervical and thoracic stimulation to explore a similar hypothesis (Martin et al., 2008; Taylor et al., 2002). Additionally, the contraction strength – stimulus response curves of TMS and LS were compared. The validation of this stimulating site will allow investigation of subcortical excitability of the corticospinal tract in the subsequent part of this Chapter.

4-2 Methods

Participants

Ten healthy, young volunteers (24 ± 4 years, 179 ± 8 cm, 77 ± 12 kg; 7 males, 3 females) participated in the study. For details about the exclusion and inclusion criteria see section 3-3.1.

Experimental design

Participants visited the laboratory on two separate occasions, a familiarisation and the experimental visit. Responses were elicited with participants sat in an isokinetic dynamometer (for greater detail, see section 3-3.2).

In the first part of the study, with the muscle at rest, TMS and LS were either delivered separately, or paired with different ISIs (Figure 4-1A). Initially, the responses to individual TMS and LS were standardised to elicit a response that was ~10-15% M_{max}, and the stimulus intensities required to produce these outputs were then applied during paired stimulation. Paired TMS and LS were delivered either with TMS preceding LS (ISIs from −16 to −2 ms, every 2 ms), both stimuli occurring at the same time (ISI of 0 ms), LS preceding TMS (ISIs from 2 to 14 ms, every 2 ms). Therefore, there were a total of 18 different stimuli, delivered separately in 10 sets, totalling 180 stimulations. The order of each set of stimuli was randomised, with pulses within each set delivered every 5-10 seconds.

In the second part of each visit, participants performed two MVCs, of which the greatest instantaneous torque was used to set guidelines for subsequent contractions. TMS and LS were then delivered separately at 10, 25, 50, 75 and 100% MVC (Figure 4-1A). The order of the type of stimulation and the contraction strength was randomised. Five stimuli were performed at each contraction intensity for each type of stimulation, to avoid the influence of decreases in muscle function at higher contraction intensities. Stimulations were delivered once the torque had plateaued at the target line. At least 60 seconds rest was given between each contraction. Initially, the responses to individual TMS and LS were standardised to ~50% M_{max} during a contraction at 50% MVC, and the stimulus intensities required to produce these outputs remained constant for all contraction intensities to investigate how different

muscle activity might affect the size of responses. Standardising evoked responses during contraction to a higher percentage of M_{max} than that used when evoking responses at rest was chosen to distinguish the evoked response from background EMG activity, and because the size of the response is known to be sensitive to change with contraction strength (Weavil et al., 2015).

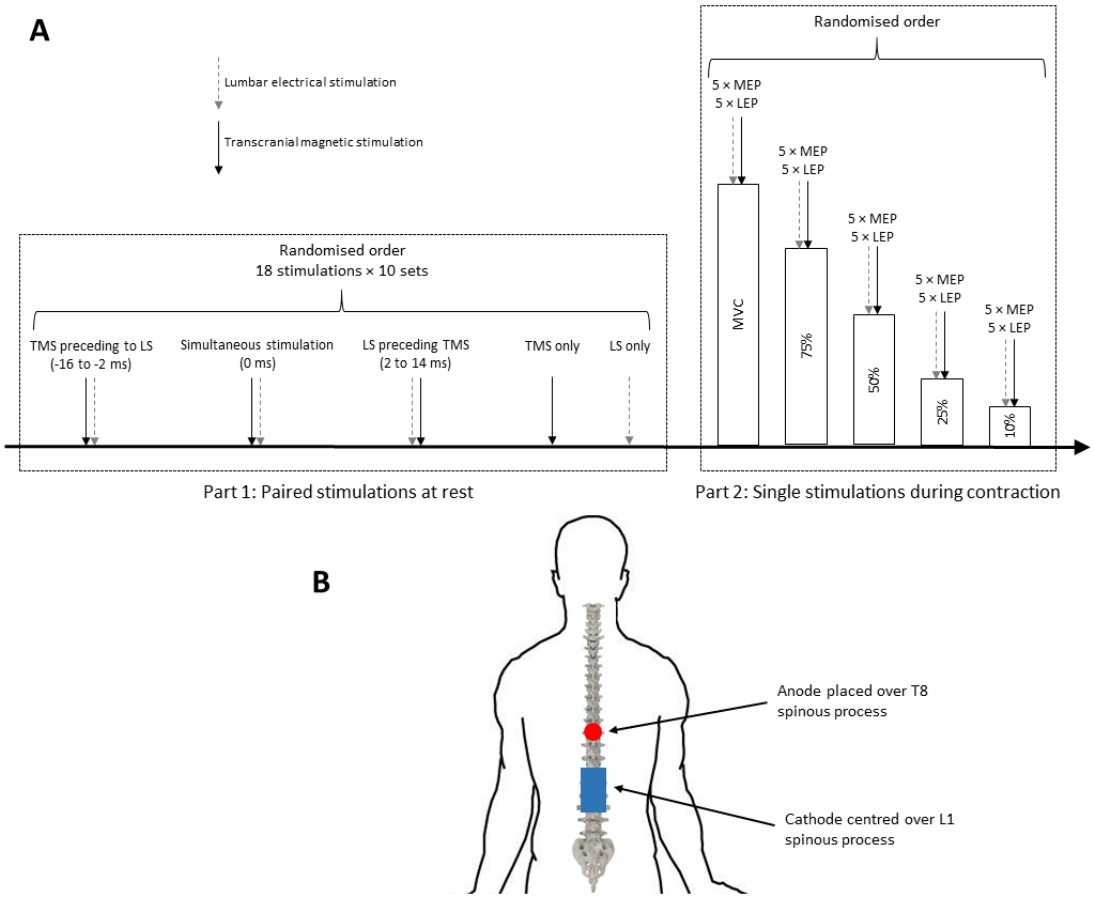

Figure 4-1. Experimental design and procedure for electrical stimulation of lumbar spinal segments. A: Experimental approach involved two parts, the first part being comprised of paired stimulation (transcranial magnetic stimulation and lumbar electrical stimulation) at rest at 16 different interstimulus intervals, and the second part consisting of single pulse magnetic and electrical stimulations at 10, 25, 50, 75 and 100% maximal voluntary contraction. B: Lumbar electrical stimulation was performed with cathode centred over L1 and anode placed over T8 spinous process. Based on modelling literature, this configuration is likely to produce the greatest electric field around the area of T10-T12 spinal segments.

Percutaneous nerve stimulation

Percutaneous stimulation of the common peroneal nerve was used to elicit M_{max} in TA (mean intensity of 47 ± 14 mA). For a detailed procedure see section 3-3.4.

Transcranial magnetic stimulation

Single pulse TMS was delivered to elicit responses in TA. For a detailed procedure see section 3-3.5. The intensity of stimulation was standardised to elicit a response equating to ~10-15% of the resting M_{max} (mean stimulation intensity of 54 ± 16%) for the first part of the study and to ~50% M_{max} during a contraction at 50% MVC (43 ± 10%) for the second part.

Electrical stimulation of the first lumbar spinous process

Lumbar evoked potentials (LEPs) were elicited with a constant-current stimulator (1 ms pulse duration; Digitimer DS7AH, Hertfordshire, UK) via self-adhesive electrodes (Nidd Valley Medical Ltd., Bordon, UK). The cathode electrode (5 × 9 cm) was centred over the first lumbar (L_1) spinous process, with the long axis of the electrode aligned to the centre of the vertebral column (Figure 4-1B). The surface area of the cathode covered two spinous processes above and below the centre point (T_{11}-L_3). A cathode of large area was chosen as it produces less discomfort and greater tolerance by participants (Kuhn et al., 2010; Ugawa et al., 1995). The bottom of the anode (circular shape; 3.2 cm diameter) was placed in the midline of the vertebral column 5 cm above the upper edge of the cathode (Ugawa et al., 1995), corresponding to the level of the eighth thoracic spinous process (T_8). Based on modelling studies, this electrode configuration was chosen as it is likely to induce the greatest electric field magnitude between T_{10} and T_{12} spinous processes due to electric field being highest between the stimulating electrodes (Kuck et al., 2017). As such, the site of greatest spinal cord activation is likely to occur between the L_1-L_5 spinal segments, corresponding to the motoneuron pool of TA (Sayenko et al., 2015; Sharrad, 1964). Similar to TMS, the intensity of stimulation was standardised to ~10-15% M_{max}

evoked in the resting position (mean intensity of 194 ± 93 mA) and to ~50% M_{max} during a contraction at 50% MVC (mean intensity of 216 ± 87 mA). The differences in the applied current are thus likely due to different standardisation of stimulus intensities for different parts of the experiment. To ensure ventral roots were not stimulated, responses were monitored for a lack of an abrupt decrease in latency and increase in response size with voluntary contraction (Taylor, 2006). Paired LS was also performed at the target stimulus intensity at an ISI of 50 ms before the start of the main recording session, with the lack of depression of the second response excluding the possibility of stimulation of dorsal roots (Danner et al., 2016; Hofstoetter et al., 2018; Roy et al., 2012). All participants reported they found LS to be tolerable.

Electromyography

EMG activity was recorded from TA muscle. For detailed procedure see section 3-3.3.

Data analysis and statistics

In the experiment assessing the interaction of LS and TMS, the data analysis was similar to that described previously by Taylor et al. (2002). Briefly, using a customised script in Spike2 (v8, CED, UK), the waveforms of individual responses to LS alone, TMS alone and paired stimulation were averaged. These are depicted in the example responses (Figure 4-2) during selected ISIs from one individual in the top three rows. Whilst in this representative response there is evidence of facilitation (highlighted grey area) at an ISI of −14 ms, for the other ISIs shown the interaction between the stimuli makes it difficult to determine if facilitation or occlusion has occurred. Thus**,** the averaged response waveform to LS alone was then temporally aligned to the LS stimulus time point of the averaged response waveform to paired stimulation and

graphically subtracted from the latter (paired – LEP; Figure 4-2, bottom row). After that, the peak-to-peak amplitude of the paired – LEP waveform was calculated and compared to the amplitude of the averaged response to TMS alone ([paired – LEP]/MEP). It has previously been suggested that the subtraction might reveal an inverted potential resulting in negative values, such as in the cases when response to LS is larger than the response to paired stimulation (Taylor et al., 2002), however, this was never the case in the present data. Paired sample T-tests were used for assessing the statistical significance of the differences in the paired – LEP amplitude relative to the MEP alone amplitude. It should be noted that individuals of different height and thus different lengths of neural pathways along with reported differences in conduction velocity between individuals (Andreassen and Arendt-Nielsen, 1987; Sadoyama et al., 1988) could confound the interpretation of the interaction of LS and TMS. For that reason, additional analyses were performed to account for this potential disparity. Firstly, the difference in MEP and LEP latency was calculated estimating the time required for the first volley elicited by TMS to reach the segmental level activated by LS. This time (rounded to the nearest 2 ms) was referred to as normalised ISI of 0 ms. Subsequently, the positive and negative normalised ISI values are indicative of the first volley evoked by TMS not having arrived at or having passed the site of descending axon activation by LS, respectively (Martin et al., 2008). Due to incomplete number of samples ($n < 10$), statistical analysis using paired sample T-test was not performed for this part of the analyses at normalised ISIs of –6, –4, and 26 and 28 ms. The variability of individual responses to TMS and LS was assessed by calculating a coefficient of variation for each series of 5 evoked potentials (MEPs or LEPs) for each individual (CV = SD of 5 evoked potentials ÷ mean of 5 evoked potentials × 100%). In the experiment assessing the responses with increased

contraction strength, peak-to-peak amplitudes of the single pulse evoked responses were calculated, averaged and normalised to M_{max}. Background EMG activity was quantified as RMS in the 100-ms epoch prior to stimulus and normalised to RMS EMG activity during an MVC. A 2 × 5 repeated measures ANOVA was performed to determine whether contraction strength-response curves and background EMG activity were different. The effect of contraction strength on MEP and LEP latencies was assessed via a one-way repeated-measures ANOVA. If F-values were found to be statistically significant, analysis was continued using pairwise comparison with Bonferroni correction. All statistical analyses were performed in SPSS (v20, SPSS Inc., Chicago, IL, USA). All data are reported as means ± standard deviations. Significance was set at alpha level of 0.05. To allow for a more nuanced interpretation of the data, Cohen's d_z were calculated as an effect size measure for statistical procedures involving paired sample T-tests. Cohen's d_z was calculated as the ratio of mean difference and standard deviation of differences, which slightly differs from traditional Cohen's *d* calculation in that it is better suited for within-subject, rather than traditional between-subject differences (Becker, 1988; Smith and Beretvas, 2009). Partial eta squared (η_p^2) were calculated as a measure of effect size for statistical procedures involving ANOVA.

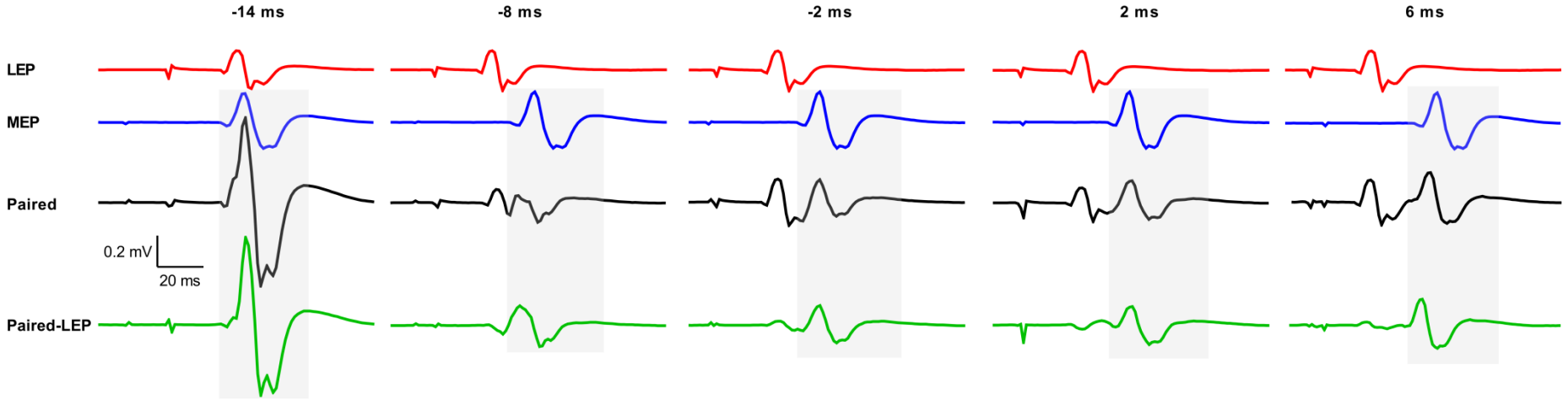

Figure 4-2. Representative traces at different interstimulus intervals (ISIs) from a single participant. In this individual, the intensity of stimulation used across all types of stimuli produced responses to individual magnetic and electrical stimuli with amplitudes corresponding to 8.9% and 14.2% of M_{max}, respectively, and latencies of 17.2 and 31.1 ms, respectively. Evoked responses to electrical stimulation of the first lumbar spinous process alone (LEP, red), magnetic stimulation of the cortex alone (MEP, blue), paired stimuli (Paired, black) and a subtracted response (Paired – LEP, green) for ISIs of −14, −8, −2, 2 and 6 ms are shown. Each trace is an average waveform of 10 responses. It is of note that the shape of the evoked response for each stimulus type is very similar. Shaded grey area is drawn for better visualisation of differences.

4-3 Results

Latencies of MEPs and LEPs at rest and across contraction intensities remained unchanged ($p \geq 0.081$; Table 4-1). The response variability was greater for MEP compared to LEPs and was reduced in an active muscle compared to rest for both evoked responses (Table 4-2).

Table 4-1. Latencies of evoked potentials in milliseconds (mean ± SD).

	LEP	MEP
Rest	17.3 ± 1.4	30.7 ± 1.6
10% MVC	17.7 ± 1.6	30.3 ± 2.0
25% MVC	17.9 ± 2.0	30.3 ± 1.9
50% MVC	17.7 ± 1.9	29.7 ± 2.2
75% MVC	17.9 ± 1.9	29.9 ± 1.8
MVC	17.9 ± 1.9	29.8 ± 2.0

Table 4-2. Variability of evoked responses (CV%; mean ± SD).

	LEP (%)	MEP (%)
Rest	30 ± 18	48 ± 9
10% MVC	16 ± 11	25 ± 13
25% MVC	17 ± 6	25 ± 19
50% MVC	17 ± 8	27 ± 20
75% MVC	21 ± 9	30 ± 19
MVC	23 ± 8	23 ± 10

Interaction of LS and TMS

Representative traces recorded in TA from one individual assessing the interaction of LS and TMS are shown in Figure 4-2. Similar individual behaviour was observed across all participants. The mean sample data shows that pairing the two types of stimuli resulted in occlusion ([Paired – LEP]/MEP < 1.0) of responses at ISIs between −8 and 14 ms (P value range = 0.001 – 0.048, d_z range = 0.5 – 1.4; Figure 4-3A). The paired responses were also facilitated at −14ms ($P = 0.038$, $d_z = 0.6$). Furthermore, six out of the ten participants also exhibited facilitation of responses ([Paired – LEP]/MEP > 1.0) at ISIs of −16 and −12 ms, respectively, but this was not statistically significant

at the group level (P = 0.195 & 0.223, d_z = 0.4 for both). For the mean sample data, individual TMS responses of 17.3 ± 6.4% M_{max} and individual LS responses of 12.6 ± 5.4% M_{max} were evoked in TA.

The effect of timing of stimuli on interaction of LS and TMS

The responses to paired stimulation were significantly occluded relative to the response to a single TMS pulse at > 2 ms before the expected arrival of the first descending volley evoked by TMS to the segmental level of LS (P value range = 0.001 – 0.033, d_z range = 0.6 – 1.2; Figure 4-3B). The paired responses were also significantly facilitated when the first descending volley evoked by TMS was at the same level as LS (P = 0.021; d_z = 0.7).

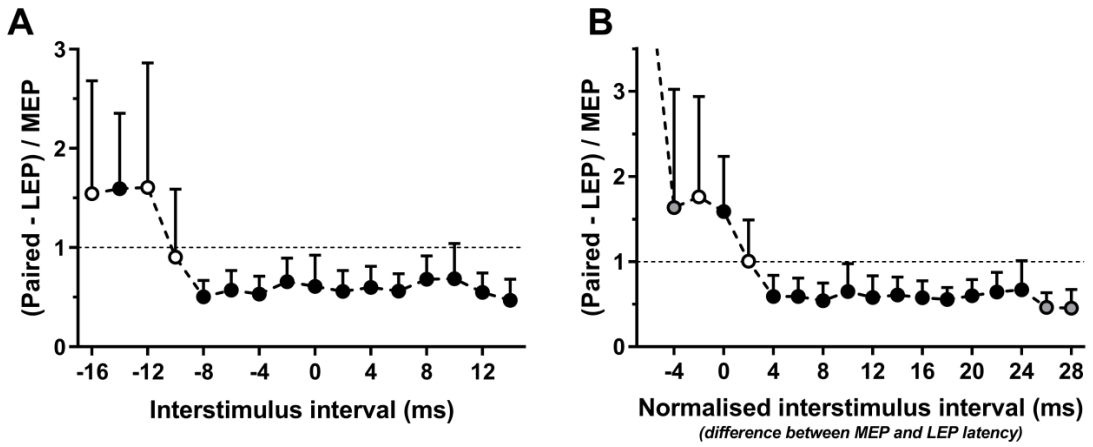

Figure 4-3. Temporal relationship of the interaction between transcranial magnetic stimulation and electrical stimulation over the first lumbar spinous process. Means and SDs are shown for the differences in peak-to-peak amplitudes of evoked responses between paired stimulation and electrical stimulus alone and expressed relative to the response to magnetic stimulation for 16 different interstimulus intervals (−16 to 14 ms, every 2 ms; A) and normalised interstimulus intervals when lumbar stimulation was delivered before (positive interstimulus intervals), at (0 ms) or after (negative interstimulus intervals) the first volley evoked by transcranial magnetic stimulation was expected to arrive at the lumbar level (B). Horizontal dashed line represents the size of the response that would be expected if there was no physiological interaction. The black filled circles indicate the response is significantly different from the expected response ($p < 0.05$). For interstimulus intervals denoted by the grey filled circles statistical analyses was not performed due to incomplete number of samples ($n < 10$). For the normalised interstimulus interval of −6 ms (B), the responses were exceptionally large (mean ratio: 4.4; $n = 1$) and lie outside the illustrated range.

Responses with increases in contraction strength

Background muscle activity increased progressively from 10 – 100% MVC ($F_{2.4, 18.3}$ = 252.0, $P < 0.001$, $\eta_p^2 = 0.97$; Figure 4-4A). MEPs and LEPs were dependent on contraction strength ($F_{1.5, 13.0} = 15.1$, $P = 0.001$, $\eta_p^2 = 0.63$), such that responses peaked at 75% MVC (Figure 4-4B). There was also a statistically significant interaction between contraction strength and type of stimulus ($F_{4, 36} = 7.7$, $P < 0.001$, $\eta_p^2 = 0.46$) with post hoc testing showing a difference between stimuli types at 10% MVC (p = 0.011).

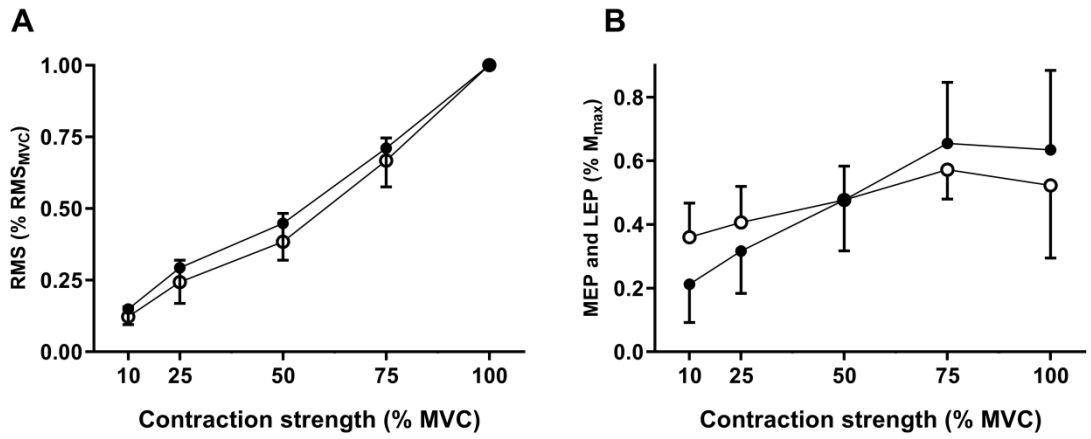

Figure 4-4. EMG activity and responses to magnetic and electrical stimuli during different contraction strengths. Root-mean-square EMG activity was calculated during 100-ms epoch prior to stimulus normalised to root-mean-square EMG activity during maximal voluntary contraction (A) and the amplitude of motor (filled circles) and lumbar (open circles) evoked potentials at different contraction strengths (C and D).

4-4 Discussion

The results of these experiments show that the response to paired magnetic cortical and electrical stimulation of the lumbar spinal segments is occluded at appropriate interstimulus intervals and that responses to TMS and LS similarly increase with increases in contraction strength with no change in onset latency. This behaviour suggests that LS and TMS activate some of the same corticospinal axons and that responses to LS are evoked transsynaptically with a monosynaptic component. Thus,

this stimulation technique has applicability as an alternative paradigm for investigating the contribution of spinal motoneuron excitability to the overall corticospinal response when lower limb muscles are targeted.

Evidence for stimulation of descending tracts

The occlusion observed with TMS being delivered before LS at intervals shorter than the difference in latencies of each stimulus alone corroborates previous findings when electrical stimulation was performed over the mastoids with arm and hand muscles targeted (Taylor et al., 2002; Ugawa et al., 1991). Similarly, when LS preceded TMS, the responses were occluded to the same degree, again confirming the findings seen when electrical stimulation was performed over the mastoids and targeting the muscles of the arm (Taylor et al., 2002). This occlusion corresponded to the timing of the stimuli when LS was delivered more than 2 ms before the expected arrival of the first descending volley evoked by TMS (Martin et al., 2008), which is consistent with collision of the descending cortical volleys of TMS with antidromic volley originating from LS. These findings indicate that LS activates some of the same axons as TMS, likely the pyramidal cells in the corticospinal tract (Taylor et al., 2002; Ugawa et al., 1991). Whilst the occluded response could have emerged due to disynaptic inhibition originating from LS-induced activation of inhibitory interneurons via cutaneous receptors of the lumbosacral region (Frigon et al., 2012), this is unlikely given the facilitation that was observed at longer ISIs when TMS preceded LS (Taylor et al., 2002). Facilitation corresponded to the first descending volley evoked by TMS having passed the segmental level of LS by more than 2 ms. This facilitation, which has been consistently shown for the aforementioned timing (Martin et al., 2008; Taylor et al., 2002; Ugawa et al., 1991), is the result of the descending volley evoked by LS arriving

at the motoneuron pool that is already excited by the TMS descending volleys (Martin et al., 2008).

In theory, it was expected that when LS was delivered prior to TMS at ISIs longer than the difference in latencies of individual stimuli, the antidromic volley would reach the cortex prior to its excitation by the magnetic stimulus, resulting in a response similar to a single-pulse TMS. However, it was found that at longer ISIs (LS preceding TMS), responses remained occluded. This behaviour is in agreement with Taylor et al. (2002), but differs to that of Ugawa et al. (1991). However, the latter employed electrical stimulation of the cortex, whilst the former stimulated the cortex with TMS, similar to the present study, suggesting that the origin of the observed depression is cortical, possibly through inhibition via collaterals of corticospinal axons (Ghosh and Porter, 1988; Krnjević et al., 1966).

There are certain factors that complicate the interpretation of the interaction between electrical stimulation of the spinal tracts and responses evoked by TMS, even if it is assumed that the pathway is purely monosynaptic (Petersen et al., 2002). Firstly, magnetic and electrical stimulation differ in their mechanism of activation of neurons, such that TMS evokes multiple descending volleys, whereas LS only elicits a single descending volley (Houlden et al., 1999; Nakamura et al., 1996; Terao et al., 2000). This makes it likely that only the first volley of TMS is affected by the collision originating from LS. Consequently, comparison of responses to paired stimulation to a single TMS response actually underestimates the occlusion as a result of collision (Martin et al., 2008). Thus, despite the interaction of the stimuli being complex, our data, in conjunction with previous work in the area (Martin et al., 2008; Taylor et al., 2002), suggests that the single volley produced by LS can occlude the response to TMS. Secondly, the observed occlusion could be a result of descending action

potentials being in a refractory state. However, this is an unlikely contributor given the observed facilitation of responses when the first descending volley evoked by TMS had passed the segmental level of LS and since occlusion occurred at ISIs far longer than the refractory period of motoneurons (> 3 ms; Day et al. 1989). Lastly, if the motoneurons are not activated monosynaptically, the paired response could be influenced by excitatory and inhibitory interneurons. However, had there been multiple synapses involved in the present study, increased excitability of motoneurons with increased contraction strength would have likely shortened the activation time of each postsynaptic cell and thus reduced the onset latency of evoked potentials (Petersen et al., 2002). This was not the case as responses to individual TMS and LS increased similarly with increased contraction strength with no change in the onset latency. The increase in response amplitude with increased contraction strength is also a good indicator that the responses were evoked transsynaptically as opposed to distal to the cell bodies (Martin et al., 2008). It is also worth noting that both LEPs and MEPs increased at a similar rate as shown previously (Maertens de Noordhout et al., 1992; Weavil et al., 2015) and peaked ≥ 75% MVC consistent with the relationship between motor unit recruitment and firing frequency of the muscles investigated (Gelli et al., 2007), which determines the probability of an evoked response (Bawa and Lemon, 1993; Brouwer et al., 1989; Jones and Bawa, 1999). The lack of a decrease of evoked responses during MVC disagrees with some experiments (Goodall et al., 2009; Mira et al., 2017), but corroborates others (Oya et al., 2008; Weavil et al., 2015). This discrepancy has been attributed to the dependency of responses with increased contractions strength on stimulus intensity (Oya et al., 2008; Weavil et al., 2015), such that the greater the stimulus intensity, the lower the probability of an evoked response with increased firing rate (Matthews, 1999).

The possibility of stimulation of other neural structures

Whilst the present data provide evidence that LS and TMS activate similar axons, i.e. the pyramidal cells in the corticospinal tract, there remains the possibility that other descending tracts might also be excited and hence be contributing to the observed effects (Ugawa et al., 1991). Of particular consideration would be those tracts located in the lateral white matter, such as rubrospinal and reticulospinal tracts (Nathan and Smith, 1982; Nathan et al, 1996), as modelling studies indicate that electric field magnitude, due to LS, is likely higher at the lateral aspects of the spinal cord where these tracts are located (Fernandes et al., 2018). Any potential effects from the rubrospinal tract can be discounted as this tract does not project below the cervical region in humans (Nathan and Smith, 1982). The reticulospinal tract does project down to the lumbar region, however, its contribution to the effects observed is likely small due to the lower density of the axons compared to corticospinal tract (Nathan et al., 1996). Thus, it appears unlikely that descending tracts other than corticospinal tract were stimulated with LS.

Though the aforementioned observations relating to a lack of changes in onset latency with increased contraction strength provide support for the monosynaptic nature of the pathway, the data from the present experiments does not completely exclude the influence of non-monosynaptic pathways. Indeed, a large propriospinal system has been shown to exist in humans that might influence corticospinal responses (Pierrot-Deseilligny, 2002). It is important to note that the corticospinal pathway as a whole encompasses not only cortical circuitry and the motoneuron pool, but also any spinal interneuronal connections (Devanne et al., 1997). TMS might activate inhibitory interneurons due to their lower threshold for activation in some muscles (Nielsen et al., 1993), reducing the excitability at the level of the motoneuron pool leading to

reduced temporal summation of the responses. Though supra-additive facilitation observed in the present experiment makes this possibility less likely, it should be noted that the lower limb muscle investigated in these experiments has been demonstrated to have di- and polysynaptic pathways (Nielsen et al., 1993; Simonetta-Moreau et al., 1999) and TA receives strong reciprocal inhibitory input (Yavuz et al., 2018). Thus, further work is required to elucidate whether responses to LS are evoked purely monosynaptically, or whether they involve an interneuronal component.

When LS is performed, there is always the possibility that nerve roots are stimulated in addition to the spinal tract. Ventral roots were unlikely to have been activated in the present experiments due to the increase in the size of responses with contraction, and a lack of abrupt decrease in latency when intensity of stimulation was increased (Taylor, 2006). Similarly, the activation of dorsal roots was unlikely given the lack of depression of the second response to paired electrical stimuli at 50 ms ISI (Danner et al., 2016; Hofstoetter et al., 2018; Roy et al., 2012). Furthermore, when dorsal roots are stimulated, the occlusion of responses to paired TMS and LS is absent, and responses to LS are not facilitated by voluntary muscle contraction (Roy et al., 2014), the opposite of which was observed in the present experiments. Thus, the possibility of having activated ventral or dorsal roots with electrical stimulation is minimal.

Variability of responses

The present data show that the CVs for LEPs at rest and during contraction are lower than MEPs (see Table 4-2). As is well established, MEPs are inherently variable due to the fluctuating nature of corticospinal and motoneuronal excitability (Ellaway et al., 1998; Kiers et al., 1993), randomness in the firing of pyramidal tract neurons and spinal motoneurons (Pitcher et al., 2003) as well as desynchronization of action

potentials (Magistris et al., 1998). The variability of responses observed in the present study is comparable to that reported previously when similar numbers of pulses were employed (Biabani et al., 2018; Brownstein et al., 2018). A greater variability in MEPs compared to LEPs can perhaps be explained by differences in the complexity of the responses to TMS as opposed to LS as discussed above. Some of the multiple volleys evoked by TMS, particularly the later, indirect waves, can fire multiple times (Edgley et al., 1997), which might contribute to the greater variability. Furthermore, greater variability of MEPs might also stem from interference signals from other cortical networks. The variability of evoked responses can be reduced by eliciting responses during a contraction (Darling et al., 2006), which is shown in the present data for both MEPs and LEPs. Due to inherent variability of evoked responses a large quantity of evoked responses are recommended to ascertain a stable index of corticospinal excitability (Brownstein et al., 2018). The present data suggests that when using LS to evoke LEPs, fewer responses might be required compared to TMS evoked MEPs.

4-5 Conclusion

Based on the occlusion of responses when the first descending volley evoked by TMS had not arrived at the segmental level, it can be concluded that electrical stimulation of the first lumbar spinous process activates some of the same corticospinal axons projecting to lower limb muscles as transcranial magnetic stimulation of the motor cortex. These responses at rest were standardised to 10-15% M_{max} and were elicited with ease in all participants. Furthermore, responses to LS grew similarly to TMS with increasing contraction strength, suggesting transsynaptic activation. All participants found stimulations to be tolerable and whilst muscle activity of upper body muscles was not measured, the experimenter did not observe shoulder and arm movements in

response to stimulation during the trials. Thus, electrical stimulation over the first lumbar spinous process can be used as an alternative method to assess corticospinal excitability at the segmental level and might be better suited when targeting lower limb muscles due to the proximity of the motoneuronal projections to the stimulating site and the ability to evoke responses in lower leg musculature at rest. As such, this method will be used in the next part of this Chapter to discern subcortical contribution to corticospinal excitability during passive ankle movement.

PART 2: CORTICOSPINAL, INTRACORTICAL AND SPINAL RESPONSES DURING PASSIVE SHORTENING AND LENGTHENING

Publications arising from this part of the chapter:

Škarabot, J., Ansdell, P., Brownstein, C.G., Hicks, K.M., Howatson, G., Goodall, S., and Durbaba, R. (2019). Corticospinal excitability of tibialis anterior and soleus differs during passive ankle movement. *Experimental Brain Research* 237, 2239-2254.

Škarabot, J., Ansdell, P., Howatson, G., Goodall, S., and Durbaba, R. (2019). Corticospinal responses during passive shortening and lengthening of tibialis anterior and soleus in older compared to younger adults. *Experimental Physiology*, DOI: 10.1113/EP088204.

4-6 Introduction

Corticospinal excitability is constantly modulated during passive and active movement. Isotonic movements modify corticospinal excitability, such that excitability tends to be lower during lengthening relative to shortening and isometric contractions (Abbruzzese et al., 1994; Duclay et al., 2014; Gruber et al., 2009), which seems to depend on the amount of Ia afferent feedback (Doguet et al., 2017). However, elucidating the direct effect of muscle length related feedback on the corticospinal tract output during dynamic contractions is challenging due to the influence of postsynaptic control mechanisms (Barrué-Belou et al., 2018; Valadão et al., 2018), and potential differences in neural drive that can influence neurophysiological responses (Abbruzzese et al., 1994; Morita et al., 2000).

Potential insight into the effect of muscle length related feedback on the corticospinal response might be gained by assessing responses during passive movement. With passive muscle lengthening, the firing of muscle spindle afferents increases proportionally to the magnitude of the stretch, but remains low during shortening of a muscle (Day et al., 2017; Matthews, 2011). This behaviour at the somatosensory receptor level might, in turn, modulate the corticospinal response. Indeed, corticospinal excitability has been shown to be reduced during passive lengthening of the wrist flexors and extensors, and has been related to the degree of muscle spindle afferent feedback (Coxon et al., 2005; Lewis et al., 2001; Lewis and Byblow, 2002). Notwithstanding these findings, the level of neural axis at which afferent-mediated changes in corticospinal output occur has not been elucidated. From a cortical perspective, intracortical inhibition is modulated during passive shortening and lengthening of the upper limbs (Lewis et al., 2001). However, despite the presence of a facilitatory corticospinal response during passive shortening of upper limb muscles

(Chye et al., 2010), the contribution of intracortical facilitatory circuits to augmented corticospinal excitability has not been considered. In addition, passive lengthening of SOL has been shown to be accompanied by greater presynaptic inhibition (Pinniger et al., 2001), whilst less is known about the effect of passive movement on subcortical output of the corticospinal tracts, which are likely devoid of classical presynaptic influence (Nielsen and Petersen, 1994). Also, far less is known about corticospinal excitability during passive movement of the lower limbs, which might differ due to disparities between facilitatory and inhibitory intracortical outputs and corticospinal projections to upper and lower limb muscles (Brouwer and Ashby, 1990; Chen et al., 1998).

The SOL and TA muscles are integral for movement about the ankle joint. From a neural perspective, TA and SOL have been shown to exhibit differences in the quantity of muscle spindles that affects the relative input from Ia afferents (Banks, 2006; De Luca and Kline, 2012), the type and the size of motor units (Burke, 1967; Dum and Kennedy, 1980), reciprocal spindle afferent input (Yavuz et al., 2018), distribution of direct corticomotoneuronal projections (Brouwer and Ashby, 1992; Brouwer and Qiao, 1995), intracortical inhibition (Lauber et al., 2018) and preferences in the input from pyramidal tract into the spinal network (Brooks and Stoney, 1971). Due to these differences in neural properties, TA and SOL might be accompanied by different corticospinal responses as a result of muscle length changes.

Healthy aging is characterised by alterations in the CNS. Some evidence suggests that aging affects sensory compared to motor axons to a greater extent (Scaglioni et al., 2002). In addition to this, older adults have been shown to exhibit alterations in cortical sensorimotor integration (Brown et al., 2018; Degardin et al., 2011; Smith et al., 2011a). As such, it is possible that corticospinal responses with modulation of muscle

spindle sensory input as a result of muscle shortening and lengthening, differs with age but remains to be investigated.

The aim of this experiment was to investigate corticospinal function of TA and SOL during passive ankle movement. Four experimental components were performed to assess 1) corticospinal modulation at different muscle lengths; 2) the contribution of cortical neurons and spinal motoneurons to the corticospinal response; 3) intracortical facilitation and inhibition; and 4) the contribution of Ia afferent input to spinal motoneurons in quiescent SOL and TA during passive ankle movement. It was hypothesised that corticospinal excitability will be dependent on the change in muscle length and muscle studied, and will be attributable to processes at both the cortical and spinal level. In addition to the abovementioned experimentation, corticospinal responses were examined in aging adults to discern whether sensorimotor processing of muscle length changes is altered in older age.

4-7 Methods

Participants

Twenty healthy, young volunteers (25 ± 4 years, 175 ± 9 cm, 78.9 ± 16.8 kg; 9 females) and ten older adults (66 ± 4 years, 177 ± 14 cm, 75.5 ± 12.7 kg; 2 females) participated in the study. Based on previous studies (Lewis et al., 2001; Lewis and Byblow, 2002), an *a priori* power analysis (Faul et al., 2007) showed six participants per group were needed to observe modulation of MEP amplitude with passive movement. For details about the exclusion and inclusion criteria see section 3-3.1.

Experimental design

The study involved four experimental components designed to investigate the effect of passive ankle motion on corticospinal excitability at different muscle lengths (Experiment 1), corticospinal and spinal excitability (Experiment 2), intracortical facilitation and inhibition (Experiment 3), and the contribution of Ia afferent input to spinal motoneurons (Experiment 4) in resting SOL and TA. Twelve participants took part in Experiment 1 (26 ± 4 years, 176 ± 9 cm, 77.8 ± 16.8 kg; 6 females). In Experiment 2, two participants did not return for further testing due to scheduling conflicts, and an additional participant was recruited (n = 11; 26 ± 4 years, 178 ± 8 cm, 81.6 ± 16.2 kg; 5 females). Due to larger heterogeneity of responses, additional participants were recruited for Experiment 3 (n = 15; 25 ± 4 years, 178 ± 9 cm, 83.1 ± 17.1 kg; 5 females). In Experiment 4, obtaining H-reflexes in resting TA proved challenging as has been previously reported (Burke, 2016; Roy and Gorassini, 2008). After screening 24 individuals, only five participants exhibited clear and consistent H-reflexes in quiescent TA to allow for comparison with SOL and took part in Experiment 4 (24 ± 3 years, 176 ± 11 cm, 72.2 ± 14.3 kg; 1 female). Individuals that took part in all four experiments were tested within six weeks of the first visit to the laboratory. To compare corticospinal responses between young and older individuals during passive movement, 10 participants over the age of 60 were recruited and took part in Experiment 5.

Experimental setup

Responses were elicited during passive ankle movement with participants sat in an isokinetic dynamometer (for greater detail, see section 3-3.2). TMS or electrical stimulation was delivered at anatomical zero (considered intermediate muscle length)

during static position and passive ankle movement. Additionally stimuli were delivered at ± 7.5° relative to anatomical zero in the part of the study examining corticospinal responses at different muscle lengths during passive ankle movement, with positive and negative degree values indicating plantar and dorsiflexion, respectively. Thus, at positive values relative to anatomical zero, the muscle was at longer and shorter length for TA and SOL, respectively, and vice versa for negative values. Based on the joint angles and movement velocity, the stimuli were delivered 2 seconds (Experiment 1-4), and 0.5 and 3.5 s after the onset of movement (Experiment 1). At least 15 s of rest was employed before each motion.

Electromyography

EMG activity was recorded from TA and SOL muscles. For detailed procedures see section 3-3.3. For SOL, the electrodes were positioned at two-thirds of the line between the medial condyle of the femur to the medial malleolus (Hermens et al., 2000).

Transcranial magnetic stimulation

Single- and paired-pulse TMS were delivered to investigate corticospinal excitability, SICI and ICF. For a detailed procedure, see section 3-3.5.

Lumbar evoked potentials

Lumbar evoked potentials were elicited using the same procedure as in Part 1 of this chapter.

Percutaneous nerve stimulation

Percutaneous nerve stimulation was performed to elicit H-reflexes in Experiment 4 in SOL and TA. To account for changes at the skin-electrode surface, M_{max} were elicited in SOL and TA and subsequently used for normalisation of the responses across Experiments 1-4 (see section 3-3.4 for a detailed procedure). In Experiments 1-4, four stimuli eliciting M_{max} in both muscles were delivered at anatomical zero. Additionally, in Experiment 1, four M_{max} were elicited at ±7.5° relative to anatomical zero.

Experimental procedures

The experimental procedures for Experiment 1-4 are summarised in Figure 4-5.

Assessment of fascicle length changes during passive ankle movement

Changes in joint angle during passive movement of muscle are usually assumed to reflect changes in the total muscle-tendon unit length. However, the proprioceptive feedback originating from muscle spindles is more closely related to changes in fascicle length than joint angle (Day et al., 2017; Matthews and Stein, 1969; Morgan et al., 2000). As modulation of corticospinal excitability has been linked to afferent feedback pertaining to changes in muscle length (Coxon et al., 2005; Lewis et al., 2001; Lewis and Byblow, 2002), it is important to establish whether changes in joint angle correspond to changes in fascicle length. Furthermore, it is important to assess the similarity of those changes between TA and SOL to ensure the corticospinal responses are not confounded by differing magnitude of afferent feedback between the two muscles.

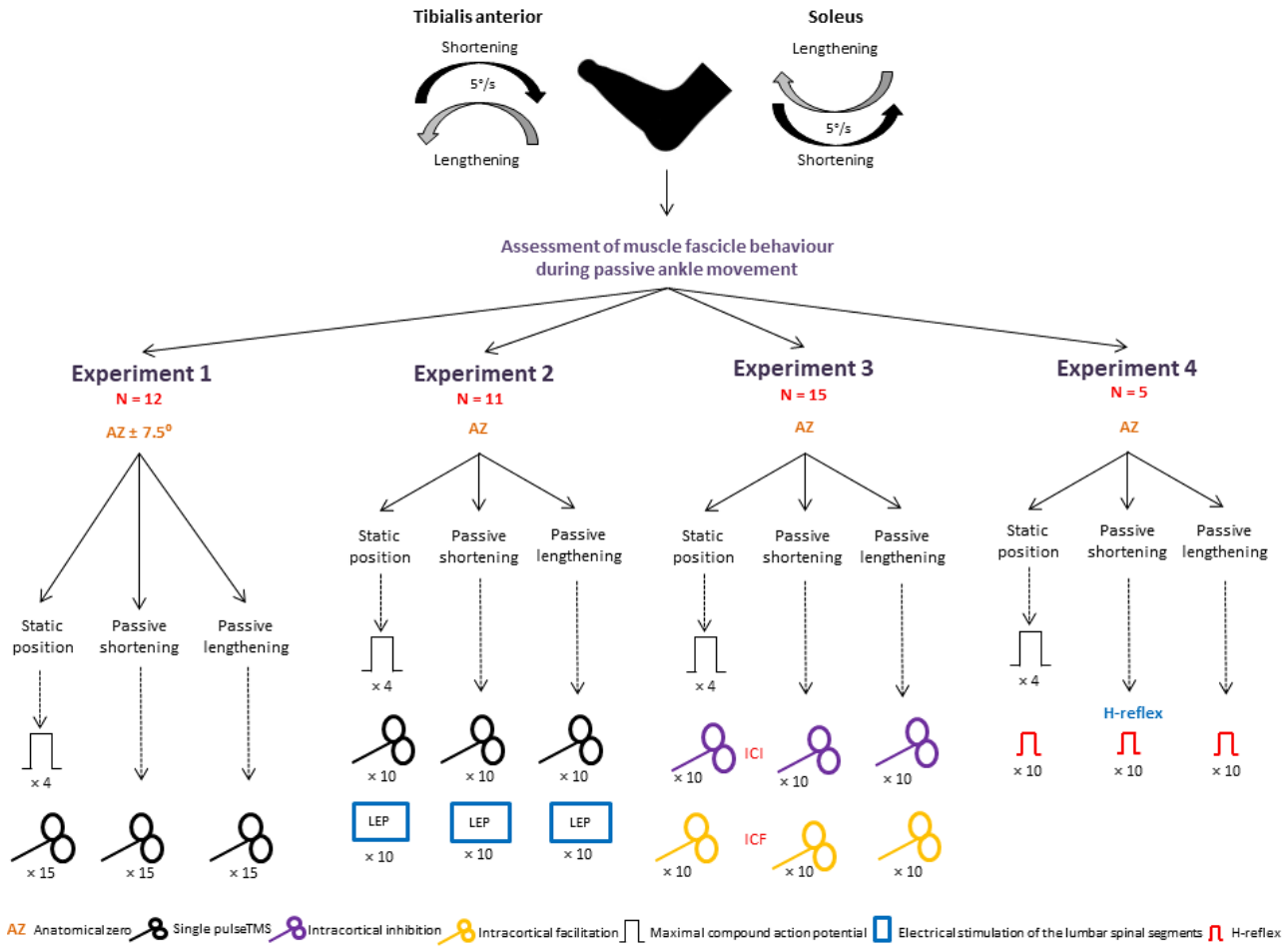

Figure 4-5. An overview of experimental procedures. ICI = intracortical inhibition, ICF = intracortical facilitation, LEP = lumbar evoked potential.

In a subpopulation of seven individuals (27 ± 3 years, 179 ± 8 cm, 84.1 ± 19.6 kg; 3 females) fascicle behaviour of the SOL and TA during 20° of passive ankle movement at $5°{\cdot}s^{-1}$ was tracked using ultrasound (see section 3-3.6 for a detailed experimental procedure and section 3-4.4 for analysis of TA). For SOL, the probe was positioned at 50% of the distance between the popliteal crease and the lateral malleolus (Valadão et al., 2018). Occasionally, this position had to be adjusted to 30% of the same reference line as only that position allowed for sufficiently clear images of fascicles (Valadão et al., 2018). Fascicle length in SOL was measured from the visible insertion of the fibre between the deep and superficial aponeurosis for SOL.

Experiment 1: Corticospinal responses at different muscle lengths during passive ankle movement

Responses in twelve individuals were assessed across nine conditions: static position and passive shortening and lengthening with single pulse TMS delivered at anatomical zero (intermediate muscle length) and at ± 7.5° relative to anatomical zero (shorter and longer muscle length depending on the muscle as explained above). The order of conditions was randomised. Intensity of TMS was standardised to 1.2 × rMT in the static position. A total of fifteen MEPs were elicited in each condition.

Experiment 2: Corticospinal and spinal motoneuronal responses during passive ankle movement

In eleven individuals, ten LEPs and ten MEPs were evoked during static position and passive ankle movement in SOL and TA (randomised order). The intensity of TMS was standardised to 1.2 × rMT. Pilot testing indicated that MEPs elicited at 1.2 × rMT in the resting position evoke a response of ~5-10% M_{max}. Thus, the stimulus intensity

of LEPs was standardised to elicit a response of ~5-10% M_{max} in the resting position (current intensity: 151 ± 54 and 163 ± 54 mA for SOL and TA, respectively). All stimuli were delivered at anatomical zero.

Experiment 3: Intracortical inhibition and facilitation during passive ankle movement

In fifteen participants, paired-pulse paradigms (SICI and ICF) were employed during static position and passive movement of the ankle to elicit responses in SOL and TA (randomised order). Ten unconditioned and ten conditioned pulses were delivered in an alternating fashion for each paired-pulse paradigm at anatomical zero.

Experiment 4: H-reflex during passive ankle movement

In five participants, H/M recruitment curves were first constructed in the anatomical zero position in both SOL and TA by gradually increasing the intensity of stimulation by 0.3 mA every 3 pulses from H-reflex threshold to M_{max}. Recruitment curves were obtained only in the static position since only the amplitude of the H-reflex, but not the slope of the H/M curve differs between passive shortening and lengthening (Pinniger et al., 2001). The H-reflex amplitude was evoked with a small M-wave of consistent size across conditions (SOL: 12 ± 6% M_{max}, TA: 8 ± 2% M_{max}; $p = 0.21$), ensuring that the same proportion of motor units were activated across conditions (Duclay and Martin, 2005), and that the H-reflex was produced on the ascending limb of the H/M recruitment curve and was thus susceptible to a change with passive ankle movement (Pierrot-Deseilligny and Burke, 2005). Ten H-reflexes were elicited in SOL and TA during static position and passive ankle movement in a randomised order. All stimuli were delivered at anatomical zero. Recordings were made separately for TA and SOL.

Experiment 5: Corticospinal responses during passive ankle movement in older adults

To assess corticospinal responses during passive movement in aging adults, ten MEPs were evoked during static position and passive movement in SOL and TA (randomised order). The intensity of TMS was standardised to 1.2 × rMT. All stimuli were delivered at anatomical zero.

Data analyses

EMG activity was visually inspected during the experiments to ensure participants maintained a relaxed muscle. If voluntary EMG activity was observed, the trial was discarded and additional trials were performed. Furthermore, RMS EMG activity was measured as per section 3-4.2. If RMS EMG was >2 SDs compared to mean baseline values, the evoked response following it was discarded. For that reason, SICI and ICF data from one participant was omitted from statistical analysis. RMS EMG across all conditions and experiments is displayed in Table 4-3. Evoked responses were analysed as per section 3-4.3.

Statistical analyses

All data are presented as means ± SD. Normality of data was assessed using Shapiro-Wilks test. If the data were not normally distributed, transformations were performed using common logarithm. A paired-sample T-test was used to assess differences in stimulus intensity at rMT (% of stimulator output; SO) between SOL and TA. Sphericity was assessed using Mauchly's test of sphericity. In the case of violation, a Greenhouse-Geisser correction was employed. A repeated measures ANOVA was used to assess differences in normalised evoked responses between resting position

and passive shortening and lengthening (within-factor – a change in muscle length). Additional factor was added to ANOVA to assess differences between stimulations performed at different lengths (within-factor – muscle length at the point of stimulation). A two-way ANOVA was used to assess differences in fascicle length with passive ankle movement (2 × direction – shortening and lengthening; 5 × joint angle). If significant F-values were found, analyses were continued using pairwise comparison with Bonferroni correction. Additionally, Pearson's class correlation and a linear regression were performed to assess the association of intracortical facilitation or inhibition to a change in MEP/M_{max} with a change in shortening or lengthening. Significance was set at an alpha level of .05. All analyses were performed using SPSS (v20, SPSS Inc., Chicago, IL, USA).

4-8 Results

Fascicle length changes during passive ankle movement

Fascicle length was modulated during passive ankle movement both in SOL ($F_{4, 24}$ = 109.9, $p < 0.001$; Figure 4-6A) and TA ($F_{4, 24}$ = 239.9, $p < 0.001$; Figure 4-6B), such that fascicle length changed linearly (Figure 4-6) with changes in joint angle throughout the 20˚ of range-of-motion ($p \leq 0.003$ and $p \leq 0.002$ for SOL and TA, respectively). Based on total change in fascicle length throughout the range-of-motion, the fascicles exhibited a similar mean change of 0.7 mm/degree and 0.6 mm/degree in SOL and TA, respectively ($p = 0.388$). In TA, fascicles were on average longer during passive lengthening (40.9 ± 4.1 mm) compared to passive shortening (39.4 ± 4.5 mm; $F_{1, 6}$ = 10.3, $p = 0.018$). However, no direction × angle interaction was found for both SOL ($F_{4, 24}$ = 1.5, $p = 0.240$) and TA ($F_{4, 24}$ = 1.2, $p = 0.357$).

Table 4-3. Root mean square EMG activity (μV; mean ±SD) in the 100 ms preceding the stimulus.

		Experiment 1			***Experiment 2***		***Experiment 3***			***Experiment 4***
		Short	*Intermediate*	*Long*	*MEP*	*LEP*	*MEP*	*SICI*	*ICF*	*H-reflex*
SOL	***STAT***	10.5 ± 0.3	10.5± 0.4	10.5 ± 0.2	10.7 ± 0.2	10.9 ± 0.6	10.6 ± 0.1	10.6 ± 0.2	10.6 ± 0.1	10.5 ± 0.1
	SHO	10.7 ± 0.2	10.7 ± 0.2	10.8 ± 0.2	10.8 ± 0.3	10.8 ± 0.2	10.7 ± 0.3	10.7 ± 0.1	10.6 ± 0.1	10.5 ± 0.1
	LEN	10.7 ± 0.2	10.7 ± 0.2	10.7 ± 0.2	10.6 ± 0.1	10.7 ± 0.2	10.6 ± 0.1	10.6 ± 0.2	10.6 ± 0.1	10.5 ± 0.1
TA	***STAT***	4.2 ± 0.5	4.2 ± 0.4	4.1 ± 0.3	4.4 ± 0.3	4.6 ± 0.4	4.2 ± 0.2	4.2 ± 0.2	4.2 ± 0.2	4.3 ± 0.1
	SHO	4.4 ± 0.6	4.4 ± 0.7	4.2 ± 0.3	4.6 ± 0.3	4.6 ± 0.2	4.5 ± 0.5	4.3 ± 0.2	4.3 ± 0.3	4.3 ± 0.1
	LEN	4.2 ± 0.3	4.3 ± 0.4	4.4 ± 0.6	4.6 ± 0.2	4.7 ± 0.3	4.3 ± 0.2	4.3 ± 0.3	4.3 ± 0.2	4.4 ± 0.1

SOL = soleus, TA = tibialis anterior; STAT = static position, SHO = passive shortening, LEN = passive lengthening, MEP = motor evoked potential, LEP = lumbar evoked potential, SICI = intracortical inhibition, ICF = intracortical facilitation

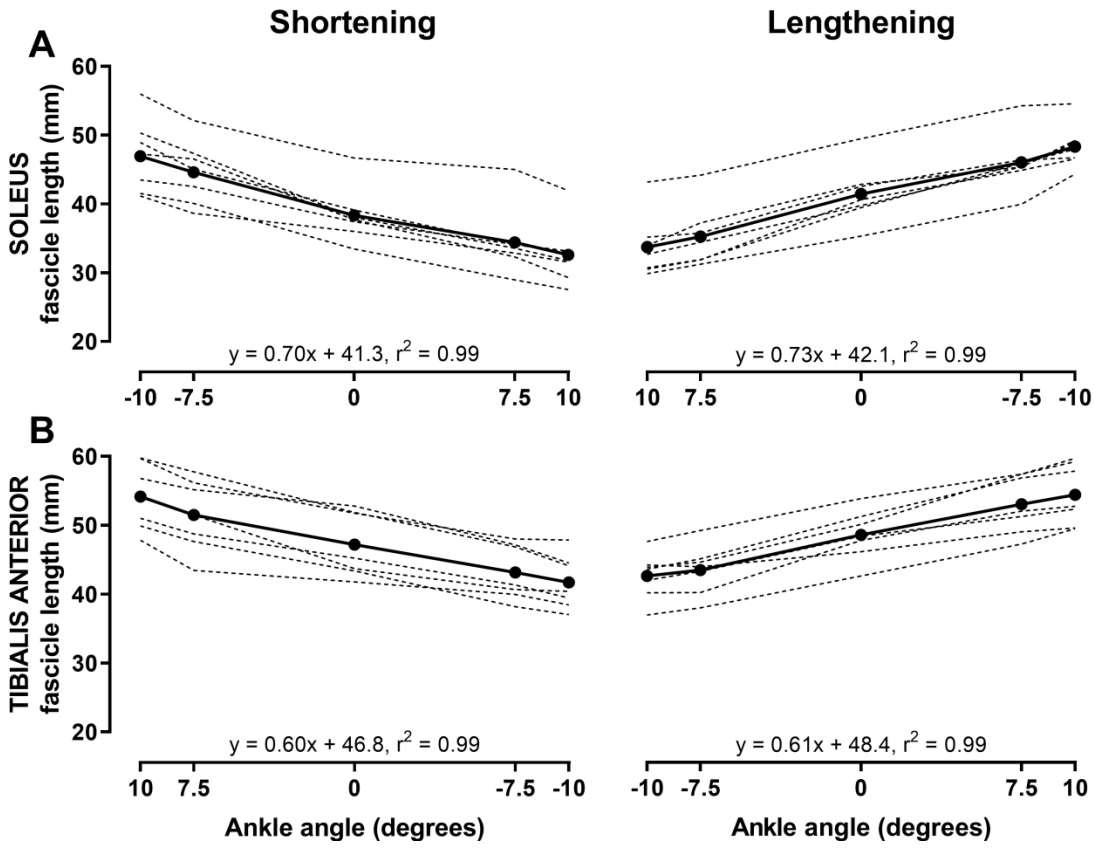

Figure 4-6. Change in fascicle length in soleus and tibialis anterior with passive movement of the ankle. Fascicle length (mm) with passive changes in the ankle joint angle during passive shortening (left panel) and lengthening (right panel) of soleus (A) and tibialis anterior (B). Fascicle length was assessed at joint angles where stimuli were delivered in subsequent experiments and are displayed on the x-axes relative to anatomical zero (ankle at 90º). Fascicles changed linearly with changes in joint angle as noted on plots. Full lines represent the sample mean, whilst dashed lines denote individual responses (n = 7).

Experiment 1: Corticospinal responses at different muscle lengths during passive ankle movement

The stimulus intensity at rMT was higher in SOL (54 ± 8% SO) compared to TA (48 ± 7% SO; t_{11} = 3.0, p = 0.012). Examples of averaged EMG recordings in SOL (A) and TA (B) in response to single pulse TMS are presented in Figure 4-7. MEP/M_{max} amplitude of SOL did not differ between the static position and during passive ankle movement (0.02 ± 0.01 vs. 0.02 ± 0.01 vs. 0.02 ± 0.01; $F_{2,22}$ = 2.3, p = 0.121), irrespective of the joint angle at the point of stimulation (0.02 ± 0.01 vs. 0.02 ± 0.01

vs. 0.02 ± 0.01 at short, intermediate and long muscle length, respectively; $F_{2,22}$ = 0.2, p = 0.787; Figure 4-7C). Conversely, a change in muscle length modulated MEP/M_{max} amplitude in TA ($F_{1.3,14.6}$ = 11.3, p = 0.003) insofar as MEP/M_{max} amplitude was greater during passive shortening (0.17 ± 0.09) compared to passive lengthening (0.09 ± 0.07; p < 0.001) and static position (0.10 ± 0.08; p = 0.023; Figure 4-7D), with no difference between passive lengthening and static position (p = 0.99). Also, MEP/M_{max} amplitude in TA was not affected by muscle length at the point of stimulation (0.12 ± 0.09 vs. 0.13 ± 0.10 vs. 0.11 ± 0.07 at short, intermediate and long muscle length, respectively; $F_{2,22}$ = 1.0, p = 0.922; Figure 4-7D).

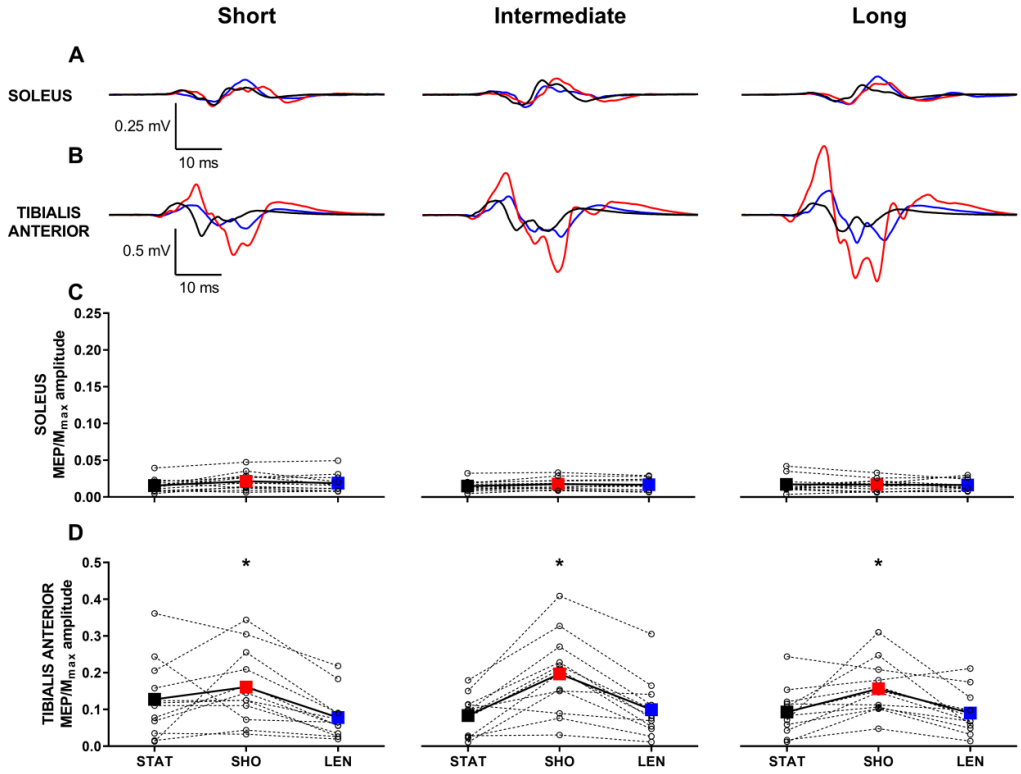

Figure 4-7. Motor evoked potentials during static position, passive shortening and lengthening in soleus and tibialis anterior with stimuli delivered at different muscle length. A, B: Averaged representative traces in response to single pulse transcranial magnetic stimulation delivered at short, intermediate and long muscle length during resting position (black line), passive shortening (red line) and lengthening (blue line) in soleus (A) and tibialis anterior (B). Each representative trace is an average of 15 waveforms. C, D: Amplitude of motor evoked potential expressed as a percentage of the amplitude of maximal compound action potential (MEP/M_{max}) during static position (STAT), passive shortening (SHO) and passive lengthening (LEN) in soleus (C) and tibialis anterior (D) at short (left panel), intermediate (centre panel) and long (right panel) muscle length. Open squares and full lines represent the sample mean, whilst open circles and dashed lines denote individual responses (n = 12). For ease of comparison, the soleus and tibialis anterior data are presented in 1:2 ratio. $^{*}p$ = 0.023 compared to static position, and p < 0.001 compared to passive lengthening.

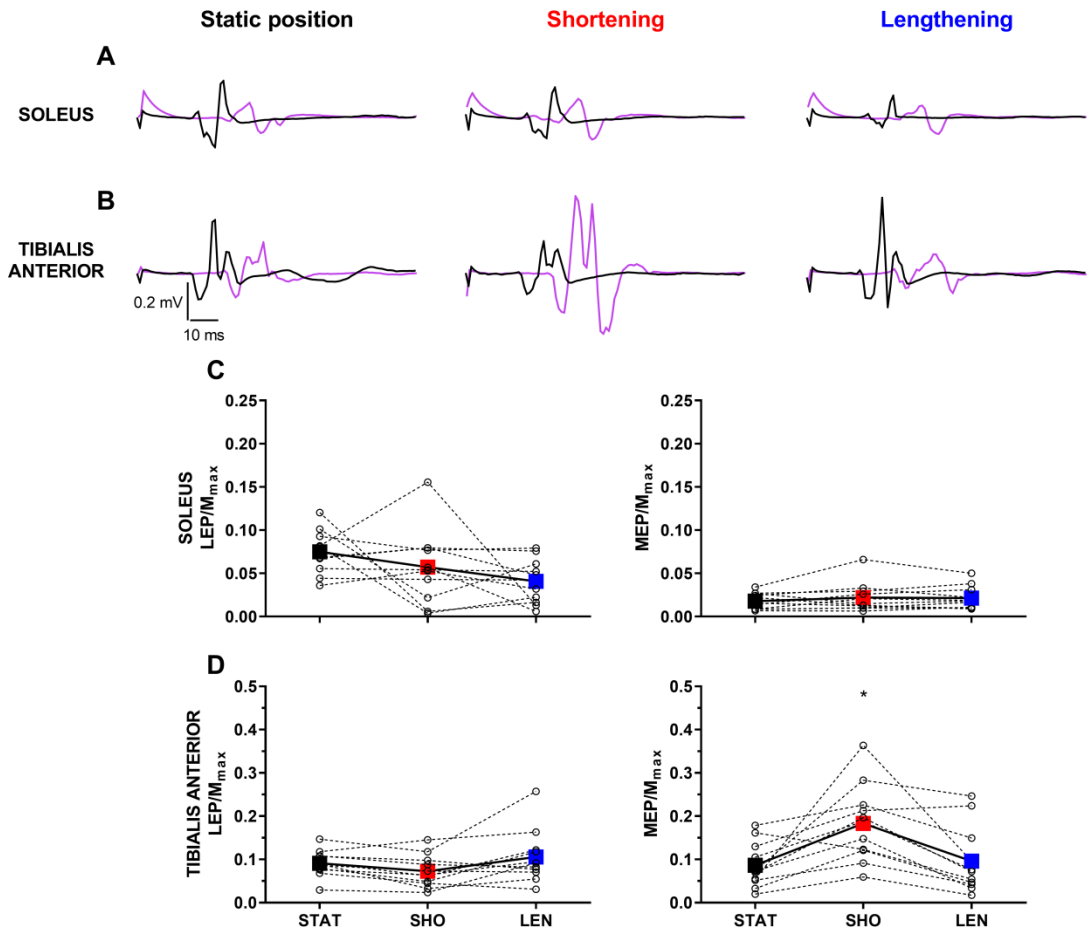

Figure 4-8. Motor evoked and lumbar evoked potentials during static position, passive shortening and lengthening in soleus and tibialis anterior. A, B: Averaged representative traces in response to electrical stimulation of the lumbar spinous processes (black line) and single pulse transcranial magnetic stimulation (violet line) during static position (left panel), passive shortening (centre panel) and passive lengthening (right panel) in soleus (A) and tibialis anterior (B). Each representative trace is an average of 10 waveforms. C, D: Amplitude of lumbar evoked potential (left panel) and motor evoked potential (right panel) expressed as a percentage of the amplitude of maximal compound action potential (LEP/M_{max} and MEP/M_{max}, respectively) during static position (STAT), passive shortening (SHO) and passive lengthening (LEN) in soleus (C) and tibialis anterior (D). Open squares and full lines represent the sample mean, whilst open circles and dashed lines denote individual responses (n = 11). For ease of comparison, the soleus and tibialis anterior data are presented in 1:2 ratio. $^{*}p < 0.005$ compared to static position and passive lengthening.

Experiment 2: Corticospinal and spinal responses during passive ankle movement

The stimulus intensity at rMT was again higher in SOL (49 ± 9% SO) compared to TA (46 ± 10% SO; $t_{10} = 3.0$, $p = 0.014$). Figure 4-8 shows examples of averaged EMG recordings in SOL (A) and TA (B) in response to single pulse TMS and electrical stimulation of descending axons at the lumbar spinal segments. These responses display similarities of MEPs in SOL across conditions (Figure 4-8A). Both MEP/M_{max}

(0.02 ± 0.01 vs. 0.02 ± 0.02 vs. 0.02 ± 0.01; $F_{2,20} = 0.72$, $p = 0.497$) and LEP/M_{max} (0.07 ± 0.02 vs. 0.06 ± 0.04 vs. 0.04 ± 0.03; $F_{2,20} = 2.95$, $p = 0.075$) were not modulated in SOL during passive ankle movement (Figure 4-8C). Similarly, LEP/M_{max} did not change in TA with passive shortening and lengthening (0.09 ± 0.03 vs. 0.07 ± 0.04 vs. 0.11 ± 0.06; $F_{2,20} = 3.63$, $p = 0.071$). However, MEP/M_{max} was modulated by a change in muscle length in TA ($F_{2,20} = 14.67$, $p < 0.001$), being greater during passive shortening (0.18 ± 0.09) compared to passive lengthening (0.10 ± 0.08; p = 0.001) and static position (0.09 ± 0.05; p = 0.003; Figure 4-8D).

Experiment 3: Intracortical inhibition and facilitation during passive ankle movement

The stimulus intensity at rMT was higher in SOL (51 ± 12% SO) compared to TA (48 ± 10% SO; $t_{13} = 4.5$, $p = 0.001$). MEP/M_{max} in SOL was not modulated with a change in muscle length (0.02 ± 0.01 vs. 0.02 ± 0.02 vs. 0.02 ± 0.01; $F_{2,26} = 1.65$, $p = 0.211$; Figure 4-9A), but was in the TA ($F_{2,26} = 15.96$, $p < 0.001$; Figure 4-9B), such that it was greater during passive shortening (0.21 ± 0.14) compared to passive lengthening (0.12 ± 0.11; $p < 0.001$) and static position (0.09 ± 0.04; $p = 0.001$). No modulation in SICI was observed in SOL (0.71 ± 0.22 vs. 0.61 ± 0.30 vs. 0.60 ± 0.23; $F_{2,26} = 0.88$, $p = 0.427$) or in TA (0.63 ± 0.25 vs. 0.55 ± 0.23 vs. 0.56 ± 0.32; $F_{2,26} = 0.63$, $p = 0.540$) during passive ankle movement, nor was ICF (SOL: 1.21 ± 0.19 vs. 1.34 ± 0.38 vs. 1.12 ± 0.24; $F_{2,26} = 1.85$, $p = 0.177$; TA: 1.29 ± 0.37 vs. 1.38 ± 0.40 vs. 1.45 ± 0.61; $F_{2,26} = 0.26$, $p = 0.777$). There was an inverse relationship between MEP/M_{max} and ICF during passive shortening of TA ($r = –0.625$, $p = 0.017$, adjusted $r^2 = 0.34$), suggesting that greater corticospinal excitability observed during passive shortening was associated with a smaller degree of intracortical facilitation (Figure 4-9C). No

other associations were found between MEP/M_{max} and SICI or ICF either in SOL or TA (Table 4-4).

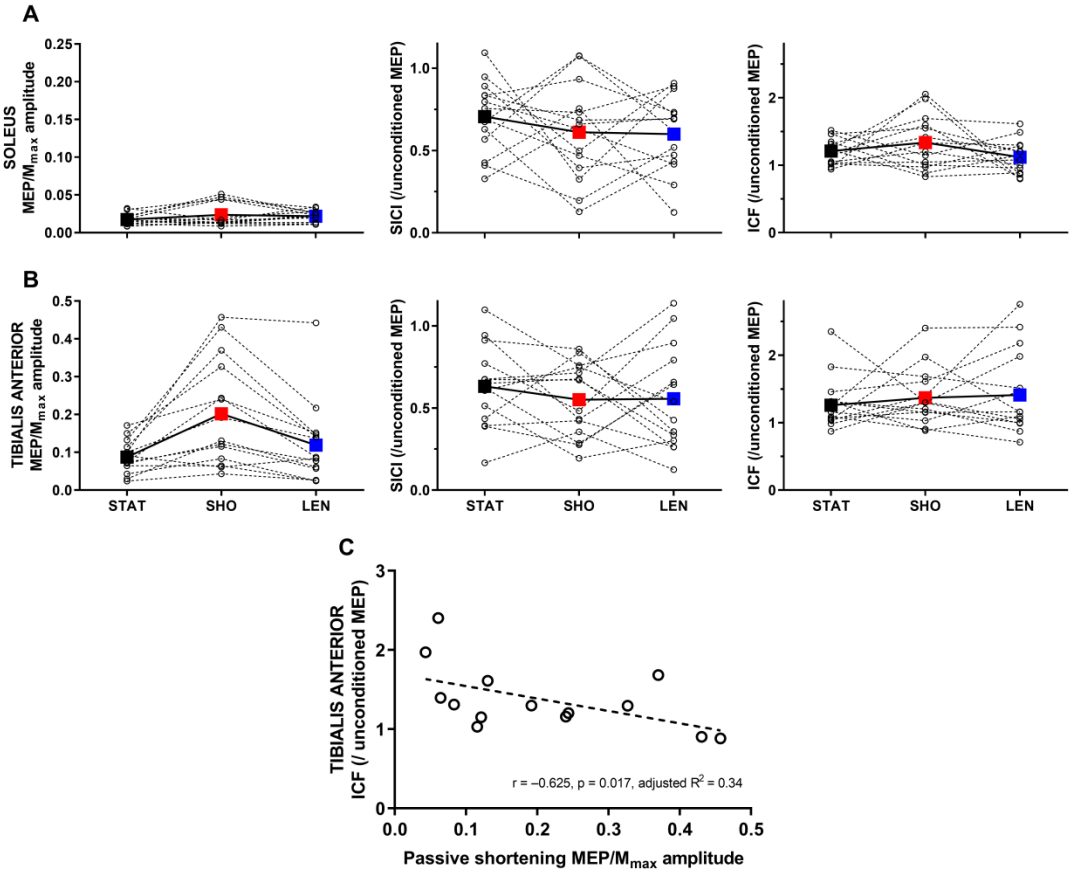

Figure 4-9. Motor evoked potentials evoked with single and paired-pulse transcranial magnetic stimulation during static position, passive shortening and lengthening in soleus and tibialis anterior. A, B: Amplitude of motor evoked potential expressed as a percentage of the amplitude of maximal compound action potential (MEP/M_{max}; left panel), short-interval intracortical inhibition (SICI; centre panel) and intracortical facilitation (ICF; right panel) expressed as a percentage of the unconditioned MEP amplitude during static position (STAT), passive shortening (SHO) and passive lengthening (LEN) in soleus (A) and tibialis anterior (B). Open squares and full lines represent the sample mean, whilst open circles and dashed lines denote individual responses (n = 14). For ease of comparison, the soleus and tibialis anterior MEP/M_{max} data are presented in 1:2 ratio. $^{*}p < 0.005$ compared to resting position and passive lengthening. C: The amplitude of motor evoked potential expressed as a percentage of the amplitude of maximal compound action potential (MEP/M_{max}) plotted against ratio of conditioned and unconditioned motor evoked potential amplitude (ICF) in response to paired-pulse transcranial magnetic stimulation with an inter-stimulus interval of 10 ms during passive shortening in TA (n = 14).

Experiment 4: H-reflex during passive ankle movement

Representative averaged traces of the H-reflex response from one individual are presented in Figure 4-10A and Figure 4-10B for SOL and TA, respectively. As clearly seen from these examples, the H-reflex responses were modulated during passive

ankle movement in SOL ($F_{1.0,\ 4.1}$ = 8.4, p = 0.043), being smaller during passive lengthening (0.40 ± 0.23) compared to passive shortening (0.56 ± 0.17; p = 0.048; Figure 4-10C). Conversely, H/M_{max} was not modulated during passive ankle movement in TA (0.04 ± 0.03 vs. 0.04 ± 0.02 vs. 0.05 ± 0.03; $F_{2,\ 8}$ = 1.6, p = 0.258; Figure 4-10D).

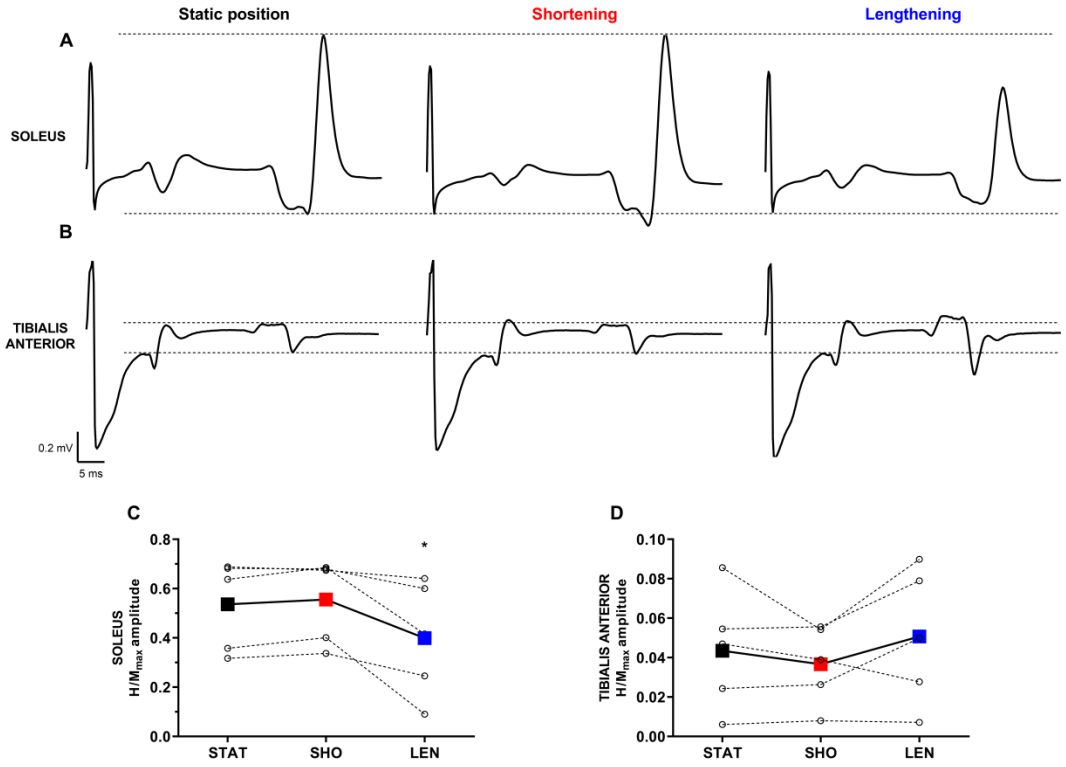

Figure 4-10. H-reflexes during static position, passive shortening and lengthening in soleus and tibialis anterior. A, B: Averaged representative traces in response to submaximal percutaneous nerve stimulation during static position (left panel), passive shortening (centre panel) and passive lengthening (right panel) in soleus (A) and tibialis anterior (B). Traces are shown from the point of stimulus and each representative trace is an average of 10 responses. Dashed lines represent the amplitude of H-reflex during static position. C, D: Amplitude of H-reflex expressed as a percentage of the amplitude of maximal compound action potential during static position (STAT), passive shortening (SHO) and passive lengthening (LEN) in soleus (C) and tibialis anterior (D). Open squares and full lines represent the sample mean, whilst open circles and dashed lines denote individual responses (n = 5). $^{*}p < 0.05$ compared to passive shortening.

Table 4-4. Associations between responses to single and paired pulse transcranial magnetic stimulation.

			MEP/M_{max}					
			SOL			TA		
			STAT	SHO	LEN	STAT	SHO	LEN
SICI (/ unconditioned MEP)	STAT	r	-0.160	-	-	-0.002	-	-
		p	0.584	-	-	0.994	-	-
	SHO	r	-	-0.042	-	-	0.077	-
		p	-	0.887	-	-	0.794	-
	LEN	r	-	-	-0.095	-	-	-0.319
		p	-	-	0.748	-	-	0.267
ICF (/ unconditioned MEP)	STAT	r	0.138	-	-	-0.284	-	-
		p	0.637	-	-	0.326	-	-
	SHO	r	-	-0.143	-	-	-0.625	-
		p	-	0.626	-	-	0.017	-
	LEN	r	-	-	-0.301	-	-	-0.433
		p	-	-	0.296	-	-	0.122

SOL = soleus, TA = tibialis anterior; STAT = static position, SHO = passive shortening, LEN = passive lengthening, MEP/M_{max} = motor evoked potential normalised to maximal compound action potential, SICI = intracortical inhibition, ICF = intracortical facilitation, r = correlation coefficient, p = significance at alpha level 0.05.

Experiment 5: Corticospinal responses during passive ankle movement in aging adult

Similar to young individuals, the stimulus intensity at rMT was higher in SOL (55 ± 8% SO) compared to TA (52 ± 6% SO; $t_9 = 3.3$, $p = 0.010$). Passive movement did not result in modulation of corticospinal responses both in SOL (0.05 ± 0.04 vs. 0.03 ± 0.02 vs. 0.04 ± 0.01; $F_{1.1,9.9} = 1.0$, $p = 0.346$) and TA (0.18 ± 0.07 vs. 0.19 ± 0.11 vs. 0.22 ± 0.11; $F_{2,18} = 0.61$, $p = 0.613$; Figure 4-11).

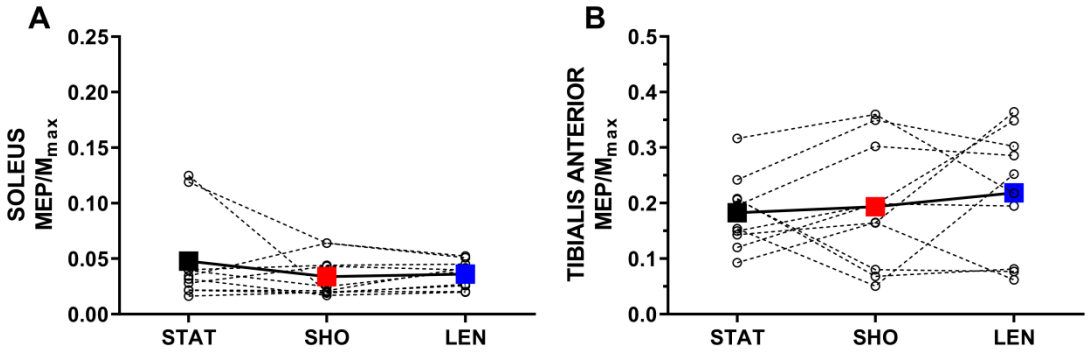

Figure 4-11. Motor evoked potentials during static position, passive shortening and lengthening in soleus and tibialis anterior in older adults. Amplitude of motor evoked potential expressed as a percentage of the amplitude of maximal compound action potential (MEP/M_{max}) during static position (STAT), passive shortening (SHO) and passive lengthening (LEN) in soleus (A) and tibialis anterior (B). Open squares and full lines represent the sample mean, whilst open circles and dashed lines denote individual responses (n = 10).

4-9 Discussion

The main finding of this study was that corticospinal excitability is modulated differently between antagonist muscles during passive ankle movement in young individuals. On the other hand, corticospinal responses were unchanged during passive movement in older adults, suggesting age-related differences in sensorimotor integration. In young, cortical excitability in TA was facilitated during passive shortening, but remained unchanged in SOL. Moreover, subcortical excitability at the lumbar spinal segmental level was not modulated in TA, suggesting a cortical and/or propriospinal contribution to the observed facilitation. These findings suggest a different intrinsic modulation of antagonist ankle muscles during passive movement in young, and altered sensorimotor integration in older adults. Results in younger individuals will be discussed first (Experiments 1-4), before returning to age-related differences.

Modulation of corticospinal excitability during passive movement is not dependent on the muscle length at the point of stimulation

The differing corticospinal response to TMS between the muscles cannot be attributed to muscle length change differences, as both muscles exhibited a similar range of fascicle shortening and lengthening during passive movement (0.7 and 0.6 mm/degree for SOL and TA, respectively). Contrary to our hypothesis, the responses were similar regardless of the muscle length at the point of stimulation. This contrasts also to previous experiments of passive wrist movement (Lewis et al., 2001; Lewis and Byblow, 2002) where corticospinal excitability was dependent on joint angle. However, direct comparison with previous experiments is difficult, due to differences in muscles tested (upper vs. lower limb), ranges of motion, and different methodologies with regard to MEP amplitude normalisation. The latter might play a role in interpreting changes in response amplitude, since electrode position variations might lead to differences in the spatial relationship between the electrode and the motor units recorded (Farina et al., 2014), which is typically reflected in M_{max} amplitude (Gerilovsky et al., 1989). The range-of-motion, and the resultant muscle length changes, could be equally important in interpreting response amplitude. Indeed, a recent study in active knee extensors showed muscle-length-dependent modulation of corticospinal excitability during lengthening contractions (Doguet et al., 2017). However, there was ~11 mm fascicle length change when moving from an intermediate to long position (Doguet et al., 2018), compared to ~5 mm seen in the present study. Thus, it seems plausible that there is a threshold of muscle length change after which increased afferent feedback is sufficient for detecting differences in corticospinal excitability.

The responses to passive ankle movement are muscle specific

The facilitation of the corticospinal response to TMS observed in TA during shortening is in agreement with studies employing passive movement in upper limb muscles (Chye et al., 2010; Coxon et al., 2005; Lewis et al., 2001; Lewis and Byblow, 2002). As LEPs and H-reflexes remained unchanged in TA, this would suggest a cortical and/or propriospinal origin of the observed facilitation. Conversely, corticospinal excitability in SOL remained unchanged with passive movement. Due to lack of published data on corticospinal excitability during passive movement in SOL, no comparison can be made with other studies. However, similar results have been obtained during active movement of SOL with comparable stimulus intensities (Duclay et al., 2011; Hahn et al., 2012; Valadão et al., 2018). Whilst LEPs remained unchanged in SOL, H-reflexes were reduced during passive lengthening. This latter finding corroborates previous studies (Duclay et al., 2011; Pinniger et al., 2001) and has been attributed to presynaptic inhibition and post-activation depression of Ia afferents (Hultborn et al., 1996). Given that LEPs are likely devoid of presynaptic influence (Nielsen and Petersen, 1994), the lack of LEP modulation in SOL during passive movement further corroborates the notion that presynaptic inhibition mediates the reduction in H-reflexes during passive lengthening.

The activity of intracortical neurons during passive ankle movement

The MEP/M_{max} facilitation observed in TA during passive shortening was not accompanied by changes in responses to paired-pulse TMS, and could be explained by greater response variability (see Figure 4-9). This is a common occurrence and might be due to different electrophysiological properties of neuronal populations sub serving the responses to SICI and ICF and inter-individual differences in synaptic

efficacy of inhibitory or excitatory interneurons (Orth et al., 2003). It was also shown that the size of the MEP/M_{max} during passive dorsiflexion negatively correlated with the ICF ratio, possibly due to the 'busy line' phenomenon, whereby glutamatergic circuitry activity is too high for conditioned MEPs to be facilitated (Ortu et al., 2008). Previous work has shown that SICI is modulated during passive wrist movements (Lewis et al., 2001), but is only evident at the transition from extension to flexion, and might be related to a sudden muscle length change and the corresponding initial burst in muscle spindle firing (Matthews, 2011). When comparing responses elicited at similar joint angles, the lack of change in SICI corroborates the finding of the previous work (Lewis et al., 2001). Thus, the present data suggest that passive muscle length changes do not modulate cortical interneuronal activity.

Cortical and propriospinal contribution to the observed corticospinal response

Increased corticospinal excitability during passive shortening in TA in the absence of LEP modulation suggests a cortical origin, associated with sensory feedback influencing the excitability of descending tracts (Meinck and Piesiur-Strehlow, 1981; Roy and Gorassini, 2008), or mediation via propriospinal inputs (Bestmann and Krakauer, 2015; Meinck and Piesiur-Strehlow, 1981).

In both primates (Herter et al., 2015; Hore et al., 1976) and humans (Goldring and Ratcheson, 1972; Shaikhouni et al., 2013), cortical neurons have been shown to be facilitated during passive shortening, whilst inhibited during passive lengthening, in line with our findings. Cutaneous and joint receptors are unlikely mediators of this behaviour due to their activation being largely restricted to the limits of movement (Burke et al., 1988), rather than throughout the movement. Thus, the primary candidates for the sensory mediated change in cortical neuronal activity are muscle

spindle afferents. This mediation might involve inhibitory inputs, either directly to motor cortical areas or through the somatosensory cortex. Indeed, in primates, hindlimb muscle stretch has been shown to result in inhibition of area 4 cortical neurons due to direct input from group II afferents (Hore et al., 1976). Furthermore, changes in TA muscle fascicle length have been shown to be tightly linked to Ia afferent sensitivity in humans (Day et al., 2017). Thus, increased corticospinal responses during passive shortening of TA might stem from decreased Ia afferent input via area 3a of the cerebral cortex (Hore et al., 1976), resulting in disinhibition of corticospinal neurons, and thus increasing corticospinal excitability (Brasil-Neto et al., 1992; Ziemann et al., 1998). The conceptual presentation of this mechanistic action is presented in Figure 4-12.

It is unclear why the augmented corticospinal response to TMS during passive shortening is specific to TA. It might stem from divergent, non-uniform distribution of direct corticomotoneuronal projections, as evidenced by short latency facilitation of firing probability of TA motor units in response to TMS, and the absence of this behaviour in SOL (Brouwer and Ashby, 1992; Brouwer and Qiao, 1995). This could have contributed to the facilitation of TA during passive shortening when corticospinal neurons may be disinhibited relative to passive lengthening (Brasil-Neto et al., 1992; Ziemann et al., 1998). There is some basis for this notion as greater facilitation during passive shortening has been observed in the wrist muscles with greater strength of corticomotoneuronal projections (Chye et al., 2010). The pyramidal tract also has a preferential input into the spinal network controlling ankle flexors, such as TA (Brooks and Stoney, 1971), which could explain the lower stimulus intensity at rMT in the present study whilst also supporting previous work (Lauber et al., 2018). Additionally, the responses in TA could be related to differing reciprocal inhibition compared to

SOL (Yavuz et al., 2018). Less reciprocal inhibition as SOL lengthens would suppress the excitatory postsynaptic potential stemming from the antagonist, allowing for reduced inhibition in corticospinal neurons in TA. Furthermore, as per H-reflex behaviour in the present study, TA appears to be influenced by presynaptic inhibitory mechanisms to a lesser extent than SOL. Thus, the facilitation observed during passive shortening of TA could be due to coupling of the lack of presynaptic influences and sensory-related facilitation of corticospinal excitability in response to movement (Schubert et al., 1997). Nonetheless, it should be noted that despite the plausibility of the abovementioned notions, this study cannot directly ascertain the mechanism of the observed behaviour.

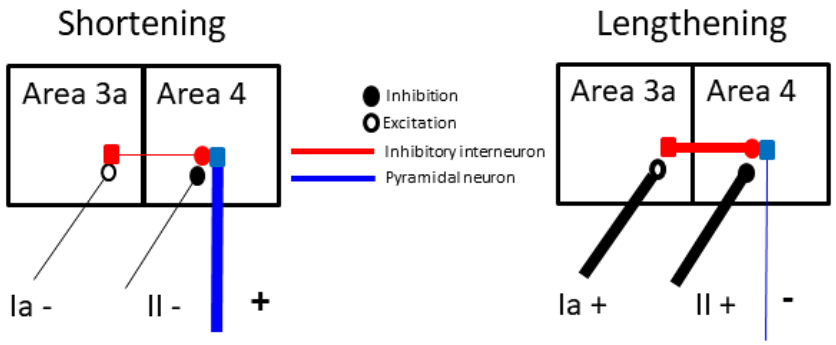

Figure 4-12. The proposed mechanism of action for changes in corticospinal excitability during passive movement in tibialis anterior. During muscle shortening, the reduced activity of muscle spindle afferent leads to disinhibition of corticospinal neurons, leading to increased response size. Conversely, during passive lengthening, the activity of muscle spindle afferents is increased causing inhibition of corticospinal neurons.

An increase in presynaptic inhibitory input to alpha motoneurons was observed during passive lengthening of SOL, with no accompanying change in MEPs and LEPs. This suggests a form of compensatory action of descending pathways during passive SOL lengthening to accommodate for reduced motoneuronal excitability. Given a lack of change in ICF and SICI, this compensation is unlikely to be intracortical in origin, pointing to the possibility of propriospinal mediation. This could occur through

facilitation of excitatory premotoneurons activated by group II afferents (Marque et al., 2005), which are likely to exhibit increased firing rate during muscle lengthening (Matthews, 2011). The specificity of this compensation to SOL is less clear, but it might again be related to asymmetrical distribution of reciprocal inhibitory input between TA and SOL (Yavuz et al., 2018).

Potential functional applications of the observed behaviour

The specificity of augmented corticospinal response in TA relative to SOL during passive shortening could reflect functional differences between these muscles. For example, during quiet standing, TA has been shown to exhibit passive fascicle length changes proportional to the sway-related changes in the ankle joint (Day et al., 2013; Di Giulio et al., 2009). The present data might thus suggest an important role of increasing corticospinal drive in this muscle during passive shortening when proprioceptive feedback originating from muscle spindles is reduced, to modulate the control signals of the antagonist via reciprocal inhibition (Di Giulio et al., 2009; Honeycutt et al., 2012).

Similar divergent responses in corticospinal excitability have also been shown in walking, whereby net facilitation was present in the TA compared to only minor modulations in SOL (Schubert et al., 1997). Comparable responses have also been observed in passive walking, i.e. air walking with assistance of the device (Kamibayashi et al., 2011, 2009). Therefore, it appears that the behaviour observed in the present experiment is intrinsic to the antagonist musculature about the ankle and occurs regardless of the extent of neural drive. It should be noted however, that despite the abovementioned extrapolations from the literature, locomotion involves greater degrees of freedom and the activation of spinal central pattern generators compared to

single joint manipulations in an isokinetic dynamometer used in the present study. Thus, further work is required to establish a more direct link between the responses observed in the present experiment and its implication in locomotion.

Corticospinal responses during passive movement in aging adults

Unlike in young adults, corticospinal responses were unchanged during passive movement, regardless of the muscle studied. This indicates that aging might lead to a decline in cortical sensorimotor integration of muscle spindle afferent input. Indeed, previous work indicates that cortical integration of cutaneous stimuli is impaired in older adults (Brown et al., 2018; Degardin et al., 2011; Smith et al., 2011a). Interestingly however, corticospinal responses when conditioned by muscle belly vibration, which is known to activate muscle spindles, have been shown to be similar between young and older adults (Brown et al., 2018). Vibration is known to preferentially activate Ia afferents (Bove et al., 2003), and as such vibration-based feedback is more likely to be integrated into area 3a of the cerebral cortex (Hore et al., 1976). Animal data also suggest that aging is accompanied by a lower degeneration rate of group Ia afferents (Vaughan et al., 2017), so age-related alterations in sensorimotor integration of information conveyed by this group of afferents are less expected. On the other hand, aging animals exhibit a significant decrease in group II afferents (Vaughan et al., 2017). Thus, the results of the present experiment might represent age-related alterations of group II afferent feedback that is known to be integrated into the area 4 of cerebral cortex (Hore et al., 1976).

Methodological considerations

The lack of modulation of corticospinal excitability during passive movement of the ankle in SOL could be due to the slow movement velocity used in the present study. Indeed, previous work using higher movement velocities has shown greater modulation in response size (Lewis et al., 2001; Lewis and Byblow, 2002), likely due to higher afferent feedback. The slower velocity was employed to ensure greater ability of relaxation and to avoid reflexive muscle activity related to passive movement, which could have confounded results (Pinniger et al., 2001). Furthermore, the relatively smaller ankle range-of-motion in the present experiment reflects the restriction and variability in joint mobility, particularly at dorsiflexion. In the upper limb, previous research has shown potentiated effects on corticospinal excitability during passive movement with greater ranges of motion (Coxon et al., 2005). Thus, future studies should explore the velocity- and muscle-length dependency of the responses.

Other limitations of the present study are the lack of repeated measures design and a small sample size in Experiment 4. With regard to the former, the significant facilitation of the response to TMS during passive shortening of TA, was replicated across three experiments (Experiment 1-3), suggesting a universal behaviour across different sample populations. As already noted, there was difficulty in obtaining H-reflexes in resting TA, corroborating previous reports (Burke, 2016; Roy and Gorassini, 2008). Despite screening 24 individuals, only five participants exhibited consistent H-reflexes in TA to allow for comparison with SOL. This small sample size does warrant caution in interpretation of the findings in Experiment 4. However, the SOL data corroborates the findings of previous work (Pinniger et al., 2001) and

suggests that presynaptic inhibition during passive lengthening is greater compared to TA.

With regard to older adults, only responses to single-pulse TMS were assessed to gain insight into age-related alterations in sensorimotor processing with muscle length changes. Because modulation of motor response during passive movement in young individuals is likely mediated by the cortex, it was assumed that sensorimotor integration is altered in aging cortical areas. However, other levels of the neural axis were not assessed. It is thus unclear whether lack of modulation in SICI, ICF and spinal excitability observed during passive movement in young would translate to older populations. However, sensorimotor integration has previously shown not to be altered in older age when SICI and ICF were assessed in response to vibration (Brown et al., 2018). Additionally, it is unclear whether an aging MTU experiences similar rate of muscle fascicle length changes during passive movement compared to young.

4-10 Conclusion

The segmental methodological approach revealed that changing muscle length modulates both corticospinal and spinal elements of the nervous system during passive movement in young, but is muscle specific. Contrary to our hypothesis, the corticospinal modulation occurred regardless of the muscle length at the point of assessment. Corticospinal excitability was facilitated in TA during passive shortening, whilst unmodulated in SOL. This suggests that neural modulation with movement should be interpreted in the context of the muscle investigated. During muscle shortening, a reduced inhibitory afferent input might explain the flexor-biased facilitation in corticospinal drive. On the other hand, older adults showed no modulation of corticospinal responses with passive movement, which might be related

to alterations of cortical sensorimotor integration of information relayed by group II afferents. These findings indicate that there could be an age-related difference in corticospinal modulation of shortening and lengthening contractions, which will be explored in the next Chapter.

CHAPTER 5: NEUROPHYSIOLOGICAL RESPONSES TO SUBMAXIMAL ISOMETRIC, SHORTENING AND LENGTHENING CONTRACTIONS

PART 1: DIFFERENCES IN NORMALISATION PROCEDURES ON ESTIMATES OF RELATIVE OUTPUT DURING SUBMAXIMAL SHORTENING AND LENGTHENING CONTRACTIONS

Publication arising from this part of the chapter:

Škarabot, J., Ansdell, P., Brownstein, C.G., Howatson, G., Goodall, S., and Durbaba, R. (2018). Differences in force normalising procedures during submaximal anisometric contractions. *Journal of Electromyography and Kinesiology* 41, 82-88.

5-1 Introduction

Due to the greater intrinsic force generating capacity of muscle fibres during lengthening contractions (Edman, 1988; Morgan et al., 2000), submaximal lengthening contractions may not be performed at an appropriate intensity relative to a maximal lengthening contraction if not normalised appropriately, i.e. relative to the specific contraction type. Whilst it seems clear that contraction type specificity should be accounted for when normalising submaximal anisometric contractions, this is not reflected in the existing literature. Indeed, there appear to be vast discrepancies regarding the procedures used for normalising submaximal forces across isometric, shortening and lengthening contractions. For example, anisometric contractions have been normalised relative to an isometric MVC (Gruber *et al.*, 2009; Kallio *et al.*, 2010), contraction-type specific MVC (Rice et al., 2015; Tallent et al., 2012) or muscle-length specific MVC (Duclay et al., 2014; Pasquet et al., 2006). Appropriate normalisation is of particular significance when stimulations are performed to assess neural responses during different contraction types. Specifically, inappropriate normalisation may result in different contraction types to be at different levels of contraction intensity-stimulus response curve of various responses (Capaday and Stein, 1987; Goodall et al., 2009; Matthews, 1986; Oya et al., 2008; Weavil et al., 2015), which could impede the understanding of the proposed unique neurophysiological response. Furthermore, appropriate normalisation is of vital importance when assessing torque variability of torque-matching tasks as the SD of torque production is dependent on relative intensity of torque production (Enoka et al., 2003).

Given the discrepancy of normalising procedures described in the literature, the purpose of this experiment was to directly assess and compare the influence of

different normalising procedures previously used under the same overall experimental conditions (i.e. the same population, dynamometer setup, contraction velocity, posture and joint positioning) in torque-matching tasks on mean torque and EMG activity during submaximal anisometric contractions. This will allow elucidation of the most appropriate normalising approach to be used in the subsequent parts of this Chapter. To visualise discrepancies and inform a hypothesis, a predictive model based on experimentally acquired different types of MVC was constructed. Based on that, it was hypothesised that the experimental data will show that normalisation to an isometric MVC or muscle-length specific MVC results in significantly lower submaximal torques and EMG activity compared to when normalised to contraction-type specific MVC.

5-2 Methods

Participants

Seven young, recreationally active men (age 25 ± 4 years; stature 179 ± 7 cm, mass 81 ± 10 kg) participated in the study.

Torque and EMG procedures

For details on torque recordings and experimental setup see section 3-3.2. For EMG procedures, see section 3-3.3. Participants performed MVCs under the following conditions: isometric contractions with the ankle at 80, 90 and 100° (corresponding to shorter, intermediate and longer muscle lengths, respectively) and anisometric contractions, i.e. isokinetic shortening and lengthening.

Experimental protocol

Participants attended the laboratory to complete a single measurement session. After initial warm-up involving individually estimated 50% submaximal isometric contractions, participants performed isometric MVCs at the pre-defined angles. Two MVCs per contraction type were performed with a rest period of 60 s between each to avoid fatigue. The greatest instantaneous value of the two attempts was recorded as the MVC. This was followed by the first part of the experiment, which involved shortening and lengthening contractions in a randomized order, set to a level equal to 50% isometric MVC at the intermediate muscle length (isometric normalisation; ISO). The second part of the experiment involved obtaining anisometric MVCs (randomised order, two trials per contraction type, 60 s rest) followed by anisometric contractions at 50% of contraction-type specific MVC (contraction-type specific normalisation; CTS). During ISO and CTS trials, participants were instructed to increase their torque level to the target torque and attempt to follow the target line as closely as possible throughout the 4-second contraction (visual feedback was provided through a monitor displaying the target torque). Finally, the muscle length-specific (MLS) submaximal anisometric contractions were performed. An example of a lengthening contraction during MLS is depicted in Figure 5-1. Lengthening contractions were initiated by matching the torque level with the line representing 50% of isometric MVC at shorter muscle length (Figure 5-1A). As muscle length increased, participants resisted the motion of the dynamometer reaching the line representing 50% of isometric MVC at an intermediate muscle length situated at the mid-point in ROM (Figure 5-1B), and finished the contraction by matching the line signifying 50% of isometric MVC at a longer muscle length (Figure 5-1C). For shortening contractions, the order of torque matching was reversed. This protocol is similar to that previously described by

Pasquet *et al.* (2006). For each condition and torque level, participants were given at least 10 practice trials, as it has been reported that variability in torque production plateaus following such practice (Hortobágyi et al., 2001b). Subsequently, experimental trials were performed of which four successful trials per condition, contraction type and contraction level were used and averaged for all measures (ISO, CTS, and MLS). A trial was deemed successful if participants produced a steady torque for 4 seconds (ISO, CTS) and produced a parabolic-like shape of torque covering the three time points at appropriate times (MLS). Due to the practice trials executed beforehand, no more than five experimental trials were needed for all participants. A minimum of 30 s rest was given between each contraction to avoid fatigue.

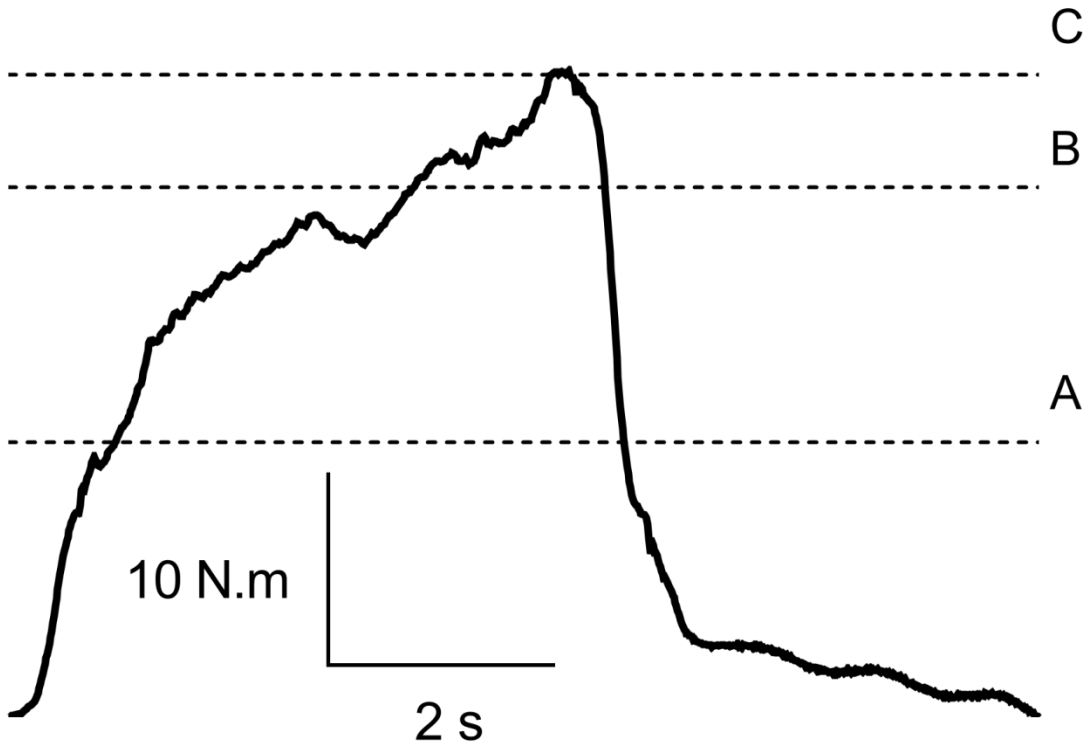

Figure 5-1. An example of submaximal lengthening contraction during muscle length specific normalisation. The motor of the device moved once participant reached the line representing 50% of the shorter muscle length isometric MVC (A). Thereafter, participants were instructed to resist the motion of the device reaching the line representing 50% of the intermediate muscle length isometric MVC about half-way throughout the range-of-motion (B), and finishing the contraction at the line representing 50% of the longer muscle length isometric MVC (C).

Data analysis

Maximal torques and corresponding RMS EMG activity were analysed first. For the purpose of better representation and visualisation of conceptual issues, the mean MVC values of the sample were then used to construct a predictive model for submaximal contractions using different normalising procedures. This was done as it allows the assessment of what torque levels are expected to emerge from different normalisation procedures which might be different to experimental values due to inaccurate torque production (Hortobágyi et al., 2001b). In the predictive model, for CTS and ISO normalization, a single value of mean percentage torque for shortening and lengthening contractions was calculated. For MLS normalization, the percentage of torque at shorter, intermediate and longer muscle lengths and the percentage of mean torque production were calculated. Whilst it is recognised that this type of normalisation will result in a parabolic torque production as per torque-angle relationship (Billot et al., 2011), for simplicity and the purposes of illustration, the percentage of mean torque production during MLS was computed assuming separate linear relationships between the shorter and intermediate lengths and between the intermediate and longer muscle lengths. For all submaximal contractions in the experiment, data from 4 successful attempts for each contraction type and intensity were analysed. Since the aim of the study was to compare the so-called 'torque-matching' task (e.g. Rice et al., 2015) across different contraction types, to allow for a valid comparison between them only the last 3.2 s of each trial was used to calculate the mean torque and RMS. The first 0.8 s of each trial was excluded from analysis which removed any issue that the acceleration phase at the start of the movement might be analysed as this was typically restricted to the initial 0.05 s of movement. Also, the deceleration component at the end of motion was not analysed as it was found to start

just after the end point of the 3.2 s analysis period. As such, the acceleration and deceleration phases of motion were ignored, allowing for a 'true' torque-matching task comparison. Mean torque is presented both in absolute and relative values, i.e. normalised to CTS MVC. The calculated RMS EMG activity was normalized to RMS EMG obtained during CTS MVC.

Statistical analysis

Data are presented as mean ± standard deviation. All analyses were performed using SPSS package (v20, SPSS Inc., USA). Statistical significance was set at an alpha level of 0.05. Normality of the data was assessed using Shapiro-Wilks test. Sphericity was assessed using Mauchly's test, and if violated, a Greenhouse-Geisser correction was employed. One-way repeated measures ANOVA was used to analyse the differences between different types of MVC. A two-way (3 × 2) ANOVA with repeated measures design was employed to analyse differences between different normalising procedures (ISO, CTS, MLS) and contraction types (shortening, lengthening). If significant F-values were found, the post hoc pairwise comparison was performed with the Fisher least significant difference test. Partial eta squared (η_p^2) was calculated to estimate effect sizes associated with main effects of ANOVA. To allow for a more nuanced interpretation of the data, Cohen's d_z effect-sizes were calculated for significant pairwise comparisons. Cohen's d_z was calculated as the ratio of mean difference and standard deviation of differences, which slightly differs from traditional Cohen's *d* calculation in that it is better suited for within-subject, rather than traditional between-subject differences (Becker, 1988; Lakens, 2013; Smith and Beretvas, 2009). One sample t-test was used to assess disparity of relative mean torque compared to the CTS MVC and the predictive model.

5-3 Results

Maximal torque production and the associated RMS EMG

Maximal torque was contraction type dependent ($F_{2.0, 11.7} = 28.8$, $p < 0.001$, $\eta_p^2 = 0.8$; Table 5-1). Post-hoc analysis showed that maximal lengthening torque was greater than any maximal isometric torque value ($p < 0.005$, d_z range = 1.2 – 2.2) or shortening maximal torque ($p = 0.001$, $d_z = 1.3$). Comparison between shortening and isometric MVC values showed a difference to shorter muscle length only ($p = 0.005$; $d_z = 1.3$). Across isometric MVC values, longer length was significantly greater compared to either intermediate ($p = 0.011$, $d_z = 0.7$) or shorter muscle length ($p = 0.001$, $d_z = 1.6$) and intermediate length was greater than shorter length ($p = 0.047$, $d_z = 0.9$). Contraction type had no effect on maximal RMS EMG values ($F_{1.3, 7.7} = 1.3$, $p = 0.306$, $\eta_p^2 = 0.2$).

Table 5-1. Torque and RMS EMG activity during different maximal contractions.

	Isometric			*Anisometric*	
	Shorter	Intermediate	Longer	Shortening	Lengthening
MVC (N·m)	37.7 ± 7.7$^{\#}$	46.4 ± 6.3	51.9 ± 4.3§	49.7 ± 5.7	63.1 ± 8.3*
RMS (mV)	0.49 ± 0.12	0.53 ± 0.13	0.46 ± 0.10	0.49 ± 0.09	0. 49 ± 0.10

MVC = maximal voluntary contraction torque; RMS = root mean square EMG activity. $^{*}p < 0.005$ compared to all others; $^{\S}p < 0.015$ compared to lengthening, intermediate length isometric and shorter length isometric; $^{\#}p < 0.05$ compared to all others.

Predictive model

A predictive model was constructed so that ISO and MLS are presented relative to CTS (Figure 5-2). For shortening contractions, mean torque level during ISO normalization is expected to be very similar compared to CTS (3% difference, Figure 5-2). With MLS normalization, whilst the mean shortening torque level is expected to

be very similar compared to CTS (4% difference; Figure 5-2), during the latter part of the contraction the torque level is smaller than expected, being 12% less at the point of cessation. For lengthening contractions, all predicted percentage torque levels for either ISO or MLS normalization are lower than expected for CTS normalization (Figure 5-2).

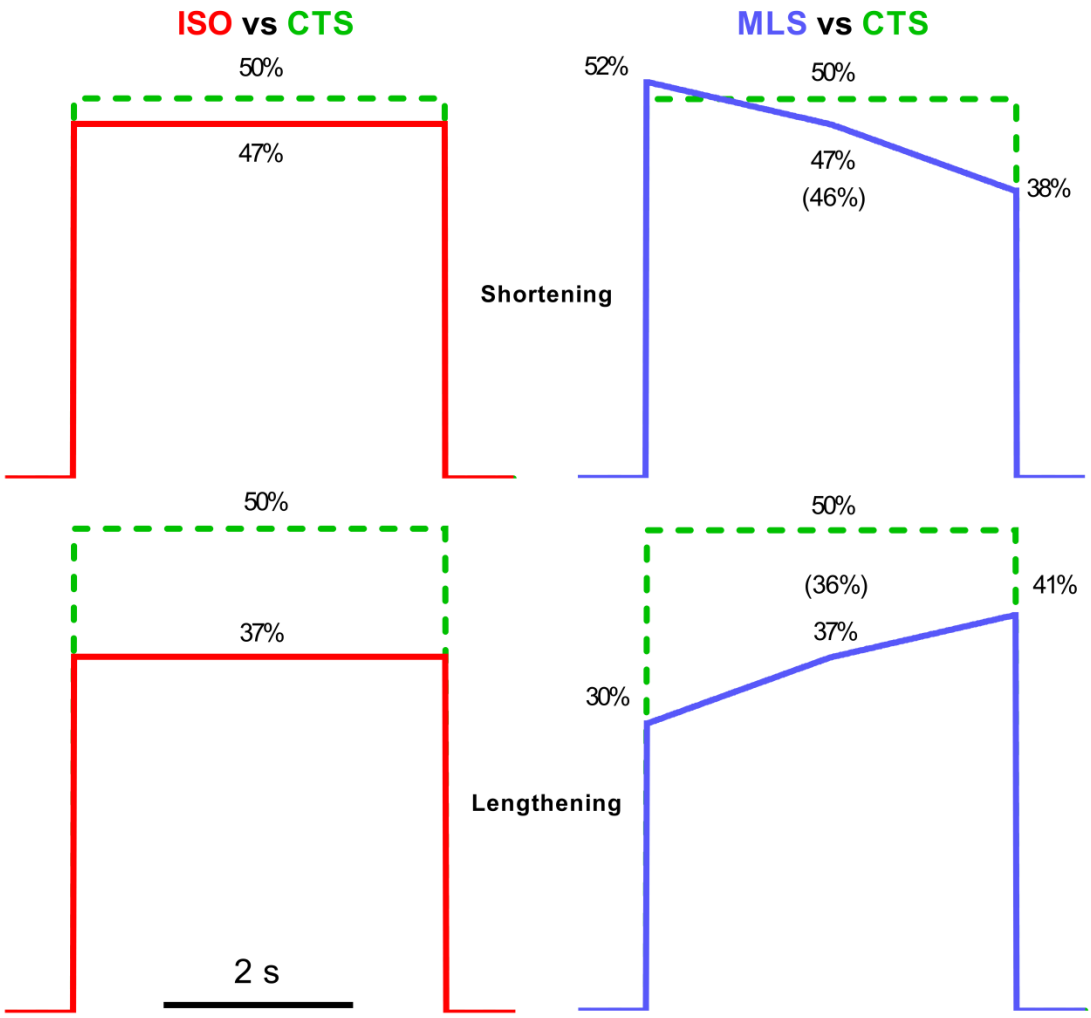

Figure 5-2. Predictive model of submaximal shortening and lengthening contractions based on experimental mean MVC values. Dotted lines represent submaximal contraction for CTS normalisation (green), whereas solid lines represent submaximal contractions for ISO (red) or MLS normalisation (blue). Percentages of torque production, relative to CTS, are depicted in all panels. For A and C, values are shown in the middle as torque production is constant. For MLS, values are presented at the onset, the midpoint and the cessation of the contraction, with mean percentage torque presented in the brackets.

Absolute mean torque and RMS EMG during submaximal contractions

There was a significant normalising procedure × contraction type interaction for mean torque during submaximal contractions ($F_{2, 12}$ = 8.3, p = 0.005, η_p^2 = 0.6). Mean lengthening torque was greater compared to shortening regardless of the normalising procedure employed (p < 0.03 for all, d_z range = 0.6 – 1.7; Figure 5-3 top row). Furthermore, CTS mean lengthening torque was greater compared to ISO (p = 0.003, d_z = 1.4) or MLS (p = 0.018, d_z = 1.0). There was a significant normalising procedure × contraction type interaction for RMS EMG ($F_{2, 12}$ = 4.5, p = 0.035, η_p^2 = 0.4). RMS EMG was greater during shortening compared to lengthening contraction regardless of the normalising procedure employed ($p \leq 0.001$ for all, d_z range = 1.9 – 3.8, Figure 5-3 bottom row). RMS EMG activity was greater during lengthening contraction for CTS compared to ISO (p = 0.s021, d_z = 0.9) or MLS (p = 0.025, d_z = 1.0).

Relative mean torque and the associated torque accuracy

With CTS normalisation, individuals were able to match the expected/predicted 50% torque level for lengthening contractions. However, relative mean shortening torque levels were significantly lower compared to the 50% predicted value (p = 0.002; Figure 5-3A2). Mean percentage torque levels for shortening and lengthening contractions with ISO normalisation were significantly smaller when compared to CTS normalisation values (p = 0.005 and p < 0.001, respectively; Figure 5-3B2), but not different from the predictive values. Relative mean lengthening torque level during MLS normalisation was significantly smaller as compared to CTS normalization (p = 0.011), but significantly greater when compared to the predictive model (p = 0.009; Figure 5-3C2).

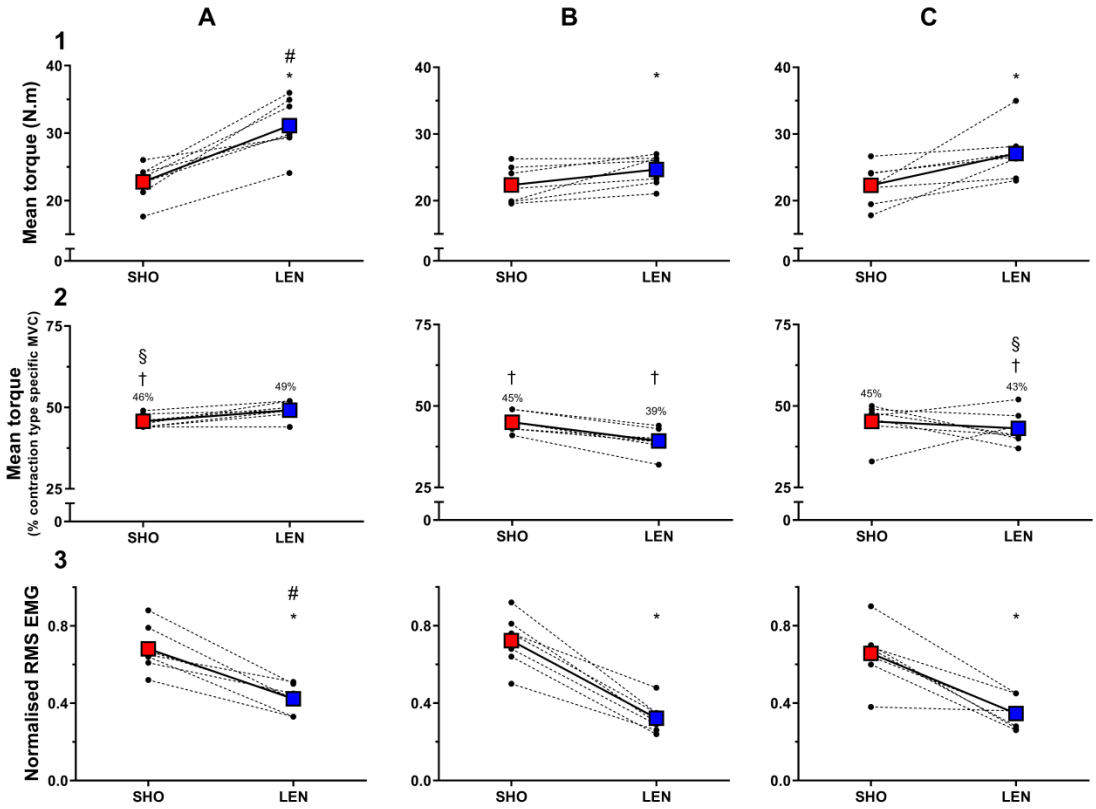

Figure 5-3. Torque and EMG activity during submaximal shortening (SHO) and lengthening (LEN) contractions. CTS, ISO and MLS normalisation relate to columns A, B & C respectively. Row 1 – Mean torque. Row 2 – Mean torque relative to CTS MVC. Row 3 – Mean RMS EMG relative to CTS RMS EMG. $^{*}p < 0.030$ compared to shortening; $^{\#}p \leq 0.025$ compared to when normalised to isometric MVC and muscle length specific isometric MVC; $^{\dagger}p < 0.015$ compared to 50% contraction-type specific maximum; $^{\S}p < 0.010$ compared to the predictive model.

5-4 Discussion

The purpose of this experiment was to present and assess the disparities in mean toque production and associated EMG activity during anisometric submaximal contractions arising from different normalising procedures that have been previously used in the literature. The main finding of the present experiment is that normalising to ISO and MLS is not an accurate approach when performing submaximal *lengthening* contractions since these two types of normalisation were characterised by significant discrepancy relative to CTS. However, the method of normalisation is less relevant

during submaximal *shortening* contractions due to the difference relative to CTS normalisation being small.

Maximal dorsiflexion lengthening torque was found to be ~27 and ~36% greater compared to shortening and isometric at intermediate length, respectively, a finding comparable to previous work (Klass et al., 2007; Reeves and Narici, 2003). As a result, with ISO normalization, submaximal lengthening torque was predicted to be 13% lower compared to CTS normalisation, which was supported by our experimental findings where the mean observed difference was 11%. Thus, submaximal lengthening contractions with ISO normalisation cannot be construed as an accurate submaximal representation of this specific contraction type when comparing contraction types. From a perspective of mechanical output, normalising different anisometric contractions to a constant value, as is the case with ISO normalisation, is similar to lifting and lowering an arbitrary absolute load. For such a task, the associated conceptual methodological issues have been highlighted previously (Duchateau and Enoka, 2016). Specifically, due to aforementioned greater capacity to produce torque during lengthening contractions, less motor unit activity is needed to produce the same absolute torque. This is supported in the present experiment by lower EMG activity observed during submaximal lengthening contractions with ISO normalization, compared to CTS. This finding could have a significant confounding effect when neural behaviour of lengthening contractions is assessed via stimulation techniques, such that with ISO and MLS normalisation, lengthening contractions at a given percentage of maximum will be at a different level of the contraction type-stimulus response curve relative to shortening and isometric contractions (Capaday and Stein, 1987; Goodall et al., 2009; Matthews, 1986; Oya et al., 2008; Weavil et al., 2015). Furthermore, submaximal lengthening contractions derived from ISO or MLS

normalisation might exhibit lower and higher standard deviation and coefficient variation of force, respectively (Enoka et al., 2003), resulting in inaccurate assessment of steadiness.

Due to the similarity in peak torque obtained during shortening and intermediate length isometric contractions, the difference between CTS and ISO normalisation procedures is less profound. Indeed, based on the predictive model this difference is 3% and experimental data supports this, with ~1% difference. Therefore, if investigations are only concerned with submaximal shortening contractions, normalisation can be performed to CTS MVC or isometric MVC at either intermediate or longer muscle lengths. The latter is valid as peak torque during anisometric contractions usually occurs at longer muscle lengths as per torque-angle relationship (Billot et al., 2011).

In an attempt to assess a similar change in torque during anisometric contractions, some researchers have normalised submaximal contractions to MLS maximal isometric contraction (Duclay et al., 2014; Pasquet et al., 2006). Theoretically, this should allow torque production to match the torque-angle curve of the muscle. However, this procedure does not appear to give a valid submaximal representative of a given anisometric contraction type for several reasons. Firstly, the capacity of a muscle to produce torque throughout ROM is unlikely to be linear. This is highlighted in the torque trace presented in our theoretical model, using a 3-point target normalisation rather than a 2-point one used previously (Duclay et al., 2014; Pasquet et al., 2006). Secondly, even though the first half of the contraction, and the mean torque production during concentric MLS appear to be close to CTS, the latter part of the contraction will eventually result in 12% smaller torque level, as per the predictive model (Figure 5-2). However, if submaximal torque production is assessed in the first

portion of the contraction or stimulations are performed at anatomical zero (e.g. Duclay et al., 2014), then MLS normalisation could still be considered accurate during *shortening* contractions. Thirdly, during lengthening contractions, the starting and finishing point of a contraction were predicted to be 20 and 9% smaller, respectively, compared to CTS, resulting in 14% smaller mean torque production throughout ROM. Our experimental findings showed that maximal torque during longer and shorter muscle length fell significantly short of maximal torque produced during lengthening and shortening contractions in the present study. This was then reflected in submaximal mean torque production and the associated EMG activity insofar as mean lengthening torque was significantly smaller compared to CTS, but still greater than predicted with our theoretical model, possibly due to overshooting the target torque (see below). Furthermore, RMS EMG activity during MLS was significantly smaller, compared to submaximal lengthening contraction using CTS. Theoretically, basing MLS normalisation on each instant of ROM should allow a complete match of the torque-angle curve, thereby making it more representative of a specific contraction type throughout the whole ROM. However, such a task might require a great degree of learning to follow a significant curvilinear torque profile rather than a steady torque level, potentially rendering it less practical. Since the aim of this experiment was to compare existing normalisation procedures used in the literature directly, a procedure whereby normalisation is performed at each instant of ROM was not investigated, but future studies should assess its effect and practicality.

In theory, when ISO and MLS normalisation are performed, mean torque during shortening and lengthening contractions should be similar, which is not accurate relative to their respective maximums due to greater torque capacity during lengthening contractions. However, it was shown that mean torque production is

greater during lengthening contraction during these two procedures, which likely stems from impaired torque accuracy. Indeed, lengthening contractions during MLS were characterised by significant overshooting of the target torque (7% greater mean torque production than predicted), which is reportedly a feature of lengthening contractions (Hortobágyi et al., 2001b). During MLS, impaired torque accuracy is likely to be even more apparent due to the target not being a constant line.

Whilst CTS normalisation appears to be the most ecologically valid normalising procedure, it is not without its limitations. Because the capacity of muscle torque is dependent on muscle length, maximal shortening and lengthening contractions are characterised by descending and ascending torque profiles, respectively. When submaximal contractions are performed, and a constant target torque is set, it is based on the peak value achieved during maximal contractions. This results in increasing and decreasing effort required to produce a given torque level throughout ROM during submaximal shortening and lengthening contractions, respectively. Differences in effort could potentially influence the associated EMG activity as when the muscle is at a shorter length greater neural drive is required to maintain the same absolute torque output. Regardless, this could be considered a representative of a real-world scenario. For example, when dorsiflexors are used to control foot drop during heel strike (Byrne et al., 2007), muscle length varies but the force produced needs to be relatively constant to prevent tripping.

5-5 Conclusion

The findings of the present experiment suggest that normalising to ISO and MLS is not an accurate approach for assessment and prescription of submaximal *lengthening* contractions based on the predictive model and the experimental data showing both

ISO and MLS normalisation resulted in significantly lower mean torque during submaximal lengthening contractions. As such, future research, particularly in the area of assessment of neural behaviour of submaximal lengthening contractions should carefully consider the appropriate normalising procedure, with CTS likely being the most accurate approach. For assessment and prescription of submaximal *shortening* contractions, normalisation to CTS MVC or isometric MVC at either intermediate or longer muscle length may be accurate and used interchangeably. Given the findings of this experiment, the CTS normalising procedure will be used for remainder of this thesis where appropriate.

PART 2: MUSCLE FASCICLE BEHAVIOUR DURING SUBMAXIMAL SHORTENING AND LENGTHENING CONTRACTIONS

5-6 Introduction

When shortening and lengthening contractions are performed, it is generally assumed that the MTU behaves congruently with changes in joint angle (Nordlund et al., 2002; Pinniger et al., 2001). However, this might be an overly simplistic view since the mechanical properties of MTU, specifically the interaction between the muscle and the tendon, depend on both the produced forces and compliance of the series elastic component (Reeves and Narici, 2003). Indeed, in cat gastrocnemius during lengthening of a muscle-tendon unit, the fascicles have been shown to be isometric or even shorten (Griffiths, 1991). Similar behaviour might also be observed in human in vivo torque production during lengthening contractions, particularly at the onset of contraction (Hahn, 2018). In such a case, the neural behaviour during isometric and anisometric contractions might not be distinct. Thus, establishing the behaviour of contractile elements of MTU during anisometric contractions is important before examining neurophysiological responses during different contraction types.

Movement velocity has also been shown to have a profound effect on H-reflex behaviour during muscle lengthening (Duclay et al., 2009; Grosprêtre et al., 2014; Nordlund et al., 2002; Romanò and Schieppati, 1987). This might be related to the greater firing of muscle spindle afferents at higher angular velocities. Given that muscle spindles lie in parallel to muscle fibres, the change in fascicle length may be a more appropriate measure of the effect of proprioceptive feedback to the central nervous system (Morgan et al., 2000). This makes it important to establish the mechanical behaviour of TA, particularly if slow movement velocity is employed like in the experiments of the present thesis. Therefore, the aim of this experiment was to assess the muscle fascicle behaviour during submaximal isometric, shortening and lengthening contractions at the angular velocity used in experiments in this Chapter.

5-7 Methods

Participants

8 young, healthy volunteers took part in the experiment (2 females; 27 ± 4 years old, 177 ± 7 cm, 79 ± 14 kg).

Experimental design

After initial warm-up consisting of submaximal isometric contractions at 50% of individual's perceived MVC, participants were instructed to perform MVC during isometric, shortening and lengthening contractions at $5°.s^{-1}$. The greatest instantaneous torque values obtained were used for normalisation of submaximal target torque levels as per the findings of Part 1 of this chapter. After that, participants were required to perform constant torque during isometric, shortening and lengthening contractions at 50% of contraction-type specific MVC during which longitudinal ultrasound images were recorded.

Experimental setup

Contractions were performed with participants sat in an isokinetic dynamometer. For a detailed procedure see section 3-3.2.

Ultrasonography

Fascicle lengthening was assessed using ultrasonography. For more details on the methodology, see section 3-3.6.

Data analysis

Fascicles were analysed every 5˚ of ankle angle throughout the 20˚ range-of-motion during shortening and lengthening contractions, and every second during isometric contractions corresponding to the timing of every 5º change during dynamic contractions. For the remainder of analytical procedures, see section 3-4.4.

Statistical analysis

All data are presented as means ± SD. Normality of data was assessed using Shapiro-Wilks test. Sphericity was assessed using Mauchly's test of sphericity. In the case of violation, a Greenhouse-Geisser correction was employed. A two-way ANOVA was used to assess differences in fascicle length during shortening and lengthening contractions (2 × direction – shortening and lengthening; 5 × angle). A one-way repeated measures ANOVA was used to investigate the differences in fascicle length across contraction types at anatomical zero and the potential differences in fascicles length during 4-second isometric contractions. If significant F-values were found, analyses were continued using pairwise comparison with Bonferroni correction. All analyses were performed using SPSS (v20, SPSS Inc., Chicago, IL, USA).

5-8 Results

Fascicles did not vary in length during isometric contractions ($F_{2.3, 16.0} = 2.7$, $p = 0.090$; Figure 5-4). However, fascicle length changed linearly with changes in joint angle during shortening and lengthening contractions ($F_{1.3, 9.1} = 94.2$, $p < 0.001$), with no differences in the slope of fascicle length changes between contraction types ($F_{4, 28} = 2.2$, $p = 0.091$). Fascicle length decreased by 36% during shortening contractions (from 48.1 ± 3.8 to 35.2 ± 3.9 mm) and increased by 34% during lengthening contractions (from 35.4 ± 3.5 mm to 47.4 ± 3.0; Figure 5-4). At anatomical zero, where

stimulations were performed in other experiments throughout the thesis, fascicle length was similar during isometric (41.1 ± 3.9 mm), shortening (40.3 ± 3.7 mm) and lengthening (41.1 ± 3.6 mm) contractions ($F_{2, 14} = 1.3$, $p = 0.292$).

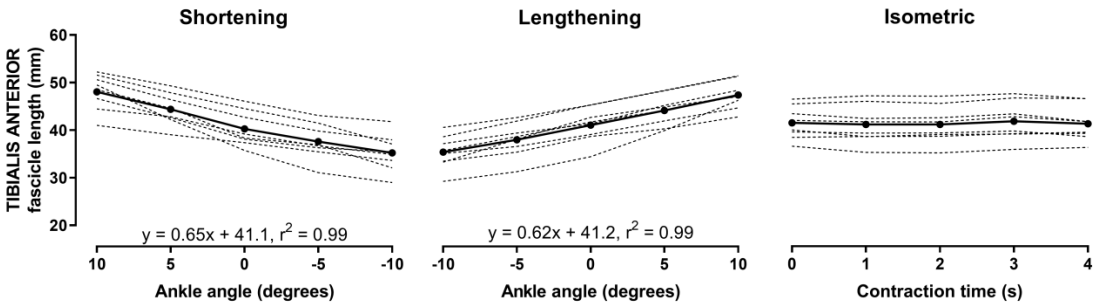

Figure 5-4. Tibialis anterior fascicle length (mm) with changes in the ankle joint angle during shortening (left panel) and lengthening (centre panel), and isometric (right panel) contractions at 50% of contraction type specific MVC. Fascicle length was assessed every 5^0 throughout the ROM for shortening and lengthening contractions and are displayed on the x-axes relative to anatomical zero (ankle at 90^0). Fascicles changed linearly with changes in joint angle as noted on plots. For isometric contractions, fascicle length was assessed every second of the contraction, corresponding to the timing of every 5^0 change during the dynamic contractions. Full lines represent the sample mean, whilst dashed lines denote individual responses.

5-9 Discussion

The results of the present experiment show that the contractile elements of the muscle behave in a distinct manner despite the slow velocity. During submaximal shortening and lengthening contractions, fascicle length decreased and increased, respectively, whilst it remained unchanged during submaximal isometric contraction. The fascicles exhibited a linear change during anisometric contractions which is in agreement with Pasquet *et al.* (2006), despite the potential variations in moment arm of the dorsiflexors during ankle movement (Maganaris et al., 1999). Importantly, the rate of fascicle length changes was similar between shortening and lengthening contractions and the fascicle length was analogous across contraction types at anatomical zero.

These findings have important implications for examining neural responses between contraction types. Firstly, since the behaviour of contractile elements of the muscle during different contraction is orthodoxical and distinct, modulation of neural

behaviour or lack of it across contraction types can be attributed to the specific contraction type. Secondly, given similar muscle length at the point of stimulus, a high degree of confidence can be ascertained that neurophysiological responses are elicited at similar muscle length. Thirdly, despite the slow velocity of movement, the fascicles did not contract quasi-isometrically, but changed in length as would be expected with higher velocities, suggesting the degree of proprioceptive feedback (Morgan et al., 2000) differed between contraction types.

5-10 Conclusion

Fascicles behave orthodoxically with changes in joint angle during submaximal contractions; shortening and lengthening during shortening and lengthening contractions, respectively, and not changing length during isometric contractions. The contractile elements of the muscle during different contraction types at $5^{\circ}.s^{-1}$ behave in a distinct manner, suggesting differences in proprioceptive feedback that might influence the behaviour of descending tracts, which will be explored in the context of aging in the next part of this Chapter.

PART 3: CORTICOSPINAL AND SPINAL MODULATION DURING SUBMAXIMAL SHORTENING, LENGTHENING AND ISOMETRIC CONTRACTIONS IN YOUNG AND OLDER ADULTS

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5-11 Introduction

The aging CNS is characterised by physiological and functional decline (Hunter et al., 2016b; McNeil and Rice, 2018; Power et al., 2013) that can lead to an impaired ability to perform daily activities and a reduced quality of life. For example, older adults often exhibit a decline in MVC force. However, the degree of strength loss might vary across different muscle groups (Doherty, 2003) and contraction type, with older adults typically displaying greater preservation of lengthening contraction strength (Power et al., 2015). Additionally, even when older adults might not present a deficit in the generation of maximal force, underlying neural changes associated with aging could still be present (McNeil et al., 2005). Force control appears to be a bigger challenge for the elderly during low (≤10% MVC) or higher (>50% MVC) intensity isometric contractions (Graves et al., 2000; Tracy and Enoka, 2002) and during shortening and lengthening compared to isometric contractions (Laidlaw et al., 2002, 2000). Furthermore, older adults exhibit a decline in force accuracy with a particular tendency to exceed the target force during lengthening contractions (Hortobágyi et al., 2001b). Whilst there are a number of age-related alterations within the muscular system (Doherty, 2003), it has been postulated that the origin of performance variability with aging is due to CNS mechanisms (Hunter et al., 2016b).

Age-related alterations in the properties of motor units have received a great deal of attention in the literature (Hunter et al., 2016b; McNeil and Rice, 2018), whereas data on the modulation of corticospinal excitability with aging is comparatively understudied. The corticospinal pathway is the main descending pathway for control of voluntary movement (Lemon, 2008), and could be impeded with aging due to the atrophy of cortical neurons (Henderson et al., 1980; Ward, 2006b) and reduction in white (Marner et al., 2003) and grey matter volume (McGinnis et al., 2011; Salat et

al., 2004), as well as instability of neuromuscular transmission (Power et al., 2016a). However, the indices of corticospinal excitability, such as the size of MEPs and the slope of input-output relationship of TMS have been shown to be similar (Hassanlouei et al., 2017; Smith et al., 2011b; Stevens-Lapsley et al., 2013) or reduced (Oliviero et al., 2006; Pitcher et al., 2003; Sale and Semmler, 2005) in older compared to young adults. These discrepancies have been suggested to be multifactorial (Hassanlouei et al., 2017), ranging from biological sex, contractile state (relaxed vs. active) and the muscle investigated (upper vs. lower limb).

The differences in corticospinal responses as a function of age have mainly been studied in upper limb muscles and might not translate well to lower limb, locomotor muscles due to differences in intracortical circuits and corticospinal projections (Brouwer and Ashby, 1990; Chen et al., 1998). With rare exceptions (e.g. Hassanlouei et al., 2017), studies have not considered locomotor muscles. Furthermore, responses have largely been explored during rest or relatively low levels of muscle activity (10% MVC; Hassanlouei et al., 2017). Lastly, investigations have focused mainly on age-related changes in corticospinal excitability during isometric contractions. As highlighted recently, isometric actions are likely to be the least functionally relevant and thus assessment of the behaviour of the corticospinal output is required during dynamic contractions in an elderly population (McNeil and Rice, 2018). Not solely relying on data examining corticospinal responses with aging in an isometric state is further supported by observations of differences in cortical sensorimotor integration between young and older adults (Chapter 4), and reduced corticospinal and spinal responses during lengthening compared to isometric contractions in younger individuals (Duclay et al., 2014, 2011; Duclay and Martin, 2005; Gruber et al., 2009). Furthermore, given that the decrements in force control with aging are likely to be

most prominent during dynamic contractions (Burnett et al., 2000; Christou et al., 2001; Laidlaw et al., 2000), it is important to elucidate whether the age-related changes in corticospinal responses are contraction-type dependent, in order to understand functional changes with aging.

It should be noted that any change in MEP size may be due to changes in the activity of cortical neurons or spinal motoneurons. Previously observed trends of reduced corticospinal excitability in older adults might, therefore, be related solely to altered activity of spinal motoneurons (Stevens-Lapsley et al., 2013). Notwithstanding its limitations (Nielsen et al., 1999), the Hoffmann (H) reflex has often been probed to investigate the spinal contribution to the overall response of the corticospinal pathway, however, in relation to aging this approach has only been applied during standing, i.e. isometric conditions (Baudry et al., 2014), and not during dynamic contractions. Based on the age-related reduction in the H-reflex, but not maximal muscle response (M_{max}) latency, it has been postulated that the age-related decrease in conduction velocity and/or synaptic transmission efficacy is more prominent for sensory afferent axons compared to the efferent axons (Scaglioni et al., 2003). However, further investigation into the changes in conduction efficacy of sensory and motor axons in aging adults is warranted, employing more robust measures, such as calculation of conduction times (Udupa and Chen, 2013).

The purpose of this study was to assess the interplay between neuromuscular performance, the associated variability and differences in corticospinal and spinal responses during submaximal contractions of different types (isometric, shortening and lengthening) between younger and older adults. It was hypothesized that corticospinal (MEP amplitude) and spinal (H-reflex amplitude) responses will be reduced during dynamic contractions, conduction times of motor and sensory

pathways will be prolonged and torque variability during dynamic contractions increased in older compared to young adults.

5-12 Methods

Participants

A total of 29 participants took part in the experiment and were split into two groups, younger (n = 15, 7 females; 26 ± 4 years old, 175 ± 6 cm, 77.5 ± 12.8 kg) and older (n = 14, 5 females; 64 ± 3 years old, 173 ± 13 cm, 75.6 ± 16.1 kg). For detailed outline of exclusion and inclusion criteria, see section 3-3.1.

Study design

The study was designed to compare corticospinal and spinal excitability during submaximal isometric, shortening and lengthening dorsiflexion between young and older adults. Participants visited the laboratory on 3 occasions, with a minimum of 48 h and a maximum of a week between sessions, at a similar time of day to limit diurnal variations (Tamm et al., 2009). The three visits included: A) familiarization, B) assessment of corticospinal excitability, and C) assessment of spinal excitability. The order of the second and third visit was pseudorandomized and counterbalanced.

Procedures

Dynamometer setup and torque recordings

For a detailed procedure of dynamometry and torque recordings see section 3-3.2.

Electromyography

The EMG activity was recorded from TA. For a detailed procedure of EMG recordings see section 3-3.3. Additionally, to monitor antagonist muscle activity electrodes were placed over SOL at two-thirds of the line between the medial condylis of the femur to the medial malleolus (Hermens et al., 2000).

Percutaneous nerve and transcranial magnetic stimulation

Percutaneous stimulation of the peroneal nerve was performed during a weak contraction of 10% isometric MVC since H-reflex in TA is difficult to evoke at rest as reported previously (Roy and Gorassini, 2008). For a detailed stimulation procedure please see section 3-3.4. M_{max} (current intensity: 54.4 ± 23.3 mA) was elicited at 10, 25 and 50% of MVC, ensuring that the responses corresponded to the torque level at the point of stimulation. To minimise the potential for inducing fatigue, M_{max} was elicited only in the isometric state at different contraction levels since M_{max} has been shown to be dependent on position (Gerilovsky et al., 1989) and contraction intensity (Lee and Carroll, 2005), but not on contraction type when elicited at the same joint angle (Tallent et al., 2013). Similarly, the H-reflex was elicited at 10, 25 and 50% of MVC. The stimulus intensity used to elicit the H-reflex produced an M-wave amplitude of 15 – 20% of contraction level specific M_{max} (Crone and Nielsen, 1989).

Single pulse TMS was performed to elicit responses in TA. For a detailed procedure see section 3-3.5. Hotspot and active MT (AMT) were determined during a weak contraction of 10% isometric MVC. As determining AMT at the contraction intensities employed in the experiment (25 and 50% MVC) would have required a high number of contractions at both intensities, it was chosen to select a lower contraction intensity to determine AMT in order to avoid the occurrence of decrements in muscle functions

as a result of performing multiple contractions at higher intensities. Furthermore, standardising the intensity of stimulation at a lower isometric contraction intensity allowed for investigation of MEP modulation with increased contraction intensity, which has been shown to be similar regardless of contraction type (Škarabot et al., 2018). During the experiment, TMS was standardised to 1.2 × AMT (stimulus output: 39 ± 10%) as it lies on the middle portion of the ascending part of the stimulus-response curve (Han et al., 2001) and is thus sensitive to changes in corticospinal excitability.

As evoked responses are sensitive to changes in muscle length (Gerilovsky et al., 1989; Lewis et al., 2001), all forms of stimulation were applied when the ankle joint was at, or passed through, anatomical zero (90°). To match the timing of stimulation, during isometric contractions stimuli were delivered at the 2-second point of a 4-second contraction.

Experimental protocol

A) familiarization

During the initial visit, participants were familiarised with the contraction tasks and stimulation techniques, i.e. PNS and TMS. This included receiving PNS at 10, 25 and 50% of isometric MVC to determine M_{max}. Familiarisation with the contraction task involved a minimum of 10 contractions performed for each contraction type (isometric, shortening and lengthening) and intensity (25% and 50% of contraction-type specific MVC), since it has been suggested that no further within- or between-day improvements in torque variability are noted after 10 trials (Hortobágyi et al., 2001b).

B) assessment of corticospinal excitability

Following a brief warm up involving individually estimated 50% submaximal isometric contractions, the session started with the determination of isometric MVC, followed by obtaining M_{max} at 25 and 50% of isometric MVC. Next, the TMS hotspot and AMT were determined whilst the participant maintained a contraction at 10% of isometric MVC. This was followed by the determination of shortening or lengthening MVC in a randomized order. Thereafter, participants performed isometric, shortening and lengthening contractions at 25 and 50% of contraction-type specific MVC in a randomised order and MEPs were recorded at each contraction type and intensity. Subsequently, the remaining contraction type was performed using the same protocol as described in the previous point. Four successful trials were recorded per contraction type and intensity and used for analysis. This number of trials was chosen as it has been shown to be the minimum required for adequate repeatability and validity of TMS (Lewis et al., 2014), whilst also minimising fatigue and has been used previously in similar experiments (Duclay et al., 2014). A trial was deemed successful if participants produced the target torque for 4 s and matched the target force level at the point of stimulation.

C) assessment of spinal excitability

The protocol for assessing spinal excitability replicated that of part B, but with replacement of TMS hotspot and AMT determination with determination of PNS stimulus intensity required to produce M-wave amplitude of 15-20% M_{max}. This stimulus intensity was then used for recording H-reflexes during the different contraction types and intensities. H-reflexes where the M-wave did not meet the criterion stated above, were discarded. As per the criteria described above, four

successful trials were recorded per contraction type and intensity and used for analysis, which ensured reliability and validity of PNS measures (Mynark, 2005), has been used previously (Duclay et al., 2014; Kallio et al., 2010), and minimised fatigue.

The levels of contraction intensity chosen allowed for comparison with other studies investigating corticospinal modulation during different contraction types (Duclay et al., 2014; Gruber et al., 2009). Furthermore, only lower and medium contraction intensities, and not maximal, were studied since they have greater functional relevance (Stein and Thompson, 2006) and are more likely to be difficult to control in older populations (Hortobágyi et al., 2001b).

With regard to the chosen contraction velocity ($5°\cdot s^{-1}$), pilot testing indicated that higher contraction velocities led to significantly greater torque variability as shown previously (Christou et al., 2003b), resulting in an inability to reliably deliver the stimuli at the desired torque output, particularly in an aging population. Since evoked responses are not only influenced by the stimulus intensity, but also the intensity of contraction (e.g. Škarabot et al., 2018b), higher contraction velocities could have led to differing relative torque levels across individuals, thus confounding comparability. Furthermore, a recent study showing differences in modulation of intracortical networks during shortening and lengthening contractions between young and older adults similarly employed a slow contraction velocity ($4°\cdot s^{-1}$; Opie and Semmler, 2016).

To minimize the decrements in muscle function due to repeated contractions, adequate rest was given where necessary. Following any MVC assessment, as well as between different contraction type sets, a minimum of 5 min rest occurred, whilst for different contraction levels within a contraction set at least 30 s was given. These rest periods have previously been shown to be sufficient to ensure that H-reflex (Howatson et al.,

2011) and MEPs (Balbi et al., 2002) have returned to resting values. The duration of each session was roughly 1.5 h.

Data analysis

Torque variability during submaximal contractions (CV_{torque}) was assessed in the 1-second epoch preceding the stimulus. The analysed time-frame ensured that the acceleration phase at the start of the movement was not included in the analysis. RMS EMG was calculated as per section 3-4.2. Evoked responses were calculated as per section 3-4.3. MEP, H-reflex and M_{max} latencies were calculated from stimulus artefact to initial deflections of the TA EMG from baseline. All latencies were measured from individual trials and subsequently averaged. Peripheral motor conduction time (PMCT) was estimated using the F-wave and M-wave obtained at 50% of isometric MVC using the standard equation: (F latency + M latency – 1)/2. To estimate central motor conduction time (CMCT), PMCT and 0.5 ms (to account for synaptic delay between corticospinal axons and alpha motoneurons) were subtracted from the MEP latency obtained during 50% of isometric MVC. Peripheral sensory conduction time (PSCT) was also estimated at 50% isometric MVC using the equation H-reflex latency – PMCT – 0.5 ms. Since conduction time to lower limbs is confounded by differences in height (Furby et al., 1992; Udupa and Chen, 2013), PMCT, CMCT and PSCT values were additionally normalised to participant height and are thus expressed as $ms \cdot m^{-1}$.

Statistical analyses

All analyses were performed using SPSS package (v20, SPSS Inc., Chicago, IL, USA). Statistical significance was set at an alpha level of 0.05. Normality was assessed using Shapiro-Wilks test. If the data were not normal, transformations were performed using common logarithm for positively skewed data. A 2-way mixed-effect intraclass correlation coefficient ($ICC_{3,1}$) model for absolute agreement (Shrout and Fleiss, 1979) and CV were used to assess between-day reliability and variability, respectively, of maximal torque and M_{max}. An ICC ≥ 0.9 was considered excellent reliability, and an ICC of 0.75-0.9 was classified as good reliability (Koo and Li, 2016). A one-sample T-test was used to assess the differences between the actual and target torque at the point of stimulation. An independent samples T-test was used to assess differences in peripheral and central motor conduction time between young and older adults. Association between torque variability and corticospinal and spinal responses was assessed using Spearman's correlation coefficient. Sphericity was assessed using Mauchly's test of sphericity. In the case of violation, a Greenhouse-Geisser correction was employed. A three-way mixed ANOVA with repeated measures design was employed to analyse differences in corticospinal and spinal responses, RMS EMG activity and CV_{torque} between contraction types, contraction intensity and age group. An additional factor of 'visit' was added to ANOVA to explore differences in MVC and M_{max} across different testing days. When significant F-values were found, the post hoc pairwise comparison was performed with a Bonferroni correction for multiple comparisons. In some cases where the F-values and p-values were similar on both days, the minimum and maximum, respectively, are reported. Data are presented as mean ± SD, unless the data had to be transformed, in which case the geometric mean ± SD are presented.

5-13 Results

Maximal torque and M_{max}

Maximal voluntary torque did not differ between the visits ($F_{1.6, 43.1} = 1.6$, $p = 0.213$) and a good to excellent between-day repeatability (ICCs > 0.8) and low variability (CV < 10%) was established for MVCs (Table 5-2). MVC was contraction type dependent ($F_{1.3, 35.4} = 139.3$, $p < 0.001$) such that isometric and shortening maximal torque were ~24% lower compared to lengthening ($p < 0.001$ for both age groups; Figure 5-5A and 5-5B), with no differences between age groups ($p = 0.158$).

Similarly, M_{max} was not different across visits ($F_{2, 54} = 3.3$, $p = 0.089$), and displayed good to excellent repeatability (ICCs > 0.8) and low variability (CV < 15%; Table 5-2). M_{max} amplitude was contraction intensity dependent ($F_{1.3, 36.3} = 38.6$, $p < 0.001$) such that it increased ~5-8% with greater contraction intensity ($p < 0.001$ for all cases; Figure 5-5C and 5-5D). Whilst a main effect of contraction intensity was found for M_{max} latency ($F_{1.7, 44.6} = 4.1$, $p = 0.031$), pairwise comparison with Bonferroni correction did not reveal any other differences (Figure 5-5E and 5-5F). PMCT was prolonged in older (11.4 ± 0.7 ms·m^{-1}) compared to younger (10.6 ± 0.6 ms·m^{-1}) adults ($t_{27} = -3.2$, $p = 0.003$).

Torque variability during submaximal contractions

CV_{torque} was dependent on contraction type ($F_{2, 54} > 246$, $p < 0.001$) and age ($F_{1, 27} > 23$, $p < 0.001$) and there was a significant contraction type × age interaction ($F_{2, 54} > 5$, $p < 0.007$). Older compared to young individuals had greater CV_{torque} during shortening and lengthening contractions ($p < 0.001$ for both; Table 5-3). In older adults, CV_{torque} was ~5-fold greater during shortening compared to isometric ($p < 0.001$) and ~1.5-fold compared to lengthening ($p = 0.001$), as well as ~3.5-fold greater

during lengthening compared to isometric contractions ($p < 0.001$). In young individuals however, CV_{torque} was ~3-fold greater during shortening and lengthening compared to isometric contractions ($p < 0.001$ for both). No association was shown between CV_{torque} and any responses to stimulation techniques (p value range = 0.067 – 0.223, r = 0.499 – 0.600).

Table 5-2. Maximal voluntary torque and maximal muscle response (mean ± SD), between-day repeatability ($ICC_{3,1}$ with 95% confidence intervals) and variability (coefficient of variation – CV%) across the three visits (familiarisation, TMS and PNS).

			ALL	YOUNG	OLDER
MVC Torque (N.m)	ISO	Fam	40.9 ± 9.9	43.3 ± 7.8	38.4 ± 11.5
		TMS	42.1 ± 10.8	46.0 ± 10.1	38.7 ± 12.5
		PNS	41.8 ± 11.7	45.4 ± 8.1	37.4 ± 11.9
		$ICC_{3,1}$	0.90 (0.82 - 0.95)	0.77 (0.55 - 0.91)	0.96 (0.91 - 0.99)
		CV (%)	6.8 ± 4.6	7.8 ± 4.8	5.6 ± 4.2
	SHO	Fam	40.8 ± 9.7	43.5 ± 9.4	37.9 ± 9.6
		TMS	41.5 ± 10.3	43.6 ± 10.1	39.2 ± 10.5
		PNS	41.6 ± 11.1	44.6 ± 10.5	38.4 ± 11.2
		$ICC_{3,1}$	0.94 (0.90 - 0.97)	0.91 (0.81 - 0.97)	0.97 (0.93 - 0.99)
		CV (%)	5.1 ± 2.9	5.8 ± 3.2	4.5 ± 2.6
	LEN	Fam	53.4 ± 12.8	55.6 ± 11.9	51.0 ± 13.7
		TMS	54.8 ± 13.6	57.4 ± 13.6	52.0 ± 13.4
		PNS	5.7 ± 1.8	57.7 ± 14.8	51.3 ± 13.9
		$ICC_{3,1}$	0.84 (0.73 - 0.91)	0.90 (0.79 - 0.96)	0.97 (0.94 - 0.99)
		CV (%)	13.0 ± 8.2	6.3 ± 3.5	4.1 ± 1.9
Mmax Amplitude (mV)	10% ISO MVC	Fam	5.5 ± 2.0	5.7 ± 2.2	5.2 ± 1.9
		TMS	5.8 ± 1.8	6.0 ± 1.7	5.4 ± 1.9
		PNS	5.7 ± 1.8	6.2 ± 1.9	5.4 ± 1.7
		$ICC_{3,1}$	0.84 (0.73 - 0.91)	0.83 (0.66 - 0.93)	0.85 (0.67 - 0.94)
		CV (%)	13.0 ± 8.2	13.4 ± 9.6	12.7 ± 6.7
	25% ISO MVC	Fam	5.8 ± 2.0	6.2 ± 2.0	5.4 ± 1.9
		TMS	6.0 ± 1.8	6.4 ± 1.9	5.7 ± 1.8
		PNS	6.2 ± 1.7	6.6 ± 1.7	5.8 ± 1.8
		$ICC_{3,1}$	0.89 (0.80 - 0.94)	0.91 (0.80 - 0.97)	0.86 (0.70 - 0.95)
		CV (%)	10.4 ± 6.8	9.3 ± 6.2	11.5 ± 7.5
	50 % ISO MVC	Fam	6.0 ± 1.9	6.3 ± 1.8	5.7 ± 2.0
		TMS	6.5 ± 1.8	6.6 ± 1.9	6.1 ± 1.7
		PNS	6.4 ± 1.7	6.6 ± 1.8	6.3 ± 1.8
		$ICC_{3,1}$	0.89 (0.82 - 0.95)	0.93 (0.84 - 0.97)	0.87 (0.72 - 0.95)
		CV (%)	8.4 ± 6.3	6.8 ± 4.4	10.1 ± 7.6

ISO = isometric, SHO = shortening, LEN = lengthening, fam = familiarisation, TMS

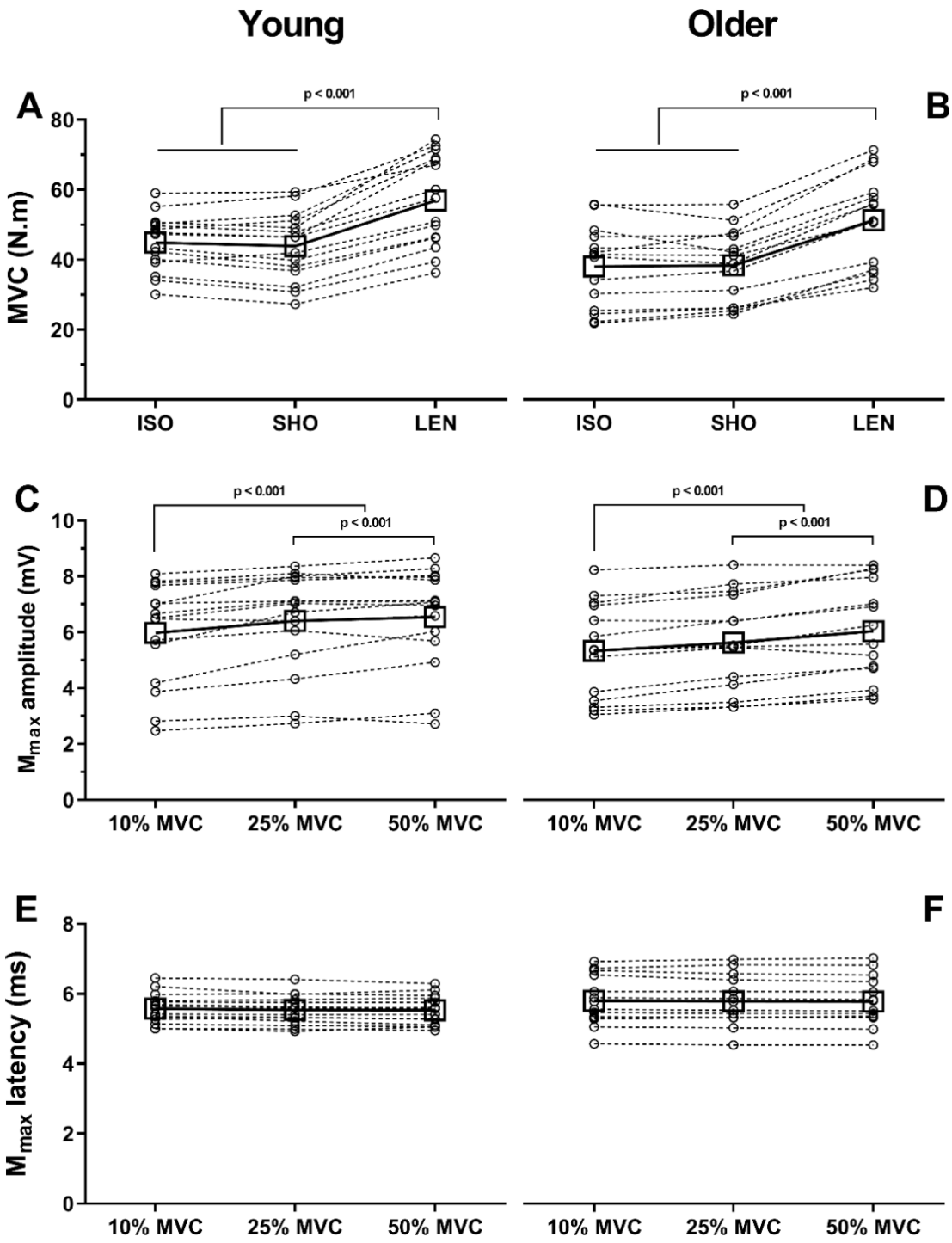

Figure 5-5. MVC torque and M_{max}. Maximum voluntary contraction torque during different contraction types (ISO – isometric, SHO – shortening, LEN – lengthening; A and B), maximal compound action potential amplitude (C and D) and maximal compound action potential latencies at different contraction intensities (E and F) in young (left panel) and older (right panel) individuals. Open circles denote individual response and open squares denote the age group mean.

Torque at stimulation and voluntary background EMG

On average, participants matched the target torque level at the point of stimulation during isometric and shortening contractions, but had a tendency to overshoot the target torque during lengthening contractions (Table 5-3).

The RMS/M_{max} during both experimental visits increased with contraction intensity ($F_{1, 27} > 166$, $p < 0.001$) and age ($F_{1, 27} > 11$, $p < 0.005$), and was modulated with contraction type ($F_{2, 54} > 14$, $p < 0.001$). Significant contraction type × age ($F_{2, 54} > 4$, $p < 0.001$) and contraction intensity × age interactions ($F_{2, 54} > 11$, $p < 0.005$) were noted. *Post hoc* testing revealed that in young individuals greater RMS/M_{max} was displayed during shortening compared to isometric ($p < 0.001$) and lengthening ($p < 0.015$) contraction, whilst in older individuals greater RMS/M_{max} was observed during shortening ($p < 0.001$) and lengthening ($p = 0.003$) compared to isometric contractions (Table 5-3).

The RMS activity of soleus during both experimental visits increased with contraction intensity ($F_{1, 27} > 20$, $p < 0.001$) and was dependent on contraction type ($F_{2, 54} > 16$, $p < 0.001$), but not on age ($F_{1, 27} > 0.2$, $p > 0.387$). There was a significant contraction type × age interaction ($F_{2, 54} > 7$, $p < 0.005$). In younger individuals antagonist RMS activity was greater during shortening compared to isometric contractions ($p = 0.001$), whilst in older individuals, antagonist RMS activity was greater during shortening ($p = 0.004$) and lengthening ($p < 0.001$) contractions compared to isometric (Table 5-3).

Table 5-3. Effect of contraction type (ISO – isometric, SHO – shortening and LEN – lengthening), intensity (25% and 50% of contraction type specific maximum) and age on voluntary torque, dorsiflexor EMG activity normalised to maximal muscle response (TA RMS) and EMG activity of the antagonist (SOL RMS), responses to percutaneous nerve stimulation (PNS; top half of table) and responses to transcranial magnetic stimulation (TMS; bottom half of table).

		25%			50%		
		ISO	SHO	LEN	ISO	SHO	LEN
CV_{torque} (%)	Young	1.8 ± 1.1‡	4.7 ± 1.8	4.3 ± 1.6	1.5 ± 0.5‡	4.7 ± 1.1	4.1 ± 1.0
	Older	2.2 ± 1.6‡	9.1 ± 2.3*#	7.7 ± 3.1*	1.7 ± 0.6‡	9.3 ± 2.4*#	8.6 ± 7.4*
Torque (% MVC)	Young	25.0 ± 0.7	24.6 ± 1.4	28.3 ± 2.7§	49.5 ± 1.0	48.0 ± 4.0	50.9 ± 7.0
	Older	25.2 ± 1.1	24.0 ± 2.0	30.4 ± 3.2§	49.6 ± 1.0	47.3 ± 2.3§	53.2 ± 3.9§
TA RMS (/ M_{max})	Young	0.014 ± 0.004	0.018 ± 0.005†	0.013 ± 0.003	0.024 ± 0.004	0.031 ± 0.006†	0.025 ± 0.008
	Older*	0.018 ± 0.005‡	0.025 ± 0.006	0.023 ± 0.008	0.029 ± 0.007‡	0.038 ± 0.011	0.035 ± 0.010
SOL RMS (mV)	Young	0.007 ± 0.001	0.009 ± 0.002$	0.008 ± 0.002	0.012 ± 0.003	0.015 ± 0.004$	0.012 ± 0.003
	Older	0.008 ± 0.002	0.010 ± 0.002$	0.010 ± 0.003$	0.011 ± 0.003	0.014 ± 0.004$	0.015 ± 0.004$
H latency (ms)	Young	33.4 ± 1.9	33.5 ± 1.8	33.4 ± 2.3	33.4 ± 1.6	33.1 ± 1.7	33.3 ± 2.2
	Older	35.0 ± 3.4	35.7 ± 3.3	34.9 ± 3.7	34.9 ± 3.7	36.1 ± 3.8	35.4 ± 3.5
M_H/M_{max}	Young	0.19 ± 0.02	0.18 ± 0.03	0.17 ± 0.02	0.19 ± 0.03	0.18 ± 0.05	0.18 ± 0.02
	Older	0.18 ± 0.01	0.17 ± 0.02	0.15 ± 0.05	0.18 ± 0.01	0.19 ± 0.02	0.18 ± 0.02
CV_{torque} (%)	Young	1.6 ± 0.5‡	4.8 ± 1.2	4.3 ± 1.3	1.3 ± 0.4‡	5.0 ± 1.0	3.7 ± 0.7
	Older	2.2 ± 1.2‡	11.1 ± 3.4*#	7.7 ± 3.7*	1.7 ± 0.7‡	9.2 ± 5.1*#	6.1 ± 3.3*
Torque (% MVC)	Young	25.1 ± 1.0	24.4 ± 1.9	28.1 ± 2.8§	49.7 ± 0.9	49.9 ± 3.2	46.6 ± 3.4§
	Older	25.1 ± 1.1	23.9 ± 2.0	30.2 ± 2.2§	49.5 ± 1.6	53.3 ± 2.9§	52.4 ± 3.1§
TA RMS (/ M_{max})	Young	0.013 ± 0.004	0.017 ± 0.005†	0.013 ± 0.003	0.024 ± 0.006	0.028 ± 0.007†	0.025 ± 0.006
	Older*	0.019 ± 0.006‡	0.027 ± 0.015	0.026 ± 0.012	0.030 ± 0.011‡	0.033 ± 0.011	0.034 ± 0.013
SOL RMS (mV)	Young	0.007 ± 0.001	0.009 ± 0.003$	0.007 ± 0.002	0.012 ± 0.004	0.014 ± 0.005$	0.013 ± 0.003
	Older	0.007 ± 0.003	0.009 ± 0.003$	0.010 ± 0.003$	0.011 ± 0.004	0.013 ± 0.004$	0.014 ± 0.005$
MEP latency (ms)	Young	29.9 ± 3.5	29.7 ± 2.3	29.1 ± 3.1	29.5 ± 4.3	28.6 ± 2.8	28.5 ± 4.3
	Older*	34.2 ± 7.9	33.4 ± 5.8	35.7 ± 7.5	31.8 ± 5.0	33.5 ± 7.3	33.1 ± 6.2

M_H/M_{max} = M-wave when H-reflex was evoked normalised to maximal muscle response; RMS = root-mean-square EMG activity.
Torque, TA RMS and SOL RMS increased with contraction intensity.
$p < 0.05$ - *compared to younger; † compared to ISO and ECC; ‡ compared to CON and ECC; #compared to ECC; §compared to target %; $compared to ISO

Responses to PNS

Representative examples of responses to PNS for a young and an older individual of similar height during 25% of contraction type specific MVC are presented in Figure 5-6A. Notably, it is clear that the older individual in these plots exhibited smaller H-reflexes as well as slightly longer latencies and this trend was similar during all contraction types at both contraction intensities.

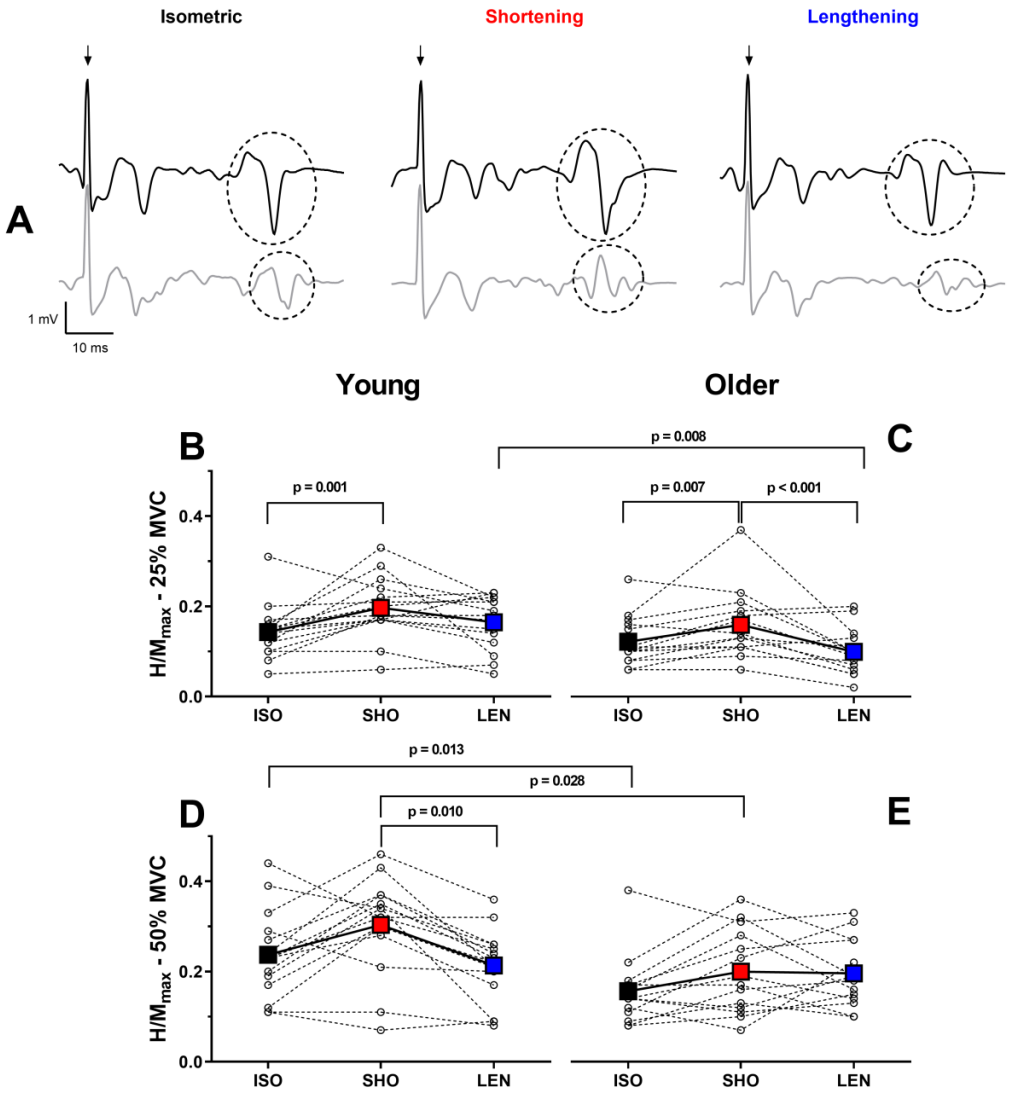

Figure 5-6. Responses to percutaneous nerve stimulation. A: Representative examples from one young (black line) and older (grey line) individual during isometric, shortening and lengthening contraction at 25% of contraction type specific MVC. The downward arrows denote the point of stimulation and the dotted circles indicate the H-reflex. Both participants were of similar height. All traces are an average of 4 waveforms. B–E: The amplitude of H-reflex normalised to maximal muscle response (H/M_{max}) in young (left panel) and older (right panel) adults at 25% (B, C) and 50% (D, E) maximal torque during isometric (ISO), shortening (SHO) and lengthening (LEN) contractions. Open circles denote individual response and open squares denote the age group mean.

M_H/M_{max} was similar across all conditions ($p \geq 0.116$; Table 5-3). H/M_{max} increased with contraction intensity ($F_{1, 27} = 58.4$, $p < 0.001$), was greater in younger adults compared to older ($F_{1, 27} = 6.0$, $p = 0.021$) and was contraction type dependent ($F_{2, 54} = 16.2$, $p < 0.001$). There was a contraction type × contraction intensity × age group interaction for H/M_{max} ($F_{2, 54} = 6.6$, $p = 0.003$). *Post hoc* analysis showed younger individuals had ~38% greater H/M_{max} compared to older during lengthening contractions at 25% of maximal torque ($p = 0.004$; Figure 5-6B & 5-6C), as well as ~33% during isometric ($p = 0.020$) and shortening ($p = 0.008$) contractions at 50% of maximal torque (Figure 5-6D & 5-6E). Furthermore, in younger individuals, H/M_{max} was ~43% greater during shortening compared to isometric contractions at 25% ($p = 0.001$; Figure 5-6B) and ~43% greater compared to lengthening contractions at 50% of maximal torque ($p = 0.010$; Figure 5-6D). Older individuals exhibited ~43% greater H/M_{max} during shortening compared to isometric ($p = 0.007$) and lengthening contractions ($p < 0.001$) at 25% of maximal torque (Figure 5-6C), but no differences were noted across contraction types at 50% of maximal torque ($p > 0.05$). H/M_{max} increased ~71% with contraction intensity in young adults during isometric ($p < 0.001$) and ~43% compared to shortening ($p = 0.001$), but not lengthening contractions ($p = 0.059$). In older adults, H/M_{max} increased ~43% with contraction intensity during isometric ($p = 0.004$) and ~25% compared to lengthening ($p < 0.001$) contractions, but not shortening ($p = 0.063$).

H-reflex latency was similar across contraction intensities and types ($p > 0.05$). Whilst the mean group values suggested H-reflex latencies were longer in the older population (Table 5-3), this difference did not reach statistical significance ($F_{1, 27} = 3.9$, $p = 0.058$). PSCT was similar between young and older individuals (8.1 ± 0.9 vs. 8.5 ± 2.0 $ms.m^{-1}$; $t_{27} = -0.5$, $p = 0.473$).

Responses to TMS

Example responses to TMS for representative participants of similar height during 25% of contraction type specific MVC are presented in Figure 5-7A. As noted by the dashed lines in the figure, older individuals exhibited longer MEP latencies and smaller amplitude of responses compared to younger individuals. Similar behaviour was observed during contractions at 50% of maximal torque.

MEP/M_{max} increased ~34% with greater contraction intensity ($F_{1, 27} = 43.7$, $p < 0.001$) and was contraction type dependent ($F_{2, 54} = 14.1$, $p < 0.001$). No differences were observed between groups ($p = 0.290$). There was a contraction type × age group interaction ($F_{2, 54} = 3.5$, $p = 0.039$). *Post hoc* testing show that in young individuals MEP/M_{max} was ~23% greater during shortening compared to lengthening ($p = 0.002$) contractions (Figure 5-7B & 5-7D). However, in older adults, MEP/M_{max} was ~43% greater during shortening ($p = 0.001$) and ~24% greater during lengthening ($p = 0.024$) compared to isometric contractions (Figure 5-7C & 5-7E).

Older individuals exhibited ~15% longer MEP latencies compared to young ($F_{1, 27} = 5.6$, $p = 0.025$; Table 5-3). As per significant interaction between contraction type and age group ($F_{2, 54} = 4.5$, $p = 0.015$), the magnitude of this difference was greater during lengthening contraction ($p = 0.006$). However, CMCT was not different between young and older adults (6.2 ± 2.1 vs. 6.9 ± 1.9 $ms \cdot m^{-1}$; $t_{27} = –0.9$, $p = 0.350$). Silent period was shorter at higher contraction intensity ($F_{1, 27} = 6.0$, $p = 0.021$), but was not dependent on contraction type or age group ($p \geq 0.188$; Table 5-3).

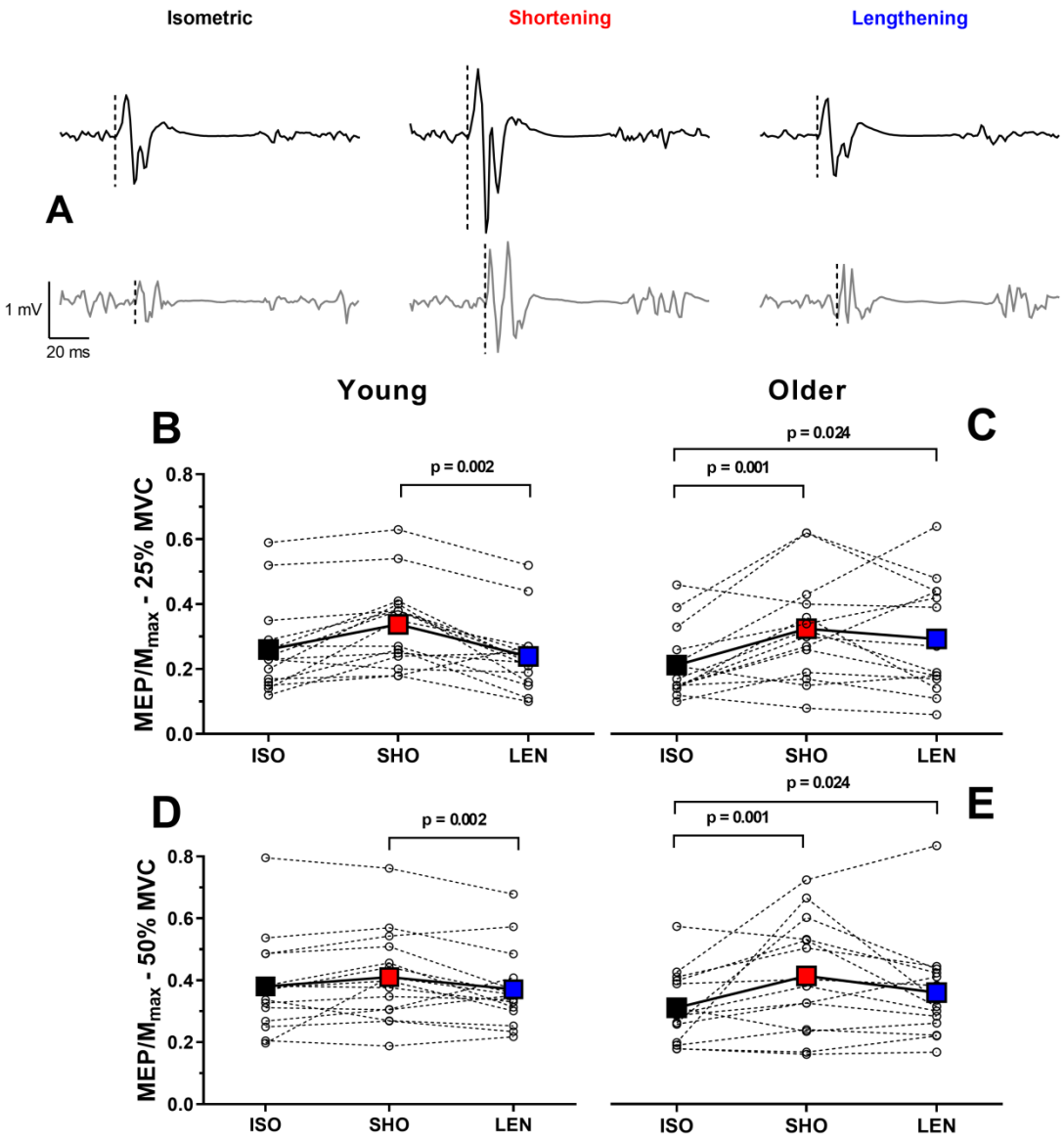

Figure 5-7. Responses to TMS. A: Representative examples from one young (black line) and older (grey line) individual during isometric, shortening and lengthening contraction at 25% of contraction type specific MVC. Each trace begins at the stimulus artefact and shows 150 milliseconds. The vertical dashed lines represent the onset of MEP (latency). Both participants were of similar height. All traces are an average of 4 waveforms. B–E: The amplitude of motor evoked potential normalised to maximal muscle response (MEP/M_{max}) in young (left panel) and older (right panel) adults at 25% (B, C) and 50% (D, E) maximal torque during isometric (ISO), shortening (SHO) and lengthening (SHO) contractions. Open circles denote individual response and open squares denote the age group mean.

The influence of background activity on the evoked responses

Modulation of responses to stimulation techniques during submaximal contractions may not just depend on torque, but also on background EMG activity (Abbruzzese et al., 1994; Gruber et al., 2009; Nordlund et al., 2002). Whilst care was taken to record responses at the same relative torque levels for each contraction type with stimulation

performed at the same joint angle in all conditions, differences in RMS/M_{max} between contraction types and age groups were still observed. Thus, the evoked responses to stimulations were additionally normalised to the pre-stimulus RMS activity ([H/M_{max}]/RMS and [MEP/M_{max}]/RMS, respectively), as has been done previously (Sidhu et al., 2012; Tallent et al., 2013).

The [H/M_{max}]/RMS did not change with contraction type ($F_{2, 54}$ = 4.6, p = 0.275), but was greater in younger individuals ($F_{1, 27}$ = 19.8, p < 0.001), and was modulated with contraction intensity ($F_{1, 27}$ = 7.6, p = 0.010). There was a contraction intensity × contraction type × age interaction ($F_{2, 54}$ = 7.4, p = 0.001). *Post hoc* analysis showed older individuals exhibited ~33% smaller [H/M_{max}]/RMS during lengthening compared to isometric (p = 0.034) and shortening (p = 0.020) contraction at 25% of maximal torque (Figure 5-8A). Furthermore, older compared to younger individuals exhibited ~44% smaller [H/M_{max}]/RMS across all contraction types and intensities (p < 0.028; Figure 5-8A-D).

Similarly, [MEP/M_{max}]/RMS was not dependent on contraction type ($F_{2, 54}$ = 1.5, p = 0.236); however, it was ~34% lower in older individuals ($F_{1, 27}$ = 15.7, p < 0.001; Figure 5-8E–H) and modulated with contraction intensity ($F_{1, 27}$ = 11.4, p = 0.002). A significant interaction between contraction intensity and age ($F_{1, 27}$ = 4.8, p = 0.037) showed that the difference in [MEP/M_{max}]/RMS with contraction intensity was only significant for younger individuals (p < 0.001).

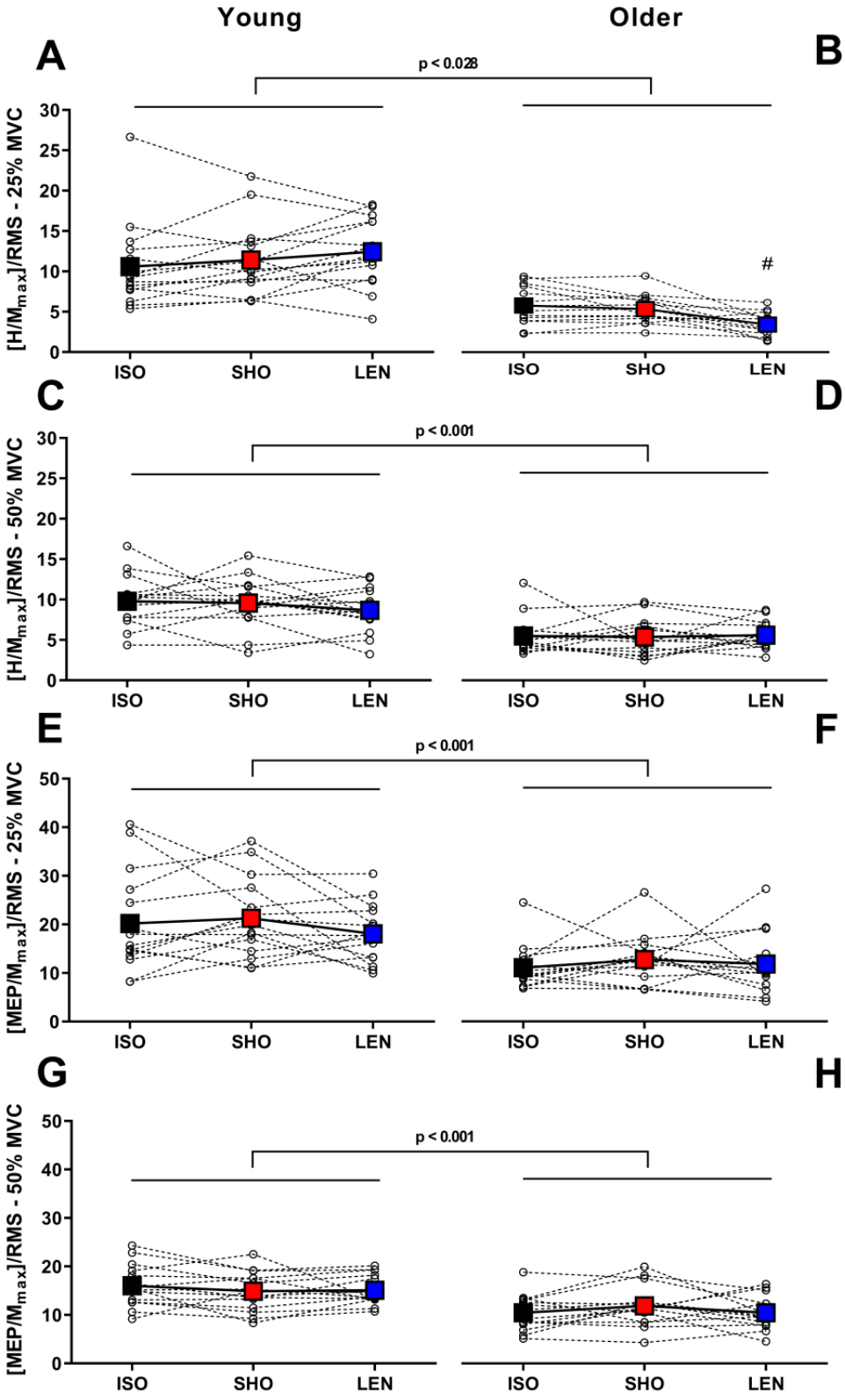

Figure 5-8. Evoked responses normalised to pre-stimulus root-mean-square EMG activity. Normalised H-reflex ([H/M_{max}]/RMS, A–D) and MEP ([MEP/M_{max}]/RMS, E–G) in young (left panel) and older (right panel) adults at 25% and 50% of maximal torque during isometric (ISO), shortening (SHO) and lengthening (LEN) contractions. Open circles denote individual response and open squares denote the age group mean. $^{\#}p < 0.010$ compared to ISO and CON.

5.14 Discussion

The present study investigated corticospinal and spinal responses in relation to neuromuscular performance variability in younger and older individuals. The novel findings are the manifestation of corticospinal and spinal changes along with increased torque variability without decrements in maximal torque production in older adults. Specifically, peripheral motor conduction times were longer, the amplitudes of MEP and H-reflexes when accounting for differences in background EMG activity were smaller, and CV_{torque} was greater in older compared to younger adults during shortening and lengthening contractions.

Maximal torque production and torque variability

Older adults were of similar strength compared to young counterparts, but their neuromuscular performance during submaximal contractions was poorer. Whilst healthy aging has been associated with the decline in peak torque production (Grabiner and Enoka, 1995; McNeil et al., 2005; Piasecki et al., 2018), this might not always be the case, particularly in the dorsiflexors (Klass et al., 2011; Kwon et al., 2011), a muscle group with similar habitual use in older age and thus, better preserved function (Abe et al., 2011; Pannérec et al., 2016). It has also been argued previously that changes in neuromuscular performance are less accurate in older adults due to greater within- and between-subject variability in performance (Degens and Korhonen, 2012; Rantanen et al., 1998), rendering the age-related changes in neuromuscular performance measures less detectable (Hunter et al., 2016b). The lack of statistical age-difference in the present study could thus be due to greater between-subject heterogeneity of older adults during MVC (see SDs in Table 5-2). This greater heterogeneity of older adults compared to the young was evident during isometric and

shortening contractions, whereas it was similar during lengthening contractions, which might stem from greater preservation of strength during lengthening contraction in older adults (Power et al., 2015). Alternatively, the average age of participants in the older group might have been too low for differences in MVC to be detected. However, the lack of dorsiflexor strength difference between young and older adults has previously been observed even when participants were over 70 years of age (Klass et al., 2011; Kwon et al., 2011). Since the loss of voluntary strength with aging seems to be related to the degree of sarcopenia (Gilmore et al., 2017; Morat et al., 2016; Piasecki et al., 2018), it seems more likely that at least some of our older individuals were pre-sarcopenic, rather than not sufficiently old per se. Another factor that could have contributed to the lack of age-related difference in MVC is uneven distribution of sexes between the young and the older sample, as the rate of age-related strength decline appears to be greater for males than females (Ditroilo et al., 2010; Wu et al., 2016).

Despite the lack of difference in MVC, older individuals displayed greater variability of torque output during submaximal shortening and lengthening contractions as well as a tendency to overshoot the target torque. The lack of relationship between maximal strength and torque variability during submaximal contractions has previously been demonstrated in older adults (Burnett et al., 2000), as well as greater torque variability during dynamic contractions (Laidlaw et al., 2000) and the tendency to overshoot the target torque (Hortobágyi et al., 2001b). This greater variability and reduced accuracy in matching the target torque is likely responsible for higher background EMG activity in older adults, a finding consistent with previous literature, especially during fine motor tasks (Keenan and Massey, 2012; Opie and Semmler, 2016). Alternatively, the greater agonist EMG activity could stem from differences in antagonist EMG activity.

Whilst our data does not demonstrate any age-group differences in antagonist EMG activity, potential differences could be masked by the lack of normalisation to the maximal EMG activity of the antagonist.

Differences in responses to stimulations as a function of age

M_{max} amplitude was not different between younger and older adults, but M_{max} increased with greater contraction intensity independent of age as shown previously (Lee and Carroll, 2005). No difference in MEP amplitude was observed between younger and older individuals during a constant torque task across contraction types. Since SP duration has been shown to be related to MEP amplitude (Orth and Rothwell, 2004), it is perhaps not surprising that no age-related difference in SP was observed as well. However, when the difference in background EMG activity between the age groups was accounted for, younger individuals displayed greater corticospinal responses compared to older regardless of contraction type, as shown previously (Oliviero et al., 2006; Sale and Semmler, 2005). The greater EMG activity, but a lack of difference in MEP/M_{max} at the same relative submaximal target torque might suggest a compensatory increase in corticospinal drive in older adults for maintenance of the desired torque level when compared to younger adults (Seidler et al., 2010). Interestingly, the representative responses in Figure 5-7A show a polyphasic waveform as shown previously in TA (Terao et al., 2000), and a slightly differing waveform shape between young and older individuals which might stem from differences in I-wave recruitment (Opie et al., 2018). However, further investigation of this behaviour is beyond the scope of this study. Reduced corticospinal responsiveness with advancing age observed in the present study might occur due to various factors, including decreased quantity of cortical neurons and their

functionality (Henderson et al., 1980; Seidler et al., 2010). However, MEP size depends on excitability of both cortical neurons and spinal motoneurons and hence the differences between younger and older adults could stem from alterations in either or both of these neural axes.

H-reflex is generally considered to be a measure of spinal excitability and can be influenced by the level of alpha motoneuron excitability, presynaptic inhibition (Capaday and Stein, 1987; Schieppati, 1987), spinal interneuronal activity (Nielsen et al., 1999; Romanò and Schieppati, 1987) and supraspinal centres (Heckman et al., 2009; Rudomin and Schmidt, 1999). The level of alpha motoneuron excitability can be linked to Ia synaptic input and hence a lower H-reflex seen in older individuals could suggest a loss of Ia axons and their synaptic input. However, in the present study this seems unlikely as the PSCT values for the older group would have been expected to have been greater in comparison to the young, which was not the case. The lower H-reflex (H/M ratio) in older individuals is consistent with an increased presynaptic inhibition in this group as previously reported (Kallio et al., 2010; Morita et al., 1995; Sabbahi and Sedgwick, 1982; Scaglioni et al., 2003). It is of interest that the greater EMG activity of the agonist observed in older adults was insufficient to compensate for the increase in presynaptic inhibition. This would suggest other changes in spinal excitability might be occurring. For instance, recent animal work has shown that aging is associated with reduced facilitatory glutamatergic inputs to the spinal cord (Maxwell et al., 2018), which could reduce motoneuronal output. Whilst this notion would suggest that the change in MEP size is more likely due to age-related alterations in the activity of the spinal motoneurons rather than cortical neurons, it should be noted that H-reflex is influenced by presynaptic inhibition, whereas MEP amplitude is not (Nielsen and Petersen, 1994). Thus, future work involving electrical stimulation of

descending pathways at a subcortical level (Taylor and Gandevia, 2004) is warranted to provide more definite conclusions.

MEP latencies were longer in older individuals, a finding previously observed in an elderly population, which might stem from age-related alterations in temporal characteristics of interneuronal (I-wave) circuitry (Opie et al., 2018). Whilst H-reflex latencies were not statistically different between groups (mean difference: 2.0 ms; $p = 0.058$), there was a trend for longer latencies in older adults, which has been previously demonstrated (Klass et al., 2011; Sabbahi and Sedgwick, 1982; Scaglioni et al., 2003). Prolonged H-reflex latency along with no difference in M_{max} latency would suggest that sensory afferent axons were more affected with aging than efferent motor axons, as previous studies have also suggested (Scaglioni et al., 2002). However, such a conclusion does not take into account the shortness of the motor pathway, and consequent shorter latency for M_{max}, making it difficult for any differences in M_{max} latency to be detected. For instance, M_{max} latency increased by the same percentage as H-reflex latency (~6%), however an absolute increase of ~0.3 ms is likely too small to be detectable. Despite no statistical difference in M_{max} latency, the difference in PMCT between young and older adults, combined with no difference in PSCT and CMCT suggests that it is the efferent motor axons that are more affected by aging. This could stem from the loss of large-diameter axons (Kawamura et al., 1977b, 1977a) as well as reductions in myelination and internodal length (Doherty and Brown, 1993; Metter et al., 1998) associated with advancing age. Collectively, the age-related differences in MEP and H-reflex amplitudes and conduction times suggest that changes occurring in CNS could be more prominent at the spinal and motoneuronal, rather than cortical level.

Relationship between functional and corticospinal changes with aging

The interesting finding in the present study is the interplay between corticospinal changes and functional alterations in older adults as they exhibited reduced corticospinal and spinal responses compared to the young, but showed divergent functional adjustments. The prolonged PMCT in older adults is suggestive of the loss of large-diameter motor axons (Kawamura et al., 1977b, 1977a). The loss of such axons could lead to sprouting of surviving alpha-motoneuron axons to reinnervate the denervated muscle fibres (Deschenes, 2011; Hepple and Rice, 2016; Luff, 1998), thus facilitating maintenance of maximal torque production. However, reinnervation leads to large motor units resulting in larger and more variable motor unit action potentials (Hourigan et al., 2015), which might contribute to increased torque variability during submaximal contractions. However, despite the plausibility of the aforementioned notion, further research is needed to test this hypothesis directly. The probability of older adults having enlarged motor units, but preserved maximal torque production in the present study suggests that they were mostly pre-sarcopenic (Gilmore et al., 2017; Piasecki et al., 2018). Whilst it has been shown previously that neural changes, such as alterations in motor unit properties, can precede the loss of maximal torque production with aging, especially in pre-sarcopenic older adults (Gilmore et al., 2017; McNeil et al., 2005; Piasecki et al., 2018), the present study extends this observation by showing there is also a decline in corticospinal and spinal responses that might occur in advance of substantial age-related maximal torque losses. Despite the preserved capacity to generate maximal torque, age-related functional alterations can still be manifested, as evidenced by increased torque variability. The latter is likely to impact activities of daily living to a greater extent than losses in maximal strength since most of the activities of daily living are submaximal, requiring fine motor

control. Thus, the assessment of age-related loss in function requires an investigation of multiple variables, rather than solely relying on measures of MVC.

Whilst older adults demonstrated reduced corticospinal and spinal responses concurrent with increased torque variability compared with young adults, no association was shown between corticospinal and/or spinal responses and torque variability. A previous study demonstrated a lack of relationship between neuromuscular performance variability and intracortical inhibitory activity (Opie and Semmler, 2016), and the present study extends this observation by suggesting a reduction in corticospinal and spinal responses in older age are not related to reductions in neuromuscular performance variability during submaximal shortening and lengthening contractions. As previously outlined, there is a possibility that a lack of relation between corticospinal responsiveness and torque variability is due to improvements in neuromuscular performance after sufficient familiarisation and repeated performance of the task throughout the experiments (Opie and Semmler, 2016) despite no observation of a systematic improvement in task performance in both experimental sessions. Alternatively, though corticospinal excitability might contribute to the common synaptic input to a greater or lesser extent depending on age, it is likely the modulation of that common input that directly determines the variability of torque output (Feeney et al., 2018).

Corticospinal and spinal modulation during isometric, shortening and lengthening contractions

No differences in CNS inhibition (SP) were noted among different contraction types regardless of age. Whilst previous results have been conflicting with some studies showing shorter SP during lengthening plantarflexion (Duclay et al., 2014, 2011;

Valadão et al., 2018), and others showed shorter SP during shortening contractions of the upper limb muscles (Opie and Semmler, 2016; Sekiguchi et al., 2007), the present study showed no change in SP across contraction types. Our findings might be related to the specific muscle investigated. For example, compared to soleus that has been studied previously (Duclay et al., 2014, 2011; Valadão et al., 2018), TA exhibits significantly shorter SPs (Lauber et al., 2018) making it less likely that the smaller modulation in SP duration across contraction types would be detected.

Lengthening contractions have been purported to be accompanied by reduced corticospinal responses (for review see Duchateau and Enoka, 2016). However, when background EMG activity was accounted for in the present study, MEPs and H-reflexes were not differently modulated across isometric, shortening and lengthening contractions. The MEP behaviour corroborates a study on soleus when comparing their responses elicited with similar relative TMS intensity to the present study (Duclay et al., 2014). Conversely, the lack of H-reflex modulation observed in the present experiment does not support reduced spinal excitability during lengthening contractions previously reported in soleus (Duclay et al., 2014; Romanò and Schieppati, 1987). This discrepancy could be the result of differences in tasks employed between experiments (Duclay et al., 2014, see also Part 1 of this chapter), slower contraction velocity (Romanò and Schieppati, 1987), and differences in EMG activity between contraction types (Duclay et al., 2014). Furthermore, the familiarisation sessions involving submaximal lengthening contractions could have led to motoneuron pool adjustments as a strategy to protect the muscle from damage (Dartnall et al., 2011; Hyldahl et al., 2017), thus potentially affecting evoked responses in the experimental session. Despite aforementioned confounding methodological factors, it is important to consider that the H-reflex behaviour might be muscle-

specific. Similar to data in the present study, no H-reflex modulation across contraction types has previously been shown for medial gastrocnemius (Duclay et al., 2011). From the perspective of spinal control, TA and SOL are known to differ in quantity of muscle spindles (~280 versus ~400; Banks, 2006; De Luca and Kline, 2012) and the strength of reciprocal spindle afferent input (4-fold greater in TA; Yavuz et al., 2018), both of which could affect the relative input from Ia afferents. Indeed, the differences between modulation of corticospinal excitability between TA and SOL were noted during passive length changes in Chapter 4 in young individuals. This suggests that the effect of processing the muscle length related sensory information on corticospinal excitability of antagonist muscles around the ankle joint might differ during both passive and active movement and between age groups. Thus, it is possible that the observed behaviour in TA in the present study is specific to the muscle investigated.

Methodological considerations

The aim of this study was not only to assess corticospinal and spinal excitability during submaximal isometric, shortening and lengthening contractions with advancing age, but also to concurrently explore functional measures of torque variability. As such, the submaximal contraction targets were based relative to an individual's maximal torque production capacity. Despite normalising to contraction type specific MVC as per Part 1 of this chapter, this approach resulted in differing background EMG activity levels across contraction types and between young and older adults. The evoked response size is known to increase concurrently with background EMG activity (Farina et al., 2014; Fuglevand et al., 1993; Rothwell et al., 1991) due to greater motoneuron excitability as a result of motoneurons being closer to their firing threshold with

augmentation of their recruitment and firing rate (Bawa and Lemon, 1993). The size of MEPs is increased with greater background EMG activity even when normalised to contraction intensity specific M_{max} (Martin et al., 2006). As such, the differences in background EMG across conditions could have confounded our interpretation of the differences in the evoked responses across contraction types and between the age groups. To account for this possibility, corticospinal and spinal responses were additionally normalised to background EMG activity as seen previously (Sidhu et al., 2012; Tallent et al., 2013). However, furture work is required to elucidate whether behaviour observed in this study is similar when the level of neural excitation is matched across conditions.

The contraction velocity chosen ($5°\cdot s^{-1}$) could be considered slow. This is a potential reason for the lack of difference observed in maximal torque production between isometric and shortening contractions (Rozand et al., 2017) as well as differences between age groups across contraction types (Power et al., 2015; Rozand et al., 2017). Furthermore, the slower velocity might have made it less likely that neural strategies during dynamic contractions will differ from isometric. The reason for the selection of this velocity was based on pilot testing that indicated difficulty in reliable delivery of stimuli during submaximal contractions at the target torque due to significant torque variability with increased contraction velocity. Moreover, the contraction velocity chosen allowed for a sufficient pre-stimulus time of torque production for a reliable analysis of torque variability. However, the data from Part 2 of this chapter showed orthodoxical shortening and lengthening of muscle fascicles with changes in joint angle during dynamic contractions at the velocity employed in the present experiment, whereas fascicles exhibited no change in length during isometric contractions, suggesting the behaviour of contractile elements during different contraction types

was indeed distinct. Fascicles were also of analogous length at the anatomical zero, confirming responses were elicited at the same muscle length. Moreover, a recent study indicated that corticospinal and spinal responses during lengthening contractions do not change with a 4-fold increase in contraction velocity (Valadão et al., 2018), suggesting that an increase in the rate of proprioceptive feedback with increases in contraction velocity does not seem to influence the potential differences in corticospinal control of different contraction types.

5-15 Conclusion

The present study showed that despite no difference in maximal torque production, corticospinal and spinal excitability were greater and peripheral motor conduction time was shorter in young compared to older adults. These findings suggest that corticospinal changes associated with aging can occur in advance of decrements in maximal torque production in TA, whereas other functional alterations, such as increased torque variability and reduced torque accuracy during submaximal dynamic contractions, are still evident. Interestingly, the present data show the pattern of corticospinal modulation of different contraction types between younger and older adults during a constant torque task in dorsiflexors is similar. This suggests that the CNS accommodates for age-related changes in order to preserve motor control of different contraction types. Additionally, the results show that in a constant torque task, corticospinal and spinal excitability of dorsiflexors are greater during shortening compared to isometric and lengthening contractions, but no differences are apparent when variances in background EMG activity are taken into account. These responses do not support the notion of a unique corticospinal control of lengthening dorsiflexion and might be task (i.e. a constant torque task) or muscle-specific (i.e. TA). The lower

corticospinal excitability during dynamic contractions in older adults might play a role in the adaptability of an aging CNS, which will be explored in the next Chapter.

CHAPTER 6: NEUROPHYSIOLOGICAL RESPONSES AND ADAPTATIONS TO A BOUT OF MAXIMAL LENGTHENING CONTRACTIONS IN YOUNG AND OLDER ADULTS

Publication arising from this part of the chapter:

Škarabot, J., Ansdell, P., Temesi, J., Howatson, G., Goodall, S., and Durbaba, R. (2019). Neurophysiological responses and adaptation following repeated bouts of maximal lengthening contractions in young and older adults. *Journal of Applied Physiology* 127, 1224-1237.

6-1 Introduction

As stated in Chapter 2, a bout of unaccustomed maximal lengthening contractions produces structural damage to muscle fibres and the extracellular matrix, resulting in impaired neuromuscular function following exercise (Newham et al., 1987). This is accompanied by an immediate reduction in voluntary and electrically-evoked force production, which persists for several days after exercise (Prasartwuth et al., 2005; Sayers et al., 2003). Despite the negative consequences that accompany a bout of unaccustomed lengthening contractions, this type of exercise provides a protective effect against subsequent bouts as evidenced by attenuation of torque loss, soreness and other muscle damage indices, referred to as the RBE (Nosaka and Clarkson, 1995).

RBE has been attributed to mechanical, cellular and neural factors (Hyldahl et al., 2017), but the latter remains comparatively unexplored. Following a bout of unaccustomed maximal lengthening contractions, a reduction in voluntary activation using motor nerve (Goodall et al., 2017; Prasartwuth et al., 2005) and TMS (Goodall et al., 2017) has been observed, suggesting a neural contribution (Howatson et al., 2007). Further evidence of neural adjustments in response to damaging exercise is exhibited by observed impairments in proprioception (Brockett et al., 1997; Carson et al., 2002), alterations in the force-EMG relationship (Carson et al., 2002; Leger and Milner, 2001; Meszaros et al., 2010; Prasartwuth et al., 2005; Semmler et al., 2007; Weerakkody et al., 2003), increased torque variability (Semmler et al., 2007; Weerakkody et al., 2003), and modulation of MU behaviour (Semmler, 2014), including increased motor unit synchronisation (Dartnall et al., 2009) and decreased recruitment threshold (Dartnall et al., 2009). However, if the damaging bout of exercise is repeated, the alterations in voluntary activation and motor unit behaviour are attenuated (Dartnall et al., 2011; Goodall et al., 2017; Howatson et al., 2007),

suggesting modulation of spinal and supraspinal inputs to the motoneuron pool. However, the exact origin of the synaptic input causing adaptation at the level of the motoneuron pool is unclear (Hyldahl et al., 2017). Animal data showed elevated brain cytokines following damaging exercise (Carmichael et al., 2005), and experiments in humans have suggested that modulation in cortical networks such as reduced intracortical inhibition (Pitman and Semmler, 2012) and increased somatosensory cortical excitability (De Martino et al., 2018) might occur. However, it remains unknown whether the abovementioned neurophysiological responses following damaging exercise would differ if the bout of exercise was repeated, when disruptions in peripheral biochemistry are expected to be smaller.

Another factor that is known to affect the neuromuscular system and could mediate the response to damaging exercise is age. Whilst older adults might exhibit a decrease in voluntary isometric strength compared to younger (Piasecki et al., 2018), there appears to be a preservation of strength during lengthening contractions in older adults as shown in Chapter 5. For this reason, employing lengthening contractions has been suggested to be a well-suited training stimulus for older adults (Roig et al., 2010). Despite this, older adults appear to exhibit a smaller manifestation of the RBE (Gorianovas et al., 2013; Lavender and Nosaka, 2006a), which could be related to the reduced capacity for neuromuscular adaptation. Indeed, healthy aging is characterised by many central nervous system (CNS) adjustments, including alterations in synaptic input during dynamic contractions originating from spinal and supraspinal centres as shown in Chapter 5. In addition, aging adults also exhibit increased inhibitory and decreased excitatory neurotransmitter activity in the motor cortex (McGinley et al., 2010; Opie and Semmler, 2016), which have been linked to a reduction in the capacity for neural adaptation (Cash et al., 2016; McNeil and Rice, 2018). This capacity might

be further impaired in older adults due to altered cortical sensorimotor integration of afferent input (Smith et al., 2011a), which is attenuated following the release of inflammatory cytokines associated with muscle damage (Carmichael et al., 2005). Thus, discerning the aetiology of neuroplastic impairment in the elderly is important for informing neurorehabilitation (Dimyan and Cohen, 2011), motor learning (Sagi et al., 2012; Stagg et al., 2011a) and adaptation to exercise (Weier et al., 2012).

The age-related alterations in CNS also result in impairments in neuromuscular function, including the ability to control force output (Enoka et al., 2003), which has important implications in performance of functional daily tasks (Feeney et al., 2018; Hamilton et al., 2019; Mani et al., 2018) and has been linked to greater incidence of falls (Carville et al., 2007). As shown in Chapter 5, older adults exhibited greater torque variability during medium intensity contractions, but this was limited to dynamic contractions. However, age-differences in torque variability during isometric contractions might be limited to lower intensities of contractions (Oomen and van Dieën, 2017). Moreover, torque variability is increased following damaging exercise in younger populations (Semmler et al., 2007; Weerakkody et al., 2003). Whilst not investigated in older population, if similar or exaggerated increases in torque variability are shown, this could have implications for performance of daily tasks, such as greater risk of falls (Carville et al., 2007), following damaging exercise.

Accordingly, the present experiment aimed to: 1) assess neurophysiological (intracortical, corticospinal and spinal) responses following damaging exercise; 2) assess the neurophysiological contribution to the RBE; and 3) compare the extent of muscle damage and the associated modulation of neurophysiological responses and torque variability between young and older adults. It was hypothesised that damaging exercise will modulate neurophysiological responses, but this modulation will be of

smaller magnitude following the repeated bout. Given the alterations in sensorimotor integration with advanced age, it was also hypothesised that older adults will exhibit a smaller RBE.

6-2 Methods

Participants

Twelve young (27 ± 5, range 21-35 years; 180 ± 7 cm, 77.2 ± 9.6 kg; 2 females) and 11 older (66 ± 4, range 61-73 years; 177 ± 13 cm, 75.3 ± 12.1 kg; 3 females) adults took part in the study. For a statistically significant interaction after an acute bout of eccentric exercise, it was estimated (alpha = 0.05, 1 – beta = 0.80) that a minimum of 4 participants per group will be needed for the variables of torque, M_{max}, MEP, SICI, LICI and ICF (Latella et al., 2019). For detailed outline of exclusion and inclusion criteria, see section 3-3.1.

Experimental protocol

Participants visited the laboratory seven times throughout the duration of the study, at the same time of day (±1 h) to limit diurnal variations (Tamm et al., 2009). The purpose of the first session was familiarisation with the experimental procedures. Following familiarisation, participants returned to the laboratory to perform a bout of maximal lengthening contractions. Before, immediately after, and 24 and 72 hours post the exercise bout, assessment of neuromuscular function was performed (Figure 6-1) including isometric MVC, followed by assessment of torque variability at 5 and 20% isometric MVC, H-reflex, and responses to TMS (randomised order). Half of the sample in each group exercised with their dominant limb, whilst the other half of the sample exercised with their non-dominant limb (pseudorandomised) as per the lateral

preference inventory (Coren, 1993) to discount the influence of limb dominance. Indeed, a separate analysis taking into account limb dominance showed no difference in measures of isometric MVC torque across all time points ($p = 0.297$). The whole protocol was repeated two weeks post the initial visit, including the exercise bout to assess the adaptive neural response.

Procedures

The bout of maximal lengthening contractions

The exercise bout consisting of maximal lengthening contractions was performed on an isokinetic dynamometer (Biodex System 4 Pro, New York, USA). For a detailed description of dynamometry, see section 3-3.2. Participants were required to perform 10 sets of 6 maximal lengthening contractions throughout 40° range of motion (from 10° dorsiflexion to 30° plantar flexion) by maximally resisting the motor of the device moving at $15°\cdot s^{-1}$ and then relaxing throughout the passive dorsiflexion (shortening) phase. A greater contraction velocity and range of motion compared to previous experiments in this thesis were chosen to better replicate a real-world scenario of resistance exercise, whereby the lengthening phase was 2 seconds in duration. The exercise was performed through the identical participant-specific range-of-motion across both bouts to match the muscle length and muscle strain between the bouts. Peak torque ($N\cdot m$) and total work (J) performed were recorded during each bout.

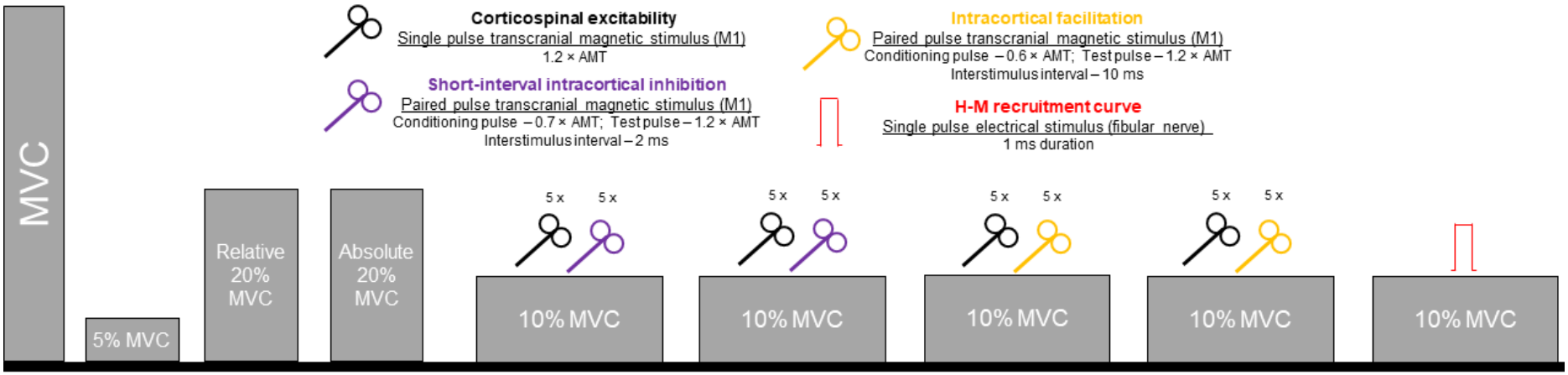

Figure 6-1. The overview of the experimental protocol. *MVC = maximal isometric voluntary contraction torque; H-M recruitment curve = H-reflex/M-wave recruitment curve; M1 = primary motor cortex; AMT = active motor threshold; SICI = short-interval intracortical inhibition; ICF = intracortical facilitation. Contractions at 5 and 10% isometric MVC were only performed with relative normalisation (see 'Constant torque task' for further details).*

Experimental setup

During the assessment of neuromuscular function, participants remained in the same dynamometer position as during exercise, but with the ankle fixed at anatomical zero (90°). Measures were performed on the limb that was exercised. This position was replicated during each visit to the laboratory to ensure consistent inferred muscle length to avoid influencing neural responses. A detailed procedure on performance of isometric MVC is described in section 3-3.2. All subsequent contractions with stimulations were performed at 10% isometric MVC (Brownstein et al., 2018).

Constant torque task

To investigate the effect of a bout of maximal lengthening contractions on torque variability, participants were asked to match the torque level to a horizontal line on the screen in front of them that was equal to 5 and 20% of isometric MVC and maintain torque production as close to the horizontal line as possible for 15 seconds. In the assessments post exercise, the constant torque task was performed with participants contracting at 5 and 20% isometric MVC. Additionally, participants performed a constant torque task at 20% isometric MVC relative to pre-exercise (absolute) isometric MVC. The absolute torque-matching at time points following exercise was only performed for 20% isometric MVC since pilot testing indicated that the difference between relative and absolute values for lower contraction intensities was within the measurement error of isometric MVC. The specific torque intensities were chosen as previous work indicated that following a bout of maximal lengthening contractions, torque variability changes are greater during low level contractions (Semmler et al., 2007). Furthermore, any differences between young and older

individuals are likely to be the greatest at, and possibly confined to, lower level contractions (Tracy and Enoka, 2002).

Surface electromyography

Electromyographic activity was recorded in TA muscle. For details, see section 3-3.3.

Transcranial magnetic stimulation

Single and paired-pulse TMS were delivered to assess corticospinal excitability, SICI and ICF. For a detailed procedure, see section 3-3.5. The hotspot was kept consistent throughout the assessments before and immediately, 24 h, and 72 h after the first bout of exercise, but was re-determined prior to the second bout of exercise. AMT was determined at the start of each individual assessment and did not differ between young and older individuals (39 ± 9 vs. 45 ± 10% SO, $p = 0.141$) or across visits ($p = 0.247$). Ten single and ten paired stimuli (randomised order) were delivered during 10% isometric MVC, with the mean of 10 responses taken as a representative value.

Percutaneous nerve stimulation

Percutaneous electrical stimuli were performed to evoke H-reflex and M-wave in TA (see section 3-3.4 for further details). The H-M recruitment curve was constructed during 10% isometric MVC by gradually increasing the intensity from H-reflex threshold by 0.5 mA to maximal H-reflex every 3 pulses. Once the H-reflex amplitude started to decrease after three consecutive increases in intensity, the amperage was increased in bigger steps (3 mA) until the EMG response plateaued. After that, the intensity was further increased by 30% to ensure supramaximal stimulation eliciting maximal compound action potential (M_{max}). The intensity required to elicit M_{max} was

lower for young, compared to older individuals (30 ± 4 vs. 50 ± 5 mA, p = 0.003), but did not differ across visits (p = 0.614).

Creatine kinase

Fingertip capillary blood samples (30 μl) were obtained at each time point and were immediately assayed for CK concentration based on reflectance in an automated system (range: 24.4 – 1500 $IU.L^{-1}$, coefficient of variation: 0.5% of reflectance; Reflotron, Roche Diagnostics, Germany). Due to technical issues, samples from 2 older individuals could not be analysed.

Data analysis

For detailed description of analysis of evoked responses, see section 3-4.3. Torque variability was quantified from the 10 seconds in the middle portion of the 15-second constant torque task (from 2.5 to 12.5 seconds) as CV_{torque}. EMG activity during torque-matching tasks was quantified as RMS in the same 10-second epoch where torque variability was assessed, and expressed relative to RMS during 500 ms around peak isometric MVC (RMS/RMS_{max}).

Statistical analyses

All analysis was performed using SPSS (v20, SPSS Inc., Chicago, IL, USA). Normality of data was assessed using Shapiro-Wilks test. If the data were not normally distributed, transformation using common logarithm was performed. Two significant outliers were identified via studentised residuals (> 3) in the young group for SICI and one for ICF, and were excluded from further analyses. Sphericity was assessed using Mauchly's test of sphericity. In the case of violation, a Greenhouse-Geisser correction

was employed. Differences in responses between groups to different bouts over time were assessed using 2 × 2 × 4 ANOVA (age × bout × time). A 2 × 2 ANOVA (age × bout) was used to assess the differences in peak lengthening MVC torque and total work performed between the two bouts of exercise. A 2 × 2 ANOVA (age × bout) was also used to assess differences between young and older adults at baseline. If significant interactions or main effects were found, the analysis was continued using pairwise comparison with Bonferroni correction. To investigate the differences in adaptability (i.e. RBE) between young and older adults, the difference in isometric MVC from baseline to 24 and 72 h post exercise for the second bout was divided by the difference in this measure following the first bout, and assessed using an independent samples t-test. To assess the neural contribution to exercise-induced disruption in neuromuscular function (isometric MVC) immediately and 24 h post-exercise, linear regression analyses were performed. Significance was set at alpha level of 0.05. Data are presented as mean ± SD, unless the data were transformed, in which case the geometric mean ± SD are presented.

Reliability

Neuromuscular responses are known to exhibit inherent variability. As such, interpreting the results within statistical measures of error has been recommended, allowing the contribution of real change and random variation to be distinguished (Furlan and Sterr, 2018). For that reason, the baseline responses from each bout were used to determine test-retest reliability of electrophysiological and mechanical variables. Typical error (TE) was calculated for the main variables of interest as the standard deviation of mean differences between the two pre-exercise values divided by the square root of 2, and was expressed as both absolute and relative (rTE) values

(percentage of the mean). Bias between the two pre-exercise scores was assessed using paired samples T-test. Reliability indices for main variables of interest are displayed in Table 6-1.

Table 6-1. Baseline differences between the age groups (mean ± SD) and reliability indices for main variables of interest.

		Bout 1	Bout 2	P	Bias	TE	$_{r}$TE (%)
		Electrophysiological variables					
M_{max} (mV)	*Young*	6.5 ± 1.8	5.8 ± 1.5	0.049	0.7	0.7	11.9%
	Older	4.9 ± 1.2	5.3 ± 1.8	0.174	-0.4	0.6	11.8%
H_{max}/M_{max}	*Young*	0.11 ± 0.06	0.12 ± 0.06	0.410	-0.01	0.03	27.3%
	Older	0.11 ± 0.06	0.11 ± 0.08	0.474	0	0.02	18.2%
MEP/M_{max}	*Young*	0.20 ± 0.10	0.21 ± 0.10	0.805	-0.01	0.08	40.0%
	Older	0.29 ± 0.11	0.26 ± 0.13	0.231	0.03	0.05	17.9%
SICI	*Young*	0.67 ± 0.21	0.78 ± 0.11	0.076	-0.11	0.13	17.8%
	Older	0.72 ± 0.20	0.72 ± 0.24	0.995	0	0.22	30.6%
ICF	*Young*	1.21 ± 0.22	1.09 ± 0.13	0.064	0.12	0.13	11.3%
	Older	1.08 ± 0.18	1.05 ± 0.19	0.585	0.03	0.22	20.4%
SP (ms)	*Young*	152 ± 28	159 ± 32	0.297	7	18	11.6%
	Older	166 ± 39	171 ± 41	0.246	-5	9	5.4%
		Mechanical variables					
Isometric MVC (N.m)	*Young*‡	59.6 ± 10.4	58.7 ± 11.4	0.964	0.9	2.9	4.9%
	Older	48.6 ± 11.8	48.0 ± 12.4	0.567	0.6	2.4	4.9%
Lengthening MVC (N.m)	*Young*	90.9 ± 22.3	90.1 ± 19.2	0.801	0.8	7.2	7.9%
	Older	78.6 ± 17.6	80.1 ± 18.4	0.356	-1.5	3.6	4.5%
Lengthening/ isometric MVC	*Young*	1.5 ± 0.2	1.6 ± 0.3	0.607	-0.1	0.1	9.3%
	Older	1.6 ± 0.3	1.7 ± 0.2	0.381	-0.1	0.1	7.6%
5% MVC (CV%)	*Young*‡	5.4 ± 1.1	5.1 ± 2.6	0.758	0.3	1.7	32.7%
	Older	7.0 ± 2.9	9.4 ± 4.3	0.081	-2.4	3.0	36.6%
20% MVC (CV%)	*Young*‡	2.2 ± 0.9	2.1 ± 0.7	0.685	0.1	0.6	27.3%
	Older	2.6 ± 0.9	3.1 ± 1.1	0.113	-0.5	0.6	20.7%

‡p < 0.05 compared to older adults (2 × 2 ANOVA); MVC = maximal voluntary isometric contraction; M_{max} = maximal compound action potential, H_{max}/M_{max} = maximal H-reflex relative to maximal compound action potential; MEP/M_{max} = motor evoked potential relative to maximal compound action potential; SICI = short-interval intracortical inhibition; ICF = intracortical facilitation; SP = silent period; TE = typical error; $_{r}$TE = relative typical error. The P-value refers to the difference between bouts (paired samples T-test).

6-3 Results

Age differences at baseline

Younger individuals displayed greater isometric MVC compared to the older ($F_{1, 21}$ = 5.2, p = 0.032, η_p^2 = 0.20; Table 6-1), and lower torque variability during an isometric constant torque task at 5% ($F_{1, 21}$ = 11.7, p = 0.003, η_p^2 = 0.36) and 20% isometric MVC ($F_{1, 21}$ = 4.4, p = 0.048, η_p^2 = 0.17). There were no age-related differences in peak lengthening MVC (p = 0.178), lengthening to isometric MVC ratio (p = 0.194), RMS/M_{max} (p = 0.065), M_{max} (p = 0.096), H_{max}/M_{max} (p = 0.787), MEP/M_{max} (p = 0.080), SICI (p = 0.672), ICF (p = 0.233) and SP (p = 0.370).

Exercise performance and markers of muscle damage

Following the initial bout of maximal lengthening contractions there was a significant reduction in isometric MVC torque ($F_{2.1, 43.8}$ = 75.8, p < 0.001, η_p^2 = 0.78), with the greatest reduction observed immediately post exercise after the initial bout regardless of age (*young*: ~28% reduction, Figure 6-2A; *older*: ~22% reduction, Figure 6-2B; p < 0.001 for both). The isometric MVC remained lower in young compared to older adults in the days following the exercise bout (*age* × *time interaction*: $F_{2.1, 43.8}$ = 6.0, p = 0.005, η_p^2 = 0.22). However, a smaller reduction in isometric MVC was demonstrated following the second bout of exercise in both groups (*bout* × *time interaction*: $F_{3, 63}$ = 4.1, p = 0.011, η_p^2 = 0.16). The RBE was similar between young and older adults as assessed by the relative difference in isometric MVC decline between bouts at 24 (90 vs. 91%; p = 0.768) and 72 hrs post-exercise (92 vs. 97%; p = 0.234).

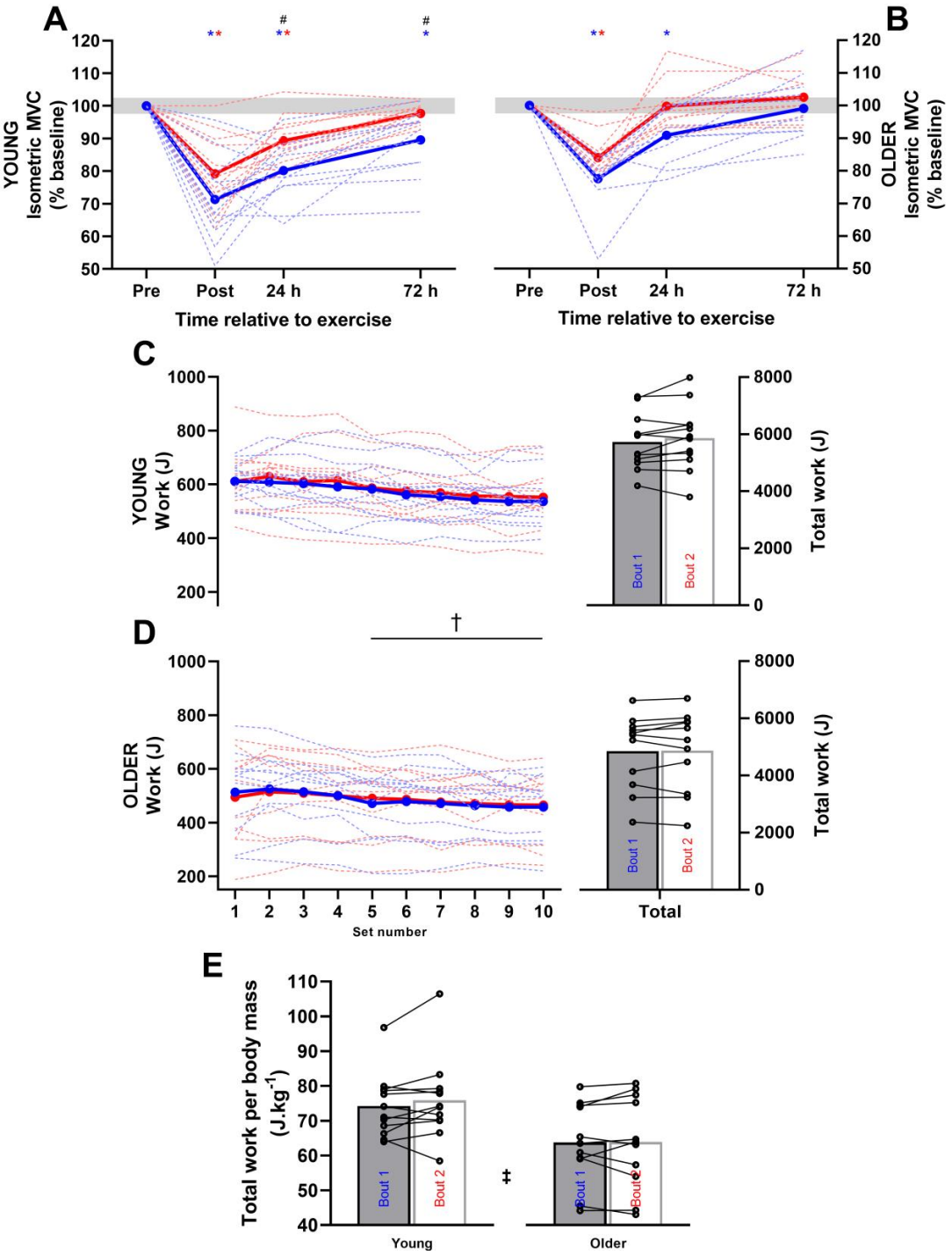

Figure 6-2. Exercise performance and recovery. Isometric maximal voluntary contraction (MVC) torque before, immediately post, and 24- and 72 hours following both bouts of exercise in young (A) and older adults (B); work performed during each set and the total work performed during each bout in young (C) and older individuals (D); and total work performed expressed relative to body mass in young and older aduls (E). Dashed lines represent individual responses whereas the full line with circles denotes the mean. The grey shaded area represents the measurement error for isometric MVC based on the difference in variability between the pre-exercise values before each bout. Data for isometric MVC are presented as a relative change, but statistical analyses were performed on raw values. *$p < 0.010$ relative to pre-exercise of 'BOUT 1', *$p \leq 0.001$ relative to pre-exercise of 'BOUT 2', #$p < 0.05$ relative to the other exercise bout (based on age × time and bout × time interactions); †$p \leq 0.021$ relative to the first set (based on main effect of time), ‡$p = 0.028$ different between the age groups (based on main effect of age).

The mean total work done across both damaging bouts in young adults was 5973 ± 1028 J, whereas older individuals did 4862 ± 1320 J. These values did not differ between the two bouts of exercise ($F_{1, 21} = 0.9$, $p = 0.360$, $\eta_p^2 = 0.40$) or age groups ($F_{1, 21} = 3.5$, $p = 0.076$, $\eta_p^2 = 0.14$; Figure 6-2C and 6-2D). During both bouts, work decreased progressively across sets (*main effect of time*: $F_{3.2, 67.2} = 24.8$, $p < 0.001$, $\eta_p^2 = 0.54$), but the decline did not differ between the groups (*age × time interaction*: $F_{3.8, 79.5} = 0.47$, $p = 0.750$, $\eta_p^2 = 0.02$). On the other hand, when total work was normalised to body mass, younger adults (75 ± 10 $J.kg^{-1}$) exhibited higher values compared to older (64 ± 12 $J.kg^{-1}$; *main effect of age*: $F_{1, 21} = 5.6$, $p < 0.028$, $\eta_p^2 = 0.21$).

An increase of CK concentration was observed following maximal lengthening contractions in both groups ($F_{1.5, 28.7} = 10.4$, $p = 0.001$, $\eta_p^2 = 0.35$). The CK kinetics were different between the two exercise bouts (*bout × time interaction*: $F_{1.3, 25.0} = 2.3$, $p = 0.014$, $\eta_p^2 = 0.25$), such that there was an increase in CK levels at 24 ($p = 0.030$) and 72 hours ($p = 0.012$) after the first bout, but only 24 hours ($p = 0.014$) and not 72 hours ($p = 0.097$) after the second bout (Table 6-2). The CK increase was also greater 24 hours following the first compared to the second bout ($p = 0.029$). There were no group differences.

Corticospinal and spinal responses and adaptation

There were no differences in prestimulus EMG activity across different time points or between the age groups ($p \geq 0.106$; Table 6-2). H_{max}/M_{max} was modulated in response to the bouts of maximal lengthening contractions (*main effect of time*: $F_{3, 63} = 3.4$, $p = 0.024$, $\eta_p^2 = 0.14$), such that it increased immediately post exercise relative to other time points ($p = 0.025 – 0.039$; Figure 6-3A and 6-3C). No differences were observed for M_{max} across all time points ($p \geq 0.287$; Table 6-2).

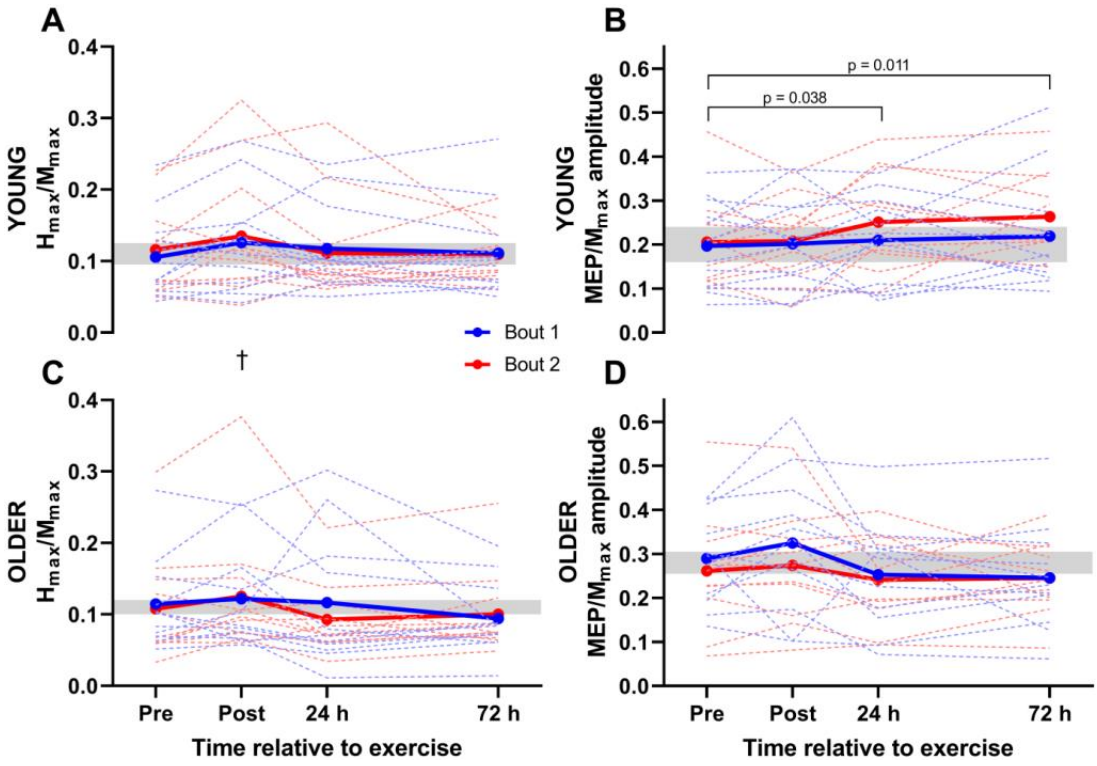

Figure 6-3. H-reflex and corticospinal excitability. The amplitude of maximal Hoffman reflex normalised to maximal compound action potential (H_{max}/M_{max}; A, C) and motor evoked potentials normalised to maximal compound action potential (MEP/M_{max}; B, D) in young (A, B) and older (C and D) adults before, immediately post, and 24- and 72 hours following both bouts of exercise. Dashed lines represent individual responses whereas the full lines with circles denote the mean. The grey shaded area represents the measurement error based on the difference in variability between the pre-exercise values before each bout. Differences in MEP/M_{max} are presented based on age× time interaction; †$p \leq 0.039$ relative to other time points (based on main effect of time).

MEP/M_{max} was modulated differently between the age groups (*age × time interaction*: $F_{3, 63} = 6.5$, $p = 0.001$, $\eta_p^2 = 0.24$) insofar as it increased at 24 ($p = 0.038$) and 72 hours ($p = 0.011$) post exercise bouts in young (Figure 6-3B), but remained unchanged in older adults ($p \geq 0.053$; Figure 6-3D). No differences in MEP/M_{max} were observed between the bouts.

Table 6-2. Maximal compound action potential, creatine kinase, intracortical facilitation and prestimulus electromyographic activity (mean ± SD).

		Time relative to exercise							
		Pre	**Post**	**24 h**	**72 h**	**Pre**	**Post**	**24 h**	**72 h**
		Bout 1				**Bout 2**			
M_{max} (mV)	*Young*	6.5 ± 1.8	6.0 ± 1.5	6.2 ± 1.8	5.9 ± 1.7	5.8 ± 1.5	5.4 ± 1.2	6.1 ± 2.1	5.6 ± 2.0
	Older	4.9 ± 1.2	4.6 ± 1.4	5.4 ± 1.8	5.4 ± 1.9	5.3 ± 1.8	5.3 ± 1.6	6.0 ± 1.7	5.3 ± 1.7
CK ($IU.L^{-1}$)	*Young*	110 ± 99	130 ± 102	367 ± 371$^{*\#}$	298 ± 291*	86 ± 38	112 ± 49	122 ± 71$^{*\#}$	160 ± 148
	Older	97 ± 59	119 ± 58	205 ± 114$^{*\#}$	200 ± 89*	115 ± 37	126 ± 41	164 ± 51$^{*\#}$	181 ± 74
ICF (/ unconditioned MEP)	*Young*	1.21 ± 0.22	1.08 ± 0.14	1.17 ± 0.20	1.05 ± 0.17	1.09 ± 0.13	1.08 ± 0.20	1.07 ± 0.11	1.09 ± 0.23
	Older	1.10 ± 0.18	1.10 ± 0.13	1.09 ± 0.13	1.04 ± 0.25	1.05 ± 0.19	1.10 ± 0.19	1.08 ± 0.19	1.07 ± 0.19
Prestimulus RMS (% M_{max})	*Young*	7.3 ± 2.2	8.2 ± 2.6	7.4 ± 2.4	7.7 ± 2.2	7.3 ± 2.9	9.0 ± 4.5	7.5 ± 2.3	8.5 ± 2.4
	Older	10.7 ± 4.9	12.1 ± 6.1	11.1 ± 7.0	10.3 ± 6.6	9.8 ± 5.8	10.8 ± 6.1	8.5 ± 3.3	9.8 ± 4.8
5% MVC **RMS (% RMSmax)**	*Young†*	8.4 ± 3.0	11.3 ± 2.4*	7.4 ± 3.5	7.0 ± 2.9	7.9 ± 2.6	11.1 ± 3.7*	7.8 ± 2.7	8.0 ± 3.1
	Older	10.9 ± 4.8	13.5 ± 5.4*	11.5 ± 4.2	11.3 ± 4.3	11.4 ± 3.6	14.7 ± 6.6*	10.6 ± 4.4	11.3 ± 4.5
$_R$20% MVC **RMS (% RMS_{max})**	*Young*	17.5 ± 6.1	19.5 ± 5.0*	16.5 ± 4.2	15.6 ± 3.5	17.4 ± 4.4	22.6 ± 4.7*	17.3 ± 3.4	19.0 ± 5.7
	Older	24.7 ± 6.7	33.5 ± 12.2*	25.7 ± 7.6	25.1 ± 7.9	23.7 ± 5.5	27.6 ± 6.8*	23.9 ± 6.9	24.2 ± 6.7
$_A$20% MVC **RMS (% RMS_{max})**	*Young†*	17.5 ± 6.1	22.7 ± 5.0*	18.2 ± 4.0	16.3 ± 3.4	17.4 ± 4.4	25.6 ± 5.4*	19.2 ± 6.0	19.2 ± 5.7
	Older	24.7 ± 6.7	36.6 ± 12.0*	27.2 ± 10.1	25.1 ± 7.9	23.7 ± 5.5	29.4 ± 5.4*	24.2 ± 6.5	24.2 ± 6.7
5% MVC **RMS (% M_{max})**	*Young*	7.2 ± 2.5	8.8 ± 2.3*	6.5 ± 3.0	6.8 ± 2.9	6.1 ± 2.7	8.3 ± 4.6*	7.4 ± 2.7	7.8 ± 3.1
	Older	10.6 ± 7.2	11.6 ± 6.8*	11.2 ± 8.4	11.2 ± 7.6	10.4 ± 5.9	12.5 ± 8.7*	8.6 ± 3.9	9.7 ± 6.0
$_R$20% MVC **RMS (% M_{max})**	*Young†*	15.0 ± 5.1	15.2 ± 4.3	14.8 ± 4.2	15.4 ± 4.5	13.5 ± 4.8	17.0 ± 8.5	16.9 ± 4.4	18.8 ± 7.1
	Older	24.7 ± 14.6	29.3 ± 18.1	24.2 ± 14.8	24.8 ± 16.3	22.2 ± 14.1	22.7 ± 11.6	19.9 ± 6.5	20.2 ± 8.1
$_A$20% MVC **RMS (% M_{max})**	*Young†*	15.0 ± 5.1	17.5 ± 4.5*	16.5 ± 4.4	16.1 ± 4.6	13.5 ± 4.8	19.1 ± 9.0*	18.8 ± 6.9	18.9 ± 7.3
	Older	24.7 ± 14.6	31.9 ± 18.8*	25.7 ± 16.3	24.8 ± 16.3	22.2 ± 14.1	24.2 ± 12.3*	19.9 ± 6.5	20.2 ± 8.1

$^{*}p < 0.050$ relative to 'Pre', $^{\#}p = 0.029$ relative to the other bout, †$p < 0.050$ compared to older (bout × time interaction); M_{max} = maximal compound action potential; CK = creatine kinase, ICF = intracortical facilitation; Prestimulus RMS (% M_{max}) = root mean square EMG activity in the 500 ms epoch prior to stimulation at 10% isometric MVC (expressed as a percentage of maximal compound action potential); RMS (%RMSmax) = root mean square EMG activity in the 10-second epoch during a constant torque task (expressed relative to root mean square EMG activity during 500 ms around peak isometric MVC); RMS (% M_{max}) = root mean square EMG activity in the 10-second epoch during a constant torque task (expressed as a percentage of maximal compound action potential); $_R$ = relative, $_A$ = absolute. N =12 for young and n = 11 for older, except for and CK in older (n = 9) and ICF in young (n = 11) (see 'Methods' for further details).

Changes in SICI were observed following the two bouts of exercise (*main effect of time*: $F_{3, 57} = 5.3$, $p = 0.003$, $\eta_p^2 = 0.22$); an increase in the ratio of conditioned to unconditioned MEP was noted immediately post ($p = 0.019$; Figure 6-4A and 6-4C), suggesting reduced intracortical inhibition.

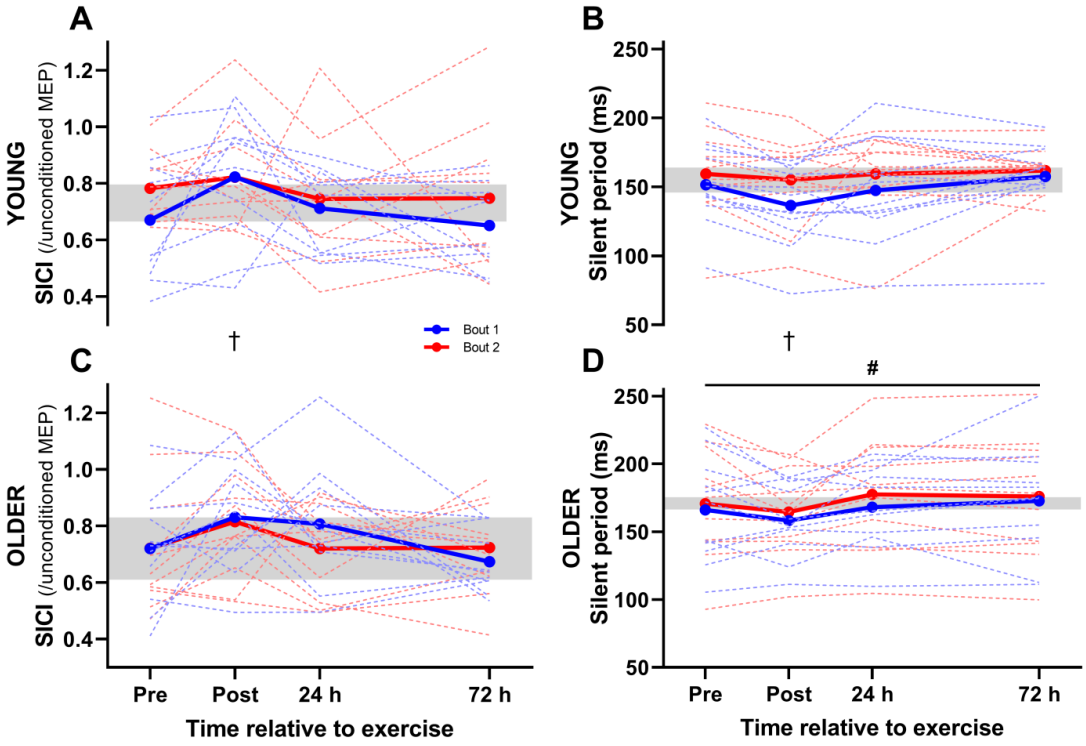

Figure 6-4. Central nervous system inhibition. Intracortical inhibition (SICI; A, C), and silent period duration (B, D) before, immediately post, and 24- and 72 hours following both bouts of exercise in young (A, B) and older adults (C, D). Dashed lines represent individual responses whereas the full line with circles denotes the mean. The grey shaded area represents the measurement error based on the difference in variability between the pre-exercise values before each bout. †$p \leq 0.044$ relative to pre-exercise (based on main effect of time); #$p < 0.05$ relative to the other exercise bout (based on main effect of bout).

For young individuals, a significant association was found between the post-exercise reduction in SICI and the extent of reduction in isometric MVC torque 24 hours post exercise ($R^2 = 0.22$, $R = -0.47$, $p = 0.036$; Figure 6-5). However, this association was not observed in older individuals ($R_2 = 0.01$, $R = -0.10$, $p = 0.645$). No other associations were shown between corticospinal and spinal responses and reductions in isometric MVC immediately post or in the days following exercise ($p \geq 0.246$). SP

was modulated in response to exercise (*main effect of time*: $F_{3, 63} = 6.3$, $p = 0.001$, $\eta_p^2 = 0.23$), such that it decreased immediately post exercise ($p = 0.044$), suggesting a reduction in inhibition (Figure 6-4B and 6-4D). This modulation of SP was greater during the first compared to the second exercise bout (*main effect of bout*: $F_{1, 21} = 6.3$, $p = 0.021$, $\eta_p^2 = 0.23$). No differences in ICF were observed ($p \geq 0.245$; Table 6-2).

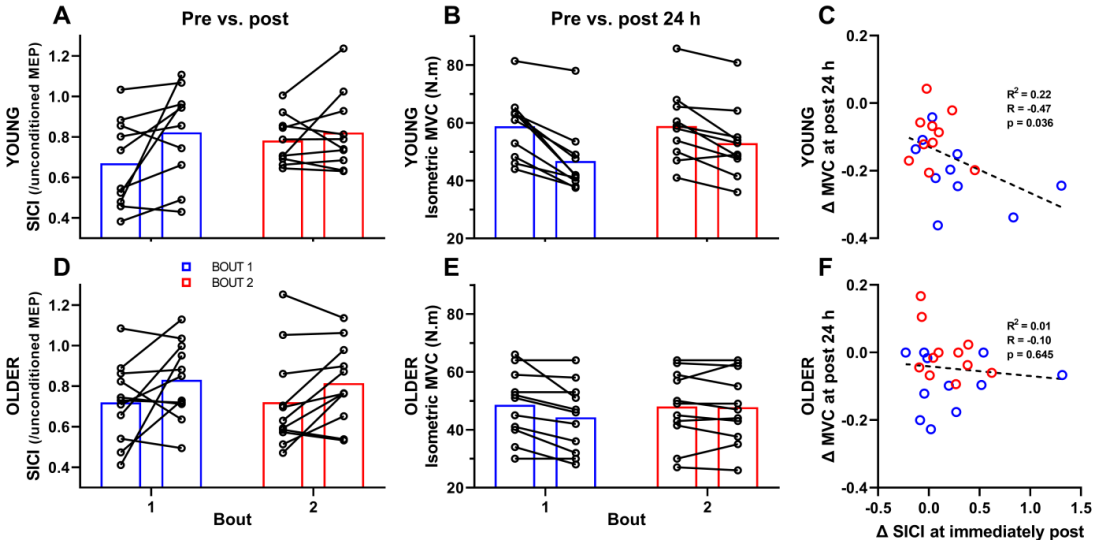

Figure 6-5. The relationship between intracortical inhibition and muscle damage. The change in short-interval intracortical inhibition immediately post exercise (A and D) was associated with the extent of muscle damage as marked by a reduction in maximal voluntary isometric (MVC) torque 24 hours post exercise (B and E), but this was only evident in young (C), and not older (F) adults.

Torque variability

Torque variability was greater in older individuals compared to young at 5% ($F_{1, 21} = 13.8$, $p = 0.001$, $\eta_p^2 = 0.40$), relative 20% ($F_{1, 21} = 12.3$, $p = 0.002$, $\eta_p^2 = 0.37$) and absolute ($F_{1, 21} = 16.8$, $p = 0.001$, $\eta_p^2 = 0.44$) 20% isometric MVC across all time points. At 5% isometric MVC, torque variability was modulated differently between the age groups across time points (*age × time interaction*: $F_{3, 63} = 3.3$, $p = 0.027$, $\eta_p^2 = 0.13$). Post hoc testing showed there was in increase in torque variability immediately post both bouts of exercise for the older group ($p = 0.007$, Figure 6-6D), whereas it

increased 24 hours following both bouts of exercise in the young ($p = 0.041$; Figure 6-6A).

At both relative (*main effect of time*: $F_{3, 63} = 6.4$, $p = 0.001$, $\eta_p^2 = 0.23$) and absolute (*main effect of time*: $F_{3, 63} = 3.4$, $p = 0.023$, $\eta_p^2 = 0.14$) 20% isometric MVC torque variability was modulated in response to exercise. At relative 20% isometric MVC, torque variability increased immediately post ($p = 0.005$) and 24 hours following both bouts of exercise ($p = 0.015$: Figure 6-6B and 6-6E), whereas it only increased immediately post exercise following both bouts of exercise at absolute 20% isometric MVC ($p = 0.012$; Figure 6-6C and 6-6F).

During a constant torque task, RMS/RMS_{max} was modulated in response to exercise at 5% (main effect of time: $F_{2.1, 43.1} = 18.0$, $p < 0.001$, $\eta_p^2 = 0.46$), relative 20% ($F_{3, 63} = 114.7$, $p < 0.001$, $\eta_p^2 = 0.41$) and absolute 20% ($F_{3, 63} = 30.5$, $p < 0.001$, $\eta_p^2 = 0.59$) of isometric MVC insofar it increased immediately post both exercise bouts ($p = 0.002$, $p < 0.001$ and $p = 0.001$, respectively; Table 6-2). Across all time points RMS/RMS_{max} was greater in older compared to younger individuals at 5% ($F_{1, 21} = 6.8$, $p = 0.017$, $\eta_p^2 = 0.24$), relative 20% ($F_{1, 21} = 14.5$, $p = 0.001$, $\eta_p^2 = 0.41$) and absolute 20% ($F_{1, 21} = 12.0$, $p = 0.002$, $\eta_p^2 = 0.37$) of isometric MVC (Table 6-2).

Similarly for RMS/M_{max} changes were noted at different time points at 5% ($F_{3, 63} = 5.4$, $p = 0.002$, $\eta_p^2 = 0.21$) and absolute 20% ($F_{2.1, 44.9} = 5.3$, $p = 0.007$, $\eta_p^2 = 0.20$) of isometric MVC during a constant torque task, increasing immediately post exercise in both bouts ($p = 0.040$ and $p = 0.004$, respectively), regardless of age. Across all time points RMS/M_{max} was greater in older compared to younger individuals at relative ($F_{1, 21} = 5.7$, $p = 0.026$, $\eta_p^2 = 0.22$) and absolute 20% ($F_{1, 21} = 4.6$, $p = 0.044$, $\eta_p^2 = 0.18$) of isometric MVC (Table 6-2).

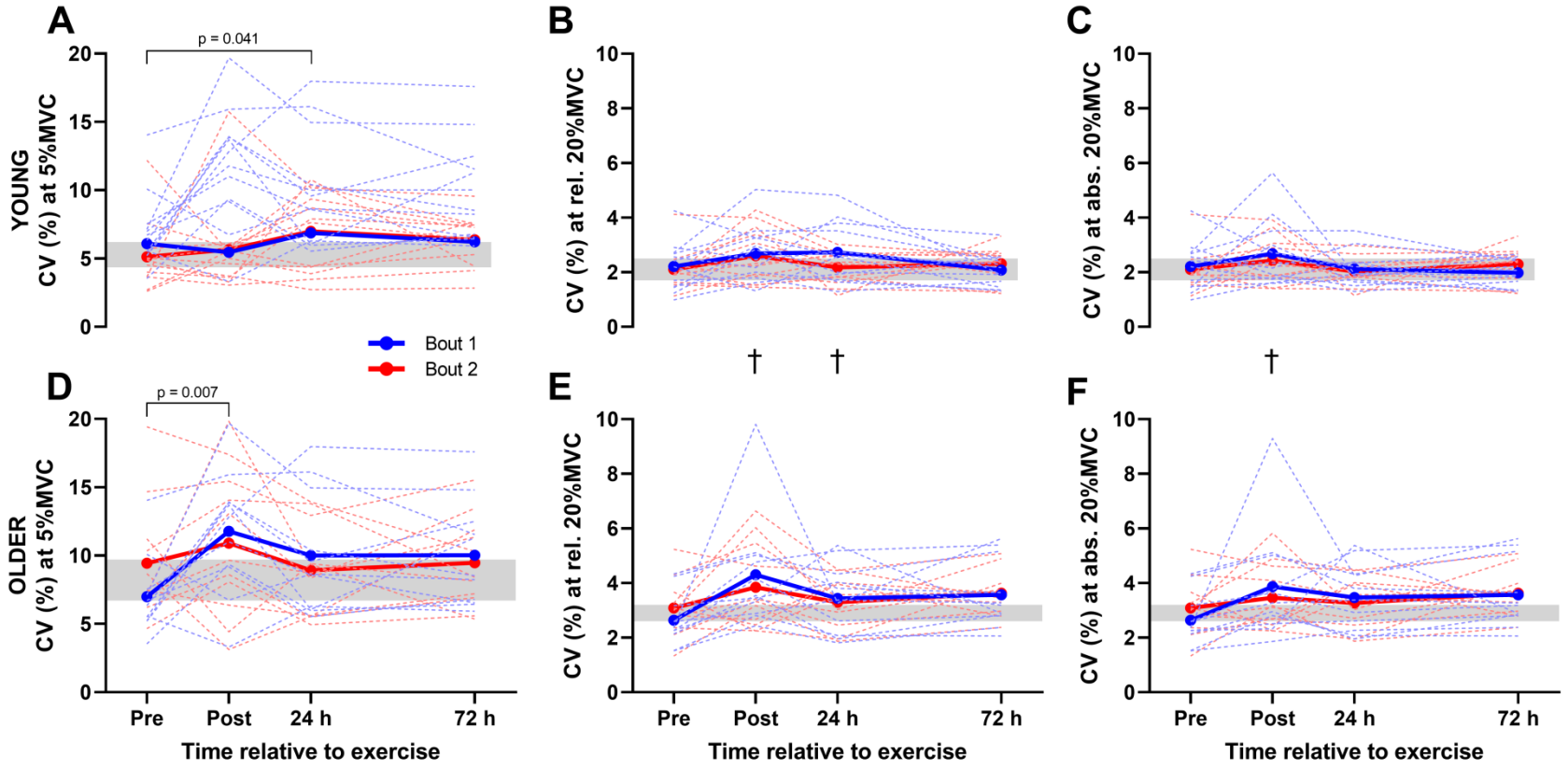

Figure 6-6. Neuromuscular performance during a constant torque task. Torque variability (coefficient of variation, CV%) during a 5 (A, D), relative (Rel.) 20 (B, E) and absolute (Abs.) 20% (C, F) of isometric maximal voluntary contraction before, immediately post, and 24- and 72 hours following both bouts of exercise in young (A, B, C) and older (D, E, F) adults. Dashed lines represent individual responses whereas the full line with circles denotes the mean. The grey shaded area represents the measurement error based on the difference in variability between the two pre-exercise values. Differences in at 5% isometric MVC are presented based on age× time interaction; †$p \leq 0.015$ relative to pre-exercise (based on main effect of time).

6-4 Discussion

The aim of this experiment was to assess corticospinal, spinal and intracortical activity in response to repeated bouts of maximal lengthening contractions in younger and older adults. A bout of maximal lengthening exercise caused a reduction in maximal torque, which was greater in young compared to older individuals. For both groups, this reduction was attenuated following a repeated bout. Corticospinal and spinal responses were modulated immediately following bouts of damaging exercise, suggesting that the observed changes were reactive to the muscle damage, rather than a protective mechanism for the repeated exercise. Older adults experienced less muscle damage, but this was not related to neurophysiological responses. These data extend our understanding about the role of the nervous system in muscle damage and the repeated bout effect throughout the life span.

Exercise-induced muscle damage and the repeated bout effect

A bout of 60 maximal lengthening contractions caused a prolonged reduction in maximal torque producing capacity regardless of age. This reduction was comparable in magnitude to that observed previously in dorsiflexors with higher volumes of exercise (150 contractions; McNeil and Rice, 2007; Power et al., 2012, 2010; Ruggiero et al., 2019). The lack of change in M_{max} suggests that this prolonged depression of maximal torque is not due to changes in sarcolemma excitability, but rather due to disruption of excitation-contraction coupling processes (Goodall et al., 2017). Twenty-four hours following exercise, isometric MVC was still reduced, suggesting that the bout of exercise was indeed damaging (Warren et al., 1999), as corroborated by the elevation in plasma CK. Furthermore, the reduction in isometric MVC was attenuated

following the repeated exercise, confirming the occurrence of RBE (Nosaka and Clarkson, 1995).

The extent of muscle damage was greater in young compared to older individuals. This cannot be attributed to the total amount of work performed because this did not differ during the two bouts of exercise between the age groups. However, older adults performed less total work per body mass, which could have contributed to less damage incurred in this group. The smaller degree of muscle damage in an aging population has been shown previously (Gorianovas et al., 2013; Lavender and Nosaka, 2006a, 2006b, 2006c) and attributed to preferential damage of type II fibres, with younger individuals often exhibiting a greater proportion of such fibres in comparison to older counterparts (Monemi et al., 1998; Naro et al., 2019). A preferential damage of type II fibres due to maximal lengthening contractions has been reported in animal (Fridén and Lieber, 1992; Lieber and Friden, 1988; Vijayan et al., 2001) as well as human studies (Fridén et al., 1983; Jones et al., 1986). However, these inferences have been questioned due to reliance on animal studies and small effect sizes in humans (Semmler, 2014). Nevertheless, the expression of RBE was similar between groups, suggesting that the adaptability of the aging neuromuscular system is preserved in response to damaging exercise of lower limbs.

Disruptions in neuromuscular performance and muscle activity

Muscle damage resulted in increased torque variability during submaximal isometric contractions immediately post exercise, as well as 24 hours after, corroborating previous work (Dartnall et al., 2008; Lavender and Nosaka, 2006a; Leger and Milner, 2001; Semmler et al., 2007). This behaviour was observed regardless of age and despite the greater torque variability of older adults at baseline, that has previously

been related to the age-related increase in variability of the common synaptic input to motoneurons (Castronovo et al., 2018; Pereira et al., 2019). Thus, our results suggest that control of muscle force is equally perturbed in young and older adults following damaging exercise.

The increased variability following damaging exercise in the young and older groups could stem from increased discharge rate variability following exercise (Dartnall et al., 2009), or increased variability of common synaptic input to motoneurons (Feeney et al., 2018) due to prolonged depression of low-frequency contractile properties (Dundon et al., 2008). At the time of increased torque variability, greater amplitude of H-reflex was also observed. This behaviour could indicate an increase in gain around the short-latency stretch reflex loop (Durbaba et al., 2013, 2005). Such an increase in gain could also contribute to concurrent increases in EMG activity post-exercise during a constant torque task (Dideriksen et al., 2015). The increased torque variability post-exercise was not dependent on exercise bout or age, suggesting it is not a variable that is adaptive in this paradigm. Overall, performance of the constant-torque task and the accompanied EMG activity, together with prolonged reduction in maximal torque producing capacity, suggest that the incurred muscle damage resulted in alterations in motoneuron pool activity.

The role of the central nervous system in the adaptive process to damaging exercise

It has been suggested that synaptic input from spinal and supraspinal centres might play a role in the adaptive process from damaging exercise (Hyldahl et al., 2017). In the present experiment, neurophysiological responses were modulated in response to damaging exercise. Maximal H-reflex increased immediately following exercise, which agrees with studies performing isometric tasks to failure (Löscher et al., 1996;

Nordlund et al., 2004; Stutzig and Siebert, 2017), but contrasts with studies showing H-reflex depression (Vangsgaard et al., 2013) or lack of change (Oza et al., 2017) following shortening and lengthening contractions. This discrepancy could be explained by methodological (Zehr, 2002) or task differences (sustained vs. intermittent, contraction intensity) among studies. As H reflex only changed transiently post-exercise, and was not altered during the 72 hrs recovery period following either bout of maximal lengthening contractions, it is likely that the changes were not involved in the adaptive process(es) underpinning the RBE, but rather reflected the acute exercise stress. The transient change, however, could stem from reduced presynaptic inhibition of Ia afferent fibres (Nordlund et al., 2004), decreased recruitment threshold of motoneurons (Dartnall et al., 2011, 2009), or a combination of these.

Changes in SICI and TMS-induced SP following damaging exercise are consistent with decreased CNS inhibition. No changes were observed in ICF, which concur with previous work following isometric contractions to task failure (Maruyama et al., 2006), but not others (Bäumer et al., 2002; Hunter et al., 2016a; Tergau et al., 2000). In young adults, the change in SICI following the first bout of damaging exercise was ~23%, similar to that observed in biceps brachii following damaging elbow flexion exercise when measured during a low intensity muscle contraction (Pitman and Semmler, 2012). However, there was only a ~5% change in SICI that was observed immediately after the second bout of exercise. This difference in the mean SICI change between the two bouts of exercise (~23 vs. ~5%) was also larger than the associated measurement error (17.8%; Table 6-1). The TMS-induced SP also showed a greater decrease in duration following the first exercise bout relative to the second. This could be related to less damage in the second bout, causing less disruption in the

characteristics of the TMS twitch (Goodall et al., 2017), which has been suggested to potentially influence SP duration (Škarabot et al., 2019). Due to the timing of post-exercise assessment, i.e. within 20 minutes of exercise, fatigue could explain modifications in CNS inhibition (Pitman and Semmler, 2012). Reductions in intracortical inhibition have been observed after fatiguing exercise with the upper (Benwell et al., 2006; Hunter et al., 2016a; Maruyama et al., 2006; Takahashi et al., 2009), but not lower limbs (Ansdell et al., 2019; Goodall et al., 2018). Furthermore, SP duration has been shown to increase post fatiguing single-joint isometric exercise (Ansdell et al., 2019; Benwell et al., 2006; Goodall et al., 2018) rather than decrease, as seen in the present study. Thus, the attenuation of the change in SICI and SP following repeated exercise suggests that this behaviour could be related to the specific exercise task employed in the present experiment, and the degree of muscle damage induced. Indeed, damaging exercise has been demonstrated to cause a near-immediate release in biochemical substrates (e.g. prostaglandin, bradykinin) and inflammation-related factors (e.g. histamine, neuropeptides; Malm et al., 1999; Pizza et al., 1995). Of particular interest is a large efflux of bradykinin that has been demonstrated immediately post damaging exercise, despite a delayed increase in CK (Blais et al., 1999), and has been shown to acutely increase the activity of group III and IV afferents (Mense and Meyer, 1988). Thus, a change in SICI following damaging exercise might be a reflection of acute alterations in afferent feedback in response to damage (Pitman and Semmler, 2012). Since muscle damage was attenuated following the second bout of exercise, it is likely that disruption to biochemical homeostasis was smaller, leading to smaller alterations in afferent feedback and thus, smaller modulation in CNS inhibitory measures. This notion is further supported by a significant association that

was observed between the reduction in intracortical inhibition and the extent of muscle damage (i.e. reduction in isometric MVC at 24 hours post-exercise) in young adults.

Interestingly, studies investigating the effect of fatigue-related group III and IV afferent feedback on neurophysiological responses showed an increase in long-interval intracortical inhibition following cycling exercise (Sidhu et al., 2018, 2017), rather than a decrease that was observed in the present study and others (Latella et al., 2019; Pitman and Semmler, 2012) following lengthening contractions. However, cycling is an activity that predominantly consists of shortening, rather than lengthening contractions. Thus, it is possible that modulation of inhibitory mechanisms is contraction-mode specific, as suggested by a recent study (Latella et al., 2019). Alternatively, afferent feedback might mediate specific inhibitory networks differently. Whilst LICI represents the activity of gamma-aminobutyric acid (GABA) B-receptors (McDonnell et al., 2006), SICI is thought to reflect GABA-A receptors (Di Lazzaro et al., 2000; Ziemann et al., 1996). Thus, increased activity of group III and IV afferents could upregulate GABA-B receptor activity causing greater inhibition, whereas GABA-A receptors could be downregulated, resulting in less inhibition.

The acute increase in biochemical substrates such as bradykinin and the associated increase in chemosensitive muscle afferents have also been shown to alter fusimotor reflexes, exciting the primary and secondary muscle spindle endings (Djupsjöbacka et al., 1995; Pedersen et al., 1997). The increased firing of muscle spindle afferents could, via inhibitory pathways, supress the corticospinal response immediately post-exercise as shown in young individuals in Chapter 4. However, at the time of return of bradykinin levels to baseline at 24 hours post-exercise (Blais et al., 1999), the inhibition will be removed, which is supported by delayed increase in corticospinal

excitability in young individuals from 24 hours post-exercise onwards in the present experiment. This delayed increase was only observed in young, but not older adults, which might be related to altered sensorimotor integration (Chapter 4).

Older adults similarly exhibited a reduction in SP duration in response to repeated bouts of damaging exercise. However, the modulation in SICI was similar between the exercise bouts (~15 vs. ~13% following bout 1 and 2, respectively), these changes were within the measurement error, and no association was noted with the extent of damage. This lack of modulation could be attributed to smaller levels of muscle damage incurred by older adults and thus, less disruption in biochemical homeostasis. Alternatively, the lack of change in SICI could be an age-specific response as older adults have been shown to exhibit attenuated afferent modulation of SICI, possibly due to altered cortical sensorimotor integration of afferent input (Smith et al., 2011a), the notion suggested in Chapter 4.

Methodological considerations

Muscle damage and RBE are complex phenomena, and thought not only to be mediated by neural factors, but also mechanical and cellular (Hyldahl et al., 2017; McHugh, 2003). Whilst the latter are equally important in adjustments of the neuromuscular system following damaging exercise, the aim of the present study was to explore neurophysiological factors, specifically processes along the corticospinal pathway. As such, the present study cannot directly ascertain the interaction between neural, mechanical and cellular mediators of muscle damage and RBE.

The time of assessment immediately post-exercise was performed within 20 minutes following a bout of maximal lengthening contractions. This makes it difficult to deduce whether immediate post-exercise modulation in neuromuscular function and

neurophysiological responses is due to damage, exercise-induced fatigue or both. Some previous investigations have performed assessments 2 hours post-exercise to try and differentiate between fatigue and damage effects on responses (Dundon et al., 2008; Pitman and Semmler, 2012). However, the available evidence also suggests that following maximal sustained isometric contractions, fatigue-related alterations in responses to TMS return to baseline values within a minute post exercise (Aboodarda et al., 2019). Nevertheless, despite not delaying the post-exercise assessment, the differential changes in certain neurophysiological responses (e.g. CNS inhibition measures) following the two exercise bouts suggest that those responses are specific to the exercise task performed in the presented experiment and are likely associated with muscle damage.

There was a significant bias in M_{max} between the two baseline scores in young individuals. Whilst some adaptation from the first bout cannot be excluded as an explanation for the observed change, it is more likely related to non-physiological factors, such as changes at the skin-electrode interface (Neyroud et al., 2015) or subtle changes in electrode placement. This is further supported by a lack of bias in measures that were normalised to M_{max}, because M_{max} represents the maximal excitation of the muscle that can be recorded, and a change in this measure will be accompanied by a corresponding change in raw amplitudes of other evoked responses (e.g. H-reflex and MEP).

The present study examined neurophysiological responses in TA following damaging dorsiflexor exercise. This specific musculature was studied due to its functional relevance to locomotion and activities of daily living, particularly in an older population, as outlined in Chapter 3. However, due to smaller muscle mass, the damaging exercise resulted in a relatively small systemic response (~2-3-fold CK

increase 24 h post-exercise) compared to previous studies in biceps brachii (~10-fold; Ref. 30). Therefore, it is possible that the present findings pertaining to afferent feedback activity actually underestimate the effect on neural inhibitory measures.

6-5 Conclusion

A bout of damaging maximal lengthening contractions caused a prolonged reduction in voluntary torque-producing capacity, which was smaller and recovered faster after the second bout of exercise, confirming the RBE. Neurophysiological responses were modulated following damaging exercise. The reduction in CNS inhibition following damaging exercise might be associated with changes in afferent feedback as a result of muscle damage, but this was observed only in young individuals, possibly due to age-related changes in cortical sensorimotor integration of afferent feedback. However, changes in neurophysiological responses were transient, not paralleling the prolonged reduction in voluntary torque producing capacity. Thus, the nervous system processes along the corticospinal pathway and within the intracortical circuitry play a limited role in the adaptive response to damaging exercise. The extent of muscle damage was smaller in older adults, but the expression of RBE was similar compared to young, and this was not related to neurophysiological responses of older individuals, contrary to our hypothesis. These data show that older adults incur less damage, but exhibit similar RBE, which has implications for exercise prescription and recovery in older age.

CHAPTER 7: GENERAL DISCUSSION

7-1 Introduction

The overall aim of this thesis was to investigate neurophysiological responses and adaptation to muscle shortening and lengthening in young and older adults. The focus was placed on dorsiflexion and the TA muscle, due to the importance of this musculature in locomotion and its link to incidence of falls in the elderly. Chapter 4 consisted of two parts. In the first part, a novel method to evoke responses in corticospinal axons at the lumbar level was developed and validated. In the second part, corticospinal responses during passive shortening and lengthening were examined using a segmental methodological approach to discern the level of neural axis at which the potential changes might occur. Additionally, corticospinal responses during passive movement were examined in older individuals and compared to the young. Chapter 5 consisted of three parts. In the first part, different existing normalisation procedures for estimates of relative torque and EMG outputs during shortening and lengthening contractions were examined, with the purpose of finding the most accurate approach for comparison of submaximal contractions of different types. In the second part, muscle fascicle behaviour was assessed during submaximal isometric, shortening and lengthening contractions, to discern whether the behaviour of contractile elements among contractions types is distinct in the experimental setup used in the present study. In the third part, age-related differences in corticospinal excitability during submaximal isometric, shortening and lengthening contractions were examined. In Chapter 6, neurophysiological responses were studied following an initial, and repeat bout of exercise involving maximal lengthening dorsiflexion in young and older adults.

The aim of this chapter is to review the main findings of this thesis and discuss them within the context of existing literature, whilst providing suggestions for future work.

A mechanistic insight into the pathways involved in regulation of muscle length in passive and active states is presented, followed by discussion of alterations in the corticospinal tract with age. Finally, the adaptability of the central nervous system during maximal lengthening contractions in the context of aging is discussed, followed by recommendations for future research. Potential functional implications of the observed neurophysiological behaviour are discussed throughout.

7-2 Summary of main findings

In Chapter 4, it was shown that passive muscle shortening and lengthening modulate corticospinal excitability in TA in young individuals, such that an increase was observed during passive shortening, whereas no modulation was noted in SOL. Based on lack of change in H-reflex amplitude, intracortical circuitry and subcortical excitability of corticospinal axons (developed and validated in Part I), it was concluded that this mediation occurs in cortical areas based on decreased inhibitory input from muscle spindle afferents. However, no change in corticospinal excitability with passive movement was observed in older adults, which might be related to impaired sensorimotor integration. In Chapter 5, it was shown that corticospinal excitability is also facilitated during submaximal shortening contractions compared to isometric and lengthening. However, this difference disappeared when responses were normalised to pre-stimulus EMG activity that differed across contraction types, despite target torque being normalised to contraction type specific maximum (as shown to be the most favourable normalisation approach in Part I of this Chapter). Normalisation of corticospinal responses to pre-stimulus EMG activity also showed reduced output of the corticospinal neurons in aging individuals during submaximal isometric, shortening and lengthening contractions, which was accompanied by prolonged

efferent conduction time and greater torque variability during dynamic contractions, despite no differences in maximal voluntary torque producing capacity. These findings might be indicative of reduced adaptability of the aging neuromuscular system in response to lengthening contractions. As such, Chapter 6 investigated neurophysiological responses and adaptation to repeated bouts of maximal lengthening contractions. The initial bout of exercise caused a prolonged reduction in voluntary torque-producing capacity, which was smaller and recovered faster after the second bout of exercise, confirming manifestation of RBE. The extent of muscle damage and the expression of RBE were smaller in older adults, but this was not related to their initial neurophysiological responses, or the changes observed following the exercise bouts. Neurophysiological responses were modulated following bouts of maximal lengthening contractions, but they were transient in nature, not paralleling the prolonged reduction in isometric MVC, suggesting that the processes along the corticospinal pathway play a limited role in the adaptive response to damaging exercise.

7-3 Corticospinal responses during muscle shortening and lengthening

Muscle shortening and lengthening modulates corticospinal tract excitability. In TA, this is the case during passive (Chapter 4) movement in young individuals and during dynamic contractions (Chapter 5) regardless of age. When background EMG differences were taken into account, no modulation of corticospinal excitability was present during active movement. This cannot be attributed to differences in fascicle shortening and lengthening during passive and active task as those were shown to be similar in Chapter 4 (passive) and Chapter 5 (active), on average ~0.6 mm per degree of joint angle change. This section will focus on different mechanisms that might

control muscle shortening and lengthening under passive and active conditions and explore the nature of purported 'unique' motor control strategy during lengthening contractions.

Differences in regulation of muscle length changes during passive and active movement – pre vs. post-synaptic mechanisms?

In Chapter 4, it was shown that corticospinal responses are facilitated during passive shortening in TA muscle compared to static position and passive lengthening in younger adults. Similarly, in Chapter 5, corticospinal responses were facilitated during submaximal shortening contractions relative to isometric and lengthening. However, shortening contractions were also accompanied by greater EMG activity. Background EMG activity affects the size of corticospinal responses (Abbruzzese et al., 1994; Gruber et al., 2009; Nordlund et al., 2002), increasing with greater EMG activity due to greater motoneuron excitability as a result of motoneurons being closer to their firing threshold with augmentation of their recruitment and firing rate (Bawa and Lemon, 1993). For that reason, normalisation of responses to background EMG activity allowed for the most valid comparison in Chapter 5. This analysis revealed lack of modulation of corticospinal responses. The question remains what are the differences between passive and active movement that could have led to what is essentially a suppression of corticospinal response during active shortening compared to passive. During active movements, a certain degree of co-contraction is bound to occur, and is also noted by antagonist (soleus) activity observed in Chapter 5. Since muscle spindle afferent activity is higher during contractions of dorsiflexors (Burke et al., 1978), SOL lengthening whilst TA is shortening could have increased Ia afferent input to corresponding Ia interneurons, and reciprocally inhibit motoneurons of TA

(Sekiguchi et al., 2003a), especially given that reciprocal inhibitory input from SOL to TA has been shown to be strong (Yavuz et al., 2018). Another mechanism that is particularly active during contraction as opposed to rest is autogenic recurrent (Renshaw cell) inhibition in motoneurons of the agonist (Pierrot-Deseilligny and Burke, 2005). However, recent research suggests that recurrent inhibition is greater during lengthening, as opposed to shortening contractions (Barrué-Belou et al., 2019, 2018), but these studies did not observe torque enhancement during lengthening contractions, thereby making the interpretation difficult (see section below for further details).

Another important consideration in the comparison between responses to passive versus active muscle shortening is the role of the gamma motoneuron system. In TA muscle in cats, an increase in gamma firing has been shown to accompany muscle shortening during locomotor activity (Taylor et al., 2006). Increased gamma firing during shortening contractions could lead to increased muscle spindle afferent feedback and hence cause inhibition at the cortical level (Chapter 4), leading to a suppression of the response. Whilst gamma motoneuron activity has not been directly recorded during dynamic contractions in humans, data from TA muscle spindle afferents during passive and active shortening and lengthening of the muscle supports the abovementioned notion, showing suppressed activity of afferents during passive shortening relative to active movement (Burke et al., 1978). As such, during passive shortening the lack of muscle spindle activity allows disinhibition in the cortex, resulting in increased corticospinal response, whereas increased gamma motoneuron firing during active shortening increases muscle spindle activity, inhibiting the cortex, and thus reducing the corticospinal response.

Interestingly, the facilitation of corticospinal responses (MEP/M_{max}) in TA during muscle shortening has been shown to occur during passive (Chapter 4) and active discrete assessments (Chapter 5), as well as during passive walking (Kamibayashi et al., 2011, 2009) and human locomotion during the swing phase (Schubert et al., 1997). This suggests that this behaviour is, at least to some extent, intrinsic to the musculature. Dorsiflexors, and by extension, the TA muscle, are involved in toe clearance (Capaday et al., 1999) and foot lift during the swing phase (Byrne et al., 2007). In both of those instances, TA is shortening. Therefore, the intrinsic increase in gain of the corticospinal output might reflect a control strategy for dorsiflexors due to their role in locomotion.

Is the activation strategy during lengthening contractions unique?

It has been purported that the activation strategy from CNS during lengthening contractions is unique (Duchateau and Enoka, 2016, 2008). Torque production during lengthening contractions is expected to be greater compared to isometric and shortening contractions, based on greater capacity of a muscle or muscle fibre to produce force during such actions (Edman, 1988; Morgan et al., 2000). However, this enhanced torque production has not always been detected in humans (Amiridis et al., 1996; Babault et al., 2001; Beltman et al., 2004; Colson et al., 2009), and studies showing 'unique' neural control of lengthening contractions have not necessarily shown torque enhancement (Hahn, 2018), thus potentially impeding interpretation of neural control of different contraction types. Indeed, when corticospinal excitability was assessed during different contraction types and torque enhancement was observed during lengthening contractions, no modulation of MEPs, cervicomedullary responses and V-waves was observed (Hahn et al., 2012). Similarly, no difference in

corticospinal excitability was noted during submaximal contractions of different types when differences in background EMG activity were taken into account (Chapter 5). Interestingly, torque enhancement of ~30% was observed during lengthening contractions compared to isometric and shortening in Chapter 5. Due to responses being normalised to contraction type specific MVC (as per Part I of Chapter 5), submaximal torque levels during lengthening contractions reflected torque enhancement. It should also be noted that when responses were compared solely during isometric and lengthening contractions (Chapter 5), in line with previous work (Gruber et al., 2009; Hahn et al., 2012; Valadão et al., 2018), no difference in corticospinal excitability between contraction types was observed. Since background EMG activity did not differ between isometric and lengthening contractions at submaximal torque levels, even without normalising to background EMG (MEP/M_{max}), showed no modulation in response size. This further reinforces the notion recently put forward by Hahn (2018), stating that corticospinal responses do not differ during lengthening contractions compared to other contraction types when torque enhancement is present. Future studies should explore this directly by grouping individuals based on the presence or absence of torque enhancement, and assessing the response to stimulations between the groups.

Alternatively, neural control of different contraction types might differ between different muscles. For example, whilst lower corticospinal excitability has been noted during lengthening contractions compared to isometric and shortening in soleus, this was not the case in gastrocnemius (Duclay et al., 2011). Similarly, Chapter 4 of this thesis showed that in young individuals, changing muscle length modulates the excitability of the corticospinal tract differently in TA and SOL, reinforcing the notion

that muscle specificity should be taken into consideration when interpreting the findings of supraspinal and spinal control of different movements.

7-3 Alterations in the central nervous system with aging

As stated in Chapter 2, literature has been equivocal regarding changes in the corticospinal tract in older age (Hassanlouei et al., 2017; Oliviero et al., 2006; Sale and Semmler, 2005; Stevens-Lapsley et al., 2013). However, these findings were previously obtained during isometric contractions only. The results of Chapter 5 suggest that an aging CNS accommodates for contraction type specific motor control, thus implying different factors might be at play. The equivocal nature of results in previous literature is also evident in response to paired-pulse TMS, with studies showing no or disparate differences in intracortical inhibitory and excitatory input between young and older adults (McNeil and Rice, 2018). The baseline scores in Chapter 6 suggest that in lower limb muscles, which have been rarely investigated, intracortical inhibitory and excitatory input is similar between young and older adults. Previous studies have also suggested that sensory axons are more affected by aging based on prolonged H-reflex latency, but lack of change in M_{max} latency (Scaglioni et al., 2003). However, the observations in Chapter 5 show a greater effect of aging on motor axons compared to sensory, based on calculation of conduction times of specific pathways, a methodological approach which is thought to be more robust (Udupa and Chen, 2013). Nevertheless, older adults seem to exhibit alterations in cortical sensorimotor integration of afferent feedback (Chapter 4). Based on previous studies showing no differences between young and older adults in cortical sensorimotor integration of increased muscle spindle firing as a result of vibration (Brown et al., 2018), it is possible that an aging CNS struggles to integrate sensory feedback from

group II afferents into the sensorimotor cortex (Chapter 4). Indeed, animal work has shown that aging is accompanied by a lower degeneration rate of group Ia afferents compared to group II afferents (Vaughan et al., 2017), which supports the notion of age-related alterations in sensorimotor integration of information originating from group II afferents.

In addition to the abovementioned alterations in the aging CNS, the findings of this thesis also suggest age-related changes in presynaptic inhibition and/or motoneuron excitability based on reduced H-reflexes concurrently with the observed reduction in MEP amplitude (Chapter 5). Furthermore, intracortical sensorimotor integration of afferent input is also attenuated with aging, based on lack of association between SICI and the extent of muscle damage as measured by reduction in MVC following repeated bouts of maximal lengthening contractions compared to younger adults (Chapter 5). The abovementioned observations are summarised in Figure 7-1.

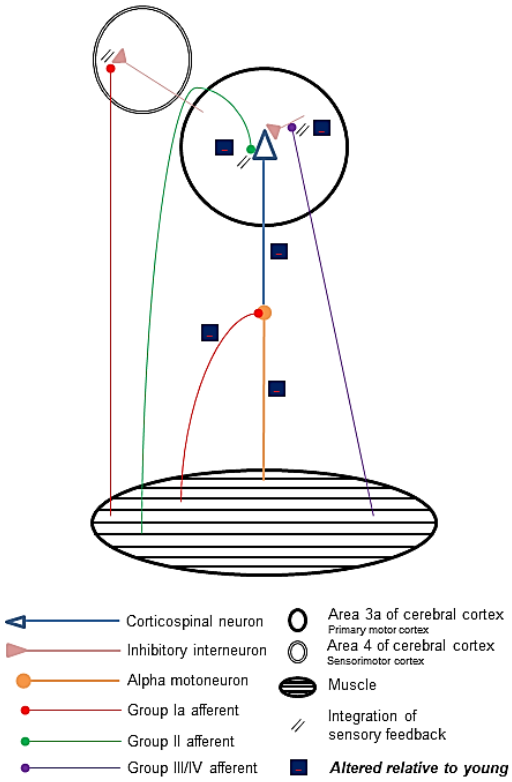

Figure 7-1. Summary of age-related alterations in various parts of the central nervous system as shown by the experimental findings of this thesis.

The remainder of this section will focus on the interplay between age-related changes in CNS and its effect on neuromuscular function, and the proposed impaired adaptability of an aging CNS.

Strength vs. central nervous system – how old is old?

Older compared to younger adults typically exhibit reduced isometric strength (Grabiner and Enoka, 1995; McNeil et al., 2005; Piasecki et al., 2018). This was not evident in Chapter 5, despite reduced corticospinal output, prolonged motor conduction time and increased torque variability. In Chapter 6 however, a reduction in isometric strength was evident in older adults. This is particularly interesting as ~80% of the sample in Chapter 6 were participants from Part III of Chapter 5, with data collected ~2 years apart (mean age of samples 64 ± 3 [range 60-71] vs. 66 ± 4 [range: 61-73] years). Whilst the decline in maximal isometric strength is expected to be smaller in dorsiflexors, due to high habitual use throughout life and thus better-preserved function (Abe et al., 2011; McNeil et al., 2007; Pannérec et al., 2016), the differences between young and older in measures of MVC could also reflect the stage of sarcopenia (McNeil et al., 2007, 2005; Piasecki et al., 2018). Thus, it seems that sufficient loss of muscle mass in TA to cause a significant decline in isometric strength occurs around the age of 65. Regardless, the data in Chapter 5 suggest that nervous system decline in older adults is observed without a decline in MVC.

The abovementioned findings raise a question as to the validity of MVC measures to classify older populations as 'old', which can be compromised for two distinct reasons. Firstly, it is possible that certain neural changes in motor axons and the corticospinal pathway occur prior to the loss of muscle mass based on Chapter 5. This could have implications for earlier detection of neuromuscular decline in older age. However,

longitudinal studies are required to directly investigate the timeframe of age-related changes in neural vs. muscular components of the neuromuscular system. Secondly, MVC might not be a sensitive enough measure to detect subtle changes in the aging neuromuscular system. Despite good repeatability of MVC measurement (Chapter 6), it has been suggested that other measures such as maximal rate of torque development are more sensitive to detect acute and chronic adjustments (Maffiuletti et al., 2016), as well as age-related decline of the neuromuscular system (McNeil et al., 2007). The relevance of the age-related isometric strength losses is also debatable, due to unlikely need to develop maximal muscle forces in activities of daily living. An argument could be made that with greater maximal strength, activities of daily living will be performed at smaller relative percentage of maximum. However, other measures such as rate of force development (Maffiuletti et al., 2016) and control of muscle force output (Hamilton et al., 2019; Mani et al., 2018) have been shown to be a better predictor of performance of functional daily tasks. Thus, it is likely such other measures are more relevant for informing adaptation of strategies to slow down the age-related degenerative process in the neuromuscular system compared to maximal isometric strength.

Regarding torque variability, an increase was noted in older adults compared to younger during dynamic contractions (Chapter 5), in line with previous observations (Laidlaw et al., 2002, 2000). However, the results of Chapter 5 did not show any difference in this measure during isometric contractions at either 25 or 50% MVC. Conversely, in Chapter 6, older adults exhibited greater torque variability during 5 and 20% of isometric MVC. Two inferences can be drawn from these results. Firstly, it is possible that the alterations in CNS are only sufficient for the age-related difference in torque variability during isometric dorsiflexion to be detected when reaching the

sarcopenic stage. Or secondly, it is possible that the differences in torque variability in relation to older age during isometric dorsiflexion are constrained to lower torque levels, i.e. below 25% MVC. Indeed, previous research has suggested that the age-difference in torque steadiness in certain muscle groups is limited to lower levels of voluntary torque (Graves et al., 2000; Tracy and Enoka, 2002). Given activities of daily living mainly occur at lower force levels, this might have significant functional applications. Unlike the relationship between torque variability and functional activities in the upper limbs of older adults (Hamilton et al., 2019; Mani et al., 2018), this link remains unexplored for lower limbs. Future studies should consider pursuing this line of investigation in dorsiflexion, perhaps by investigating the relationship between torque steadiness and stair climbing and descent, single limb stance, and different balance tasks, for example.

Corticospinal and intracortical responses – adaptability potential of the neuromuscular system?

Age-related changes in corticospinal responses have been equivocal in the literature, with studies showing either reduced output of corticospinal neurons in older individuals (Oliviero et al., 2006; Pitcher et al., 2003; Sale and Semmler, 2005), or no change (Hassanlouei et al., 2017; Smith et al., 2011b; Stevens-Lapsley et al., 2013). The reason for discrepancies among studies has been suggested to be multifactorial (Hassanlouei et al., 2017), ranging from biological sex, contractile state (relaxed vs. active) and the muscle investigated (upper vs. lower limb). The latter has been investigated recently, with results showing reduced corticospinal responses in a hand muscle, but no change in quadriceps in a sample of older adults (Rozand et al., 2019). However, it is not clear whether this is a lower vs. upper limb discrepancy per se, or

just the case of projections to different muscles being affected differently. For example, in TA, a lower limb muscle, corticospinal excitability was found to be reduced in older adults in Chapter 5. Importantly, assessments have previously only been performed during isometric contractions. Chapter 5 showed a reduced output of the corticospinal pathway regardless of contractions type. This suggests that age-related changes accommodate for specific control of different contractions types. Given corticospinal excitability was similar across contraction types when differences in background EMG activity were taken into account, other factors could explain why some studies have not observed a difference between young and older adults. One factor is contraction intensity. Previous studies have either assessed responses during rest (e.g. Oliviero et al., 2006) or during a low level contraction, i.e. 10% MVC (e.g. Hassanlouei et al., 2017). Chapter 5 provides the first investigation of age-related differences in corticospinal excitability with higher levels of contraction strength. Indeed, in Chapter 6, when responses were assessed at 10% MVC, no difference in corticospinal excitability was noted between young and older adults. This lack of difference suggests that young and older adults might differ in the gain of corticospinal output. With increasing contraction intensity, corticospinal responses tend to increase to a certain level, upon which they either plateau or exhibit a slight decline (Goodall et al., 2009; Martin et al., 2006; Todd et al., 2003; Weavil et al., 2015). This gain of the corticospinal tract has largely been related to the relationship between MU recruitment and discharge rate in modulation of muscle force output. Thus, corticospinal responses tend to increase whilst additional MUs are being recruited, whereas they plateau or even slightly decrease when MU firing frequency increases are largely responsible for further increases in muscle force (Bawa and Lemon, 1993; Brouwer et al., 1989; Gelli et al., 2007; Jones and Bawa, 1999). For TA, recruitment

of MUs is the primary modulator of force output up until ~90% of MVC (Desmedt and Godaux, 1977; Van Cutsem et al., 1998) and this does not seem to differ across contraction types (Škarabot et al., 2018). As such, it is conceivable that differences in MU properties between young and older adults might explain the difference in corticospinal responses at different contraction strengths. Indeed, older adults exhibit significant modifications in the MU, including the loss in number (Campbell et al., 1973; McNeil et al., 2005; Tomlinson and Irving, 1977) and increase in MU size, as well as decrease in discharge rate (Dalton et al., 2010; Kirk et al., 2018; Klass et al., 2008).

Intracortical and excitatory inhibitory neuron activity was not shown to differ between young and older adults as assessed in Chapter 6. A recent meta-analysis noted only a 'slight reduction' for SICI and ICF with older age, but this was not statistically significant, and large variability between studies was noted (Bhandari et al., 2016). The paired-pulse paradigms were assessed during a weak contraction in Chapter 6, and corroborate a study that assessed responses in an active hand muscle (McGinley et al., 2010). However, these authors also observed a difference between young and older adults in a relaxed state (McGinley et al., 2010). Therefore, age-related responses in SICI and ICF might be limited to relaxed muscle. Stimulus parameters might also be responsible for lack of observed differences. For example, when fixed stimulus intensity for a test pulse is used, the ratio of conditioned and unconditioned response might be constrained by the test response size (Cirillo et al., 2018; Vucic et al., 2006). For that reason, the so-called threshold-tracking approach has been used, whereby the size of the response to test (e.g. 2 mV) and conditioned stimuli (e.g. 0.2 mV) are standardised and the stimulus output required to achieve that is tracked (Cirillo et al., 2018; Vucic et al., 2006). A recent study using a threshold-tracking approach showed

a reduction in SICI in older individuals, without changes in GABA concentration in M1 as measured by magnetic resonance spectroscopy (Mooney et al., 2017). However, the differences were also only evident when ISI was 1 ms (Mooney et al., 2017). Inhibition at 1 ms is thought to reflect extrasynaptic GABA-A activity (Stagg et al., 2011b), whereas at 2-3 ms, SICI is meant to reflect synaptic activity (Ziemann et al., 1996), suggesting that the age-related changes might be constrained to the extrasynaptic GABA-A receptor activity. Based on the above, the results of Chapter 6 suggest that synaptic GABA-A activity in relation to TA is similar between young and older adults.

The question remains what the functional relevance of differing corticospinal and intracortical responses is in young and older adults. These variables have been implicated in motor learning and thus, adaptability potential of the neuromuscular system (Cash et al., 2016; McNeil and Rice, 2018). For this reason, investigation in Chapter 6 was conducted to assess whether age-related changes in the CNS output would affect the adaptability of an older neuromuscular system in response to repeated bouts of maximal lengthening contractions, but this was not the case. Maximal lengthening contractions are a gross motor action however, and it could be that the measures of cortical inhibition and facilitation are solely relevant for fine motor skills. However, in Chapter 5, no correlation was observed between age-related differences in corticospinal excitability and torque variability. Changes in corticospinal excitability and intracortical inhibition are also thought to be potent indicators of motor learning potential. However, a recent analysis of eleven studies did not show any associations between changes in corticospinal excitability and intracortical inhibition, and the magnitude of motor skill acquisition (Berghuis et al., 2017). On the other hand, cortical reorganisation with aging has been proposed to have a significant

effect on postural control, however before definitive conclusion can be made, direct experimentation is required (Papegaaij et al., 2014). Further work is needed to establish a relationship between the relevancy of cortically-mediated changes and behavioural outcomes after exercise and motor learning throughout the life span.

7-5 The adaptability of the central nervous system to maximal lengthening contractions with age

It has to be noted that investigation of neural control of shortening and lengthening usually involves assessment of discrete points in time during such behaviour of a muscle (e.g. investigations in Chapter 4 and 5). However, in real-world scenarios, muscle shortening or lengthening is usually performed in a cyclical fashion. An example of such activity is resistance exercise. Even when solely lengthening contractions are performed during resistance exercise, as has been recommended in older adults as a superior strategy to promote increases in the output of the neuromuscular system (LaStayo et al., 2003c), the movement is performed in a repetitive, cyclical fashion.

The role of central nervous system in repeated bout effect

The RBE, whereby the reduction of MVC following damaging exercise is attenuated with repeated bouts of exercise (Nosaka and Clarkson, 1995), is thought, at least in part, to be mediated by neural factors (Hyldahl et al., 2017). An early study showed changes in median frequency of EMG signal during the second bout of exercise using lengthening contractions, implying a neural contribution to the phenomenon (Howatson et al., 2007). Subsequent studies have also found that alterations of adjustments in motoneuron pool activity. For example, greater synchronisation of MU

activity has been shown in the week following the first bout of damage, which has been interpreted as a strategy of the nervous system to facilitate coordination between synergists, and thus distributing the mechanical stimulus over a greater proportion of MUs in subsequent bouts (Dartnall et al., 2011). Interestingly, reduced MU threshold and increased synchronisation following the first bout was shown to be absent after the second bout (Dartnall et al., 2011). Whilst there clearly is some neural contribution to RBE, the origin of the synaptic input from supraspinal and spinal components has not been investigated (Hyldahl et al., 2017). In Chapter 6, it was shown that the contribution of corticospinal processes to the RBE is limited, and the modulation of those responses is more likely to be reactive to changes in damage-associated increase in the activity of chemosensitive afferents. This conclusion was based on observations that modulation of neurophysiological responses was limited to the period post-exercise, whereas responses at 24 and 72 hours post-exercise were similar to baseline. In contrast, four weeks of resistance exercise involving maximal lengthening contractions have been shown to induce increases in corticospinal excitability, volitional drive from supraspinal centres (as assessed by V-wave) and increased H-reflex amplitude (Duclay et al., 2008; Tallent et al., 2017). This suggests that adaptations in corticospinal and spinal activity require longer periods of exercise for their manifestation.

Less muscle damage, but similar repeated bout effect – should exercise and recovery prescription differ for older adults?

Whilst isometric strength is often reduced in older adults, strength during lengthening contractions seems to be preserved (Power et al., 2015), as also shown in Chapter 5 and Chapter 6. As such, resistance exercise involving lengthening contractions has

been suggested to be a superior training strategy for increasing muscle strength and mass in the elderly as well as their functional ability (LaStayo et al., 2003b; Reeves et al., 2009). However, research on how older adults respond and adapt to a novel exercise stimulus involving lengthening contractions has been limited, particularly in relation to age-related alterations in CNS function. The results from Chapter 6 suggest that older adults incur smaller muscle damage for the same workload during exercise involving maximal lengthening contractions compared to younger individuals. However, older adults seem to experience similar RBE compared to young. This suggests that older adults might be able to tolerate greater volumes of resistance exercise involving maximal lengthening contractions. Thus, exercise prescription may involve higher volumes of exercise in older individuals, even in the initial stages of a novel exercise programme involving maximal lengthening contractions.

7-6 Future considerations

The results of this thesis open many research questions that could be explored in the future. Whilst some have already been considered throughout this chapter, additional ones that have been identified are presented here.

With regards to the motor control of shortening and lengthening contractions, the relative contribution of pre and post-synaptic mechanisms remains unclear. Corticospinal responses have been shown to differ during lengthening contractions when stimulations were performed at different joint angles, suggesting a large contribution of muscle spindle afferents to the observed responses during lengthening contractions (Doguet et al., 2017). Based on the results of Chapter 4 of this thesis, changing muscle length modulates corticospinal excitability relative to the extent of muscle spindle afferent firing in young individuals. However, modulation of

corticospinal responses seems absent during active shortening and lengthening as per results of Chapter 5. Corticospinal responses have also been shown to not differ during dynamic contractions at different muscle fascicle velocities (Valadão et al., 2018). These two findings are suggestive of the involvement of postsynaptic mechanisms in the control of dynamic contractions. The primary candidate for postsynaptic control is the inhibition of the Renshaw cells. Indeed, recent work suggests that the inhibitory input through the Renshaw cell is profoundly modulated during lengthening contractions (Barrué-Belou et al., 2019, 2018). Future work could examine, in a single experiment, the relative contribution of presynaptic, that is – muscle spindle afferent feedback, and postsynaptic, e.g. Renshaw cell inhibition, to the supraspinal control of dynamic contractions. This could be achieved by performing ultrasound recordings during different contraction velocities, as muscle fascicle behaviour is tightly linked to Ia afferent activity (Day et al., 2017), concurrent with a measure of Renshaw cell inhibition. The latter can be assessed with a paired H-reflex technique (Barrué-Belou et al., 2018). However, recent advances in technology, such as high-density EMG recordings, have allowed a more direct assessment (Özyurt et al., 2019). Due to transparent nature of some high-density EMG electrodes, this would also allow concurrent recording of muscle fascicle behaviour and Renshaw cell activity. However, this methodology would first need to be validated during dynamic contractions. With regard to presynaptic control, a challenge for future research is also the development of non-invasive recordings that would allow the distinction between group Ia and II muscle spindle afferents. Based on animal work (Hore et al., 1976), in conjunction with findings of Experiment 4, group II afferent activity might have a more direct effect on cortical structures. As such, being able to assess the relative

contribution of group Ia and II afferents during muscle shortening and lengthening would provide further detail to the picture of mechanistic control of movement.

This thesis primarily focused on the role of corticospinal pathway in response to muscle shortening and lengthening in relation to aging. Whilst the corticospinal pathway is regarded as the main conduit of activation signals for human movement, other descending tracts also contribute. Particularly interesting is the reticulospinal tract. In the absence of pathology, reticulospinal is secondary to the corticospinal tract in human motor control (Baker, 2011). However, reticulospinal tract is thought to be involved in the control of posture, reaching movement and performance of gross motor tasks (Baker, 2011), which are relevant in an aging adult. The vast majority of data on the reticulospinal tract is available from primate recordings, however, investigations using startle reaction and click sound stimulation have also provided human data (Aguiar and Baker, 2018; Baker and Perez, 2017). One potential avenue of research, on top of investigating the role of reticulospinal tract in various human movements that remain understudied, is the investigation of changes in this tract with aging.

The experiment in Chapter 6 assessed responses before, and in the days following exercise. However, the modulation of neurophysiological responses was not assessed during exercise involving maximal lengthening contractions, but rather before, immediately after, and 24 and 72 hours post-exercise. Given that neural changes as assessed by frequency analysis of surface EMG recordings during exercise have been shown when bouts of exercise were repeated (Howatson et al., 2007), future investigations should consider recordings responses during exercise. Furthermore, given the literature has suggested adjustments in the motoneuron pool activity, specifically a shift in MU activity among synergists (Dartnall et al., 2011), MU activity could be assessed during repeated bouts of damaging exercise, perhaps with the use

of high-density EMG recordings that allow a large population of MUs to be assessed (Merletti et al., 2008).

The experiments in the present thesis used mixed-sex samples to study corticospinal responses and compare young and older adults. However, in young populations, profound sex differences exist in CNS, with potent effects of oestrogen and progesterone on modulation of intracortical inhibition and fatigability (Ansdell et al., 2019). For that reason, females were only tested if using hormonal contraceptives where CNS function has been shown to be stable (Ansdell et al., 2019). However, the literature is less clear about sex differences in relation to aging and CNS function. In females, sex hormones fluctuate naturally throughout the lifespan (Brown, 2011), which has the potential for differing responses in females during menopause (Del Río et al., 2018). Additionally, it is unclear how hormone-replacement therapy, that has potential anti-catabolic effects (Qaisar et al., 2013), might affect neuromuscular function in both males and females (Cheng et al., 2003; Finni et al., 2011). Future research should thus explore interaction between age and sex in relation to CNS function.

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APPENDICES

APPENDIX 1 – Example of participant information sheet

Neurophysiological responses and adaptation following a bout of eccentric exercise in young and older adults

Participant Information Sheet

You are being invited to take part in this research study. Before you decide it is important for you to read this leaflet so you understand why the study is being carried out and what it will involve.

Reading this leaflet, discussing it with others or asking any questions you might have will help you decide whether or not you would like to take part.

What is the Purpose of the Study

The aim of this study is to examine the nervous system adaptations to lengthening contractions in young individuals and compare it with the adaptive response of older individuals whose potential for adaptation might be reduced. Additionally, the effect of exercise involving lengthening contractions on steadiness of force production and its adaptive response will be investigated.

Why have I been invited?

It is important that we assess as many people as possible and you have indicated that you are interested in taking part in this study.

Also, you are a person with no intracranial plates or pacemakers in your body or history of neurological disorders and are between the ages of 18 and 35 or are older than 60**.**

Do I have to take part?

No, participation in the study is individual's own decision. This information sheet is given to you to help you make that decision. If you do decide to take part, you can withdraw from the study whenever you choose, without giving the reason for doing so. You are also free to leave the study before its completion. Not participating or leaving the study before its completion will not affect you in any way.

What are the possible benefits of taking part?

By taking part in this study you will help us understand the capacity of older adults for adaptation to lengthening exercise. This might lead to better understanding and optimization of strategies to improve functional ability of the elderly.

What will happen if I take part?

If you decide to take part you will be asked to visit the Biomechanics laboratory in Sport Central of Northumbria University on the agreed dates and times 7 times. During each session, you will be sat on a chair designed to measure the force output. The first session will be a familiarization session where you will be habituated with the experimental protocol, which should last approximately an hour. About two weeks after that you will return to the laboratory for your first exercise session. Baseline neuromuscular assessment will be performed first, which will involve painless stimulations to the top of your head and painless stimulations to your skin over a nerve. Both techniques are considered non-invasive and test behavior of the nervous system. You may experience a twitch of the leg or arm as a result of these stimulations. Additionally, a capillary blood sample will be taken from your finger tip to measure a marker of muscle soreness.

After the baseline assessment you will be required to perform the exercise bout involving 10 sets of 6 repetitions of maximal lengthening contractions. After that, the baseline assessment will be repeated again. This session will last approximately 1.5 hours.

You will then be asked to return to the laboratory 1 and 3 days after the exercise session. During those visits, only baseline assessment will be performed, which will last approximately 30-40 minutes.

A week after the last session you will return to the laboratory again to repeat the exercise session as described above and then have your baseline assessment performed 1 and 3 days after.

Throughout the period of testing (two 4-day blocks) and the day before the initial visit you will be required to avoid strenuous physical activity and refrain from alcohol intake. Furthermore, you are asked to avoid caffeine intake in the hours of the day preceding the testing.

What are the possible disadvantages of taking part?

The experiment requires a degree of physical exertion, however, it will likely not, besides some small temporary fatigue, cause any issues related to your musculoskeletal system. Lengthening contractions may result in muscle soreness, but this usually subsides within a few days. In an unlikely event of bruising and high fewer in the days following the experiment, the participants will be advised to consult their GP.

The stimulation techniques used in the experiment are considered safe. It is extremely unlikely that you will have an adverse reaction to stimulation to the top of your head. In rare cases repetitive pulse stimulation to the top of one's head has induced seizures in subjects, however, this study will only be using single and paired-pulse. To further reduce the risk you will have no history or current signs of neurological disorders and no metal material in your body. Any loose objects or potential hazards will be removed from the laboratory before testing begins to ensure in the extremely unlikely event you do have a seizure the risk of injury is minimised. There is a slight risk of fainting, however, the investigator will be vigilant when monitoring you during the test and will take appropriate action if you are showing signs or symptoms of fainting. Participants may experience temporary hearing changing from the noise generated by the pulses to the top of your head. This will subside within a few hours of leaving the laboratory. Occasionally, a muscle tension-type headache and slight discomfort at the site of stimulation can occur. However, the former usually subsides within a day of leaving the laboratory, while the latter subsides immediately after cessation of the stimulus.

Stimulation to the skin of the body part under assessment is a completely safe method of investigating spinal excitability. You will feel a short, sharp "tingle" during each stimulation. All stimulations will be low level and any discomfort will immediately cease after the stimulation. To reduce any skin irritations, electrode gel will be applied to the appropriate area.

The responses recorded during the experimental sessions will be kept confidential and anonymous (see below for details).

Note that if you decide to cease participation during the experiment, you can do so without having to give us the reason why.

Will my taking part in this study be kept confidential and anonymous?

Yes. Your name will not be written on any of the data we collect. The written information you provide will have an ID number, not your name. Your name will not be written on the recorded files, and your name will not appear in any reports or documents resulting from this study. The consent form you have signed will be stored separately from your other data. The data collected from you in this study will be confidential. The only exception to this confidentiality is if the researcher feels that you or others may be harmed if information is not shared.

How will my data be stored, and how long will it be stored for?

All paper data, including the questionnaires and your consent forms will be kept in locked storage. All electronic data, including the recordings from your interview, will be stored on the University U drive, which is password protected. Any personal information will be destroyed after 3 years. All data will be stored in accordance with University guidelines and General Data Protection Regulation.

What categories of personal data will be collected and processed in this study?

Any indirectly collected personal data in this study, including contact details or health data as per the questionnaire, will be kept on a password protected computer and destroyed upon completion of your participation.

What is the legal basis for processing personal data?

The legal basis for processing the personal data required for the purposes of this study is that the research is being conducted in the public interest.

Who are the recipients or categories of recipients of personal data, if any?

Apart from the research team, there will be no other recipients of personal data.

What will happen to the results of the study and could personal data collected be used in future research?

The general findings might be reported in a scientific journal or presented at a research conference, however the data will be anonymized and you or the data you have provided will not be personally identifiable, unless we have asked for your specific consent for this beforehand.

Who is Organizing and Funding the Study?

The study is organized and funded by Northumbria University.

Who has reviewed this study?

The Faculty of Health & Life Sciences Research Ethics Committee at Northumbria University have reviewed the study in order to safeguard your interests, and have

What are my rights as a participant in this study?

As per General Data Protection Regulation you have the right to access a copy of the information comprised in your personal data upon submission of a Subject Access Request, the right in certain circumstances to have inaccurate personal data rectified; and the right to object to decisions being taken by automated means. Furthermore, if you are dissatisfied with the University's processing of personal data, you have the right to complain to the Information Commissioner's Office.

Contact for further information:

Jakob Škarabot: jakob.skarabot@northumbria.ac.uk

Dr. Rade Durbaba (supervisor): rade.durbaba@northumbria.ac.uk

Name and contact details of the Data Protection Officer at Northumbria University: Duncan James (**dp.officer@northumbria.ac.uk**

APPENDIX 2 – Examples of consent forms

CONSENT FORM

Project Title: **Neurophysiological responses and adaptation following a bout of eccentric exercise in young and older adults**

Principal Investigator: **Jakob Škarabot**

	please tick or initial where applicable
I have carefully read and understood the Participant Information Sheet.	☐
I have had an opportunity to ask questions and discuss this study and I have received satisfactory answers.	☐
I understand I am free to withdraw from the study at any time, without having to give a reason for withdrawing, and without prejudice.	☐
I agree to take part in this study.	☐

I also consent to the retention of this data under the condition that any subsequent use also be restricted to research projects that have gained ethical approval from Northumbria University.	☐

Signature of participant..... Date.....…….. (NAME IN BLOCK LETTERS).....…….
Signature of Parent / Guardian in the case of a minor .....
Signature of researcher..... Date.....…….. (NAME IN BLOCK LETTERS).....…….

Project Title: **Neurophysiological responses and adaptation following a bout of eccentric exercise in young and older adults**

Principal Investigator: **Jakob Škarabot**

I agree that the following tissue or other bodily material may be taken and used for the study:

Tissue/Bodily material	Purpose	Removal Method
Capillary blood	*Level of creatine kinase assessment*	*Via automated lancet delivery device*

I understand that if the material is required for use in any other way than that explained to me, then my consent to this will be specifically sought. I understand that I will not receive specific feedback from any assessment conducted on my samples, but should any kind of abnormality be discovered then the investigator will contact me.

Signature of participant.....
Date.....……..

Signature of Parent / Guardian in the case of a minor

.....
Date.....……..

Signature of researcher.....
Date.....……..

APPENDIX 3 – Neurological PAR-Q

Participant Screening (Neurological PAR-Q)

Name _____Phone number: _____Date_____

How old are you? _____ Weight _____ Height _____

Please answer the following questions:

Have you ever broken a bone in your arm and/or hand? YES NO

Have you ever broken a bone in your leg and/or foot? YES NO

Do you have pain in your arms and your hands? YES NO

Do you have pain in your legs and your feet? YES NO

Have you ever been diagnosed with a neurological disorder, particularly epilepsy? YES NO

Have you ever been diagnosed with a brain disorder such as Parkinson's disease? YES NO

Have you ever had a stroke? YES NO

Do you have any metal objects in your head? YES NO

Are you taking any medications that you know would affect neuronal conduction? YES NO

Do you have a pacemaker? YES NO

Have you had any operations involving your heart? YES NO

Do you have a metal plate in the skull, metal objects in the eye or skull (for example after brain surgery or shrapnel wounds)? YES NO

Are you pregnant or seeking to become pregnant in the near future? YES NO

Have you ever had an allergic reaction to shaving or adhesive on electrodes? YES NO

The information I have given is correct to the best of my knowledge at the time of completion.

Signature of
Participant.....Date.....
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APPENDIX 4 – Lateral preference inventory

Lateral Preference Inventory

Please answer the following question by ticking the appropriate box:

	L	***R***	***Either***
With which hand do you draw?	☐	☐	☐
Which hand would you use to throw a ball to hit a target?	☐	☐	☐
In which hand would you use an eraser on paper?	☐	☐	☐
Which hand removes the top card when you are dealing from a deck?	☐	☐	☐
With which foot would you kick a ball to hit a target?	☐	☐	☐
If you wanted to pick up a pebble with your toes, which foot would you use?	☐	☐	☐
Which foot would you use to step on a bug?	☐	☐	☐
If you had to step up onto a chair, which foot would you place on the chair first?	☐	☐	☐

The information I have given is correct to the best of my knowledge at the time of completion.

Signature of Participant.....Date.....
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