IMPACT OF FATIGUING EXERCISE ON CORTICOSPINAL EXCITABILITY AND MOTOR PERFORMANCE IN YOUNG AND OLDER ADULTS

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CONTENTS

ABSTRACT 7
DECLARATION 9
ACKNOWLEDGEMENTS 10
1. LITERATURE REVIEW 11
1.1. MOTOR CONTROL 12
1.1.1. Human motor cortex 12
1.1.2. Transcranial Magnetic Stimulation 13
1.1.2.1. Physiology underlying TMS 14
1.1.2.2. Application of TMS 16
1.1.2.3. Single pulse TMS 16
1.1.2.4. Paired pulse TMS 17
1.1.3. Intracortical inhibition and γ-aminobutyric acid (GABA) 18
1.2. CENTRAL FATIGUE, TMS AND CORTICOSPINAL EXCITABILITY 20
1.2.1. Central fatigue measures via voluntary activation 20
1.2.2. Corticospinal excitability during single-joint fatiguing contractions 22
1.2.3. Intracortical Inhibition (SICI, LICI and SP) during Fatigue 24
1.3. AGEING, INTRACORTICAL INHIBITION AND FATIGUE 25
1.3.1. Age-related changes in the central nervous system 26
1.3.1.1. The brain 26
1.3.1.2. The motor unit 28
1.3.2. Effects of fatiguing exercise in older adults 29
1.3.3. Intracortical inhibition in older adults 32
1.4. MOTOR PERFORMANCE, AGEING AND FATIGUE 35
1.4.1. Plasticity, Motor Performance and Ageing 36
1.4.2. Motor Performance and Fatigue 39
1.5. NEUROMODULATION, MOTOR PERFORMANCE AND FATIGUE 40
1.5.1. Transcranial direct current stimulation 40
1.5.2. TDCS, motor performance and ageing 42
1.5.3. TDCS and fatigue 45
1.6. SUMMARY AND AIMS 48
2. SINGLE JOINT FATIGUING EXERCISE DECREASES LONG BUT NOT SHORT-INTERVAL INTRACORTICAL INHIBITION IN OLDER ADULTS 53
2.1. ABSTRACT 53
2.2. INTRODUCTION 54
2.3. METHODS 56

2.3.1. Experimental set-up and EMG recordings 57
2.3.2. Experimental protocol 57
2.3.3. Peripheral nerve stimulation 60
2.3.4. Transcranial magnetic stimulation 60
2.3.5. Intracortical Inhibition 61
2.3.6. Data analysis 62
2.3.7. Statistical analysis 63
2.4. RESULTS 64
2.4.1. Baseline Measures 64
2.4.2. SICI Session 67
2.4.3. LICI Session 70
2.5. DISCUSSION 73
2.5.1. Main findings 73
2.5.2. Fatigability in young and older adults 73
2.5.3. Change in MEP measures of corticospinal excitability 74
2.5.4. SICI and LICI post fatiguing exercise in a resting muscle 76
2.5.5. Methodological Considerations 77
2.5.6. Conclusion 78
3. SUSTAINED SUBMAXIMAL ISOMETRIC FATIGUING EXERCISE OF THE ELBOW FLEXORS HAS NO AGE-RELATED EFFECT ON $GABA_B$ MEDIATED INHIBITION 83
3.1. ABSTRACT 83
3.2. INTRODUCTION 84
3.3. METHODS 86
3.3.1. Experimental set-up 87
3.3.2. Electrical Recordings 87
3.3.3. Experimental protocol 88
3.3.4. Electrical stimulation of the brachial plexus 92
3.3.5. Transcranial magnetic stimulation 92
3.3.6. Data analysis 95
3.3.7. Statistical analysis 95
3.4. RESULTS 96
3.4.1. Biceps Brachii 97
3.4.1.1. Force and EMG 97
3.4.1.2. Corticospinal excitability measures 100
3.4.1.3. Triceps Brachii 103

3.5. DISCUSSION 104
3.5.1. Main findings 104
3.5.2. Fatigability 105
3.5.3. $GABA_B$ mediated inhibition 106
3.5.4. Corticospinal excitability 109
3.5.5. Conclusion 110
4. SUSTAINED SUBMAXIMAL ISOMETRIC FATIGUING EXERCISE OF THE ELBOW FLEXORS HAS NO AGE-RELATED EFFECT ON MOTOR PERFORMANCE 114
4.1. ABSTRACT 114
4.2. INTRODUCTION 115
4.3. METHODS 116
4.3.1. Experimental set-up 118
4.3.2. Electrical Recordings 118
4.3.3. Fitts Movement Task 120
4.3.4. Experimental protocol 121
4.3.5. Data Analysis 125
4.3.6. Statistical Analysis 125
4.4. RESULTS 125
4.4.1. Baseline measures 125
4.4.2. Time to Task Failure and Force 126
4.4.3. Simple Movement Time Task 127
4.5. DISCUSSION 129
4.5.1. Main findings 129
4.5.2. Short Control Contraction 129
4.5.3. Sustained single joint fatigue 131
4.5.4. Age related difference in MT 132
4.5.5. Conclusion 133
5. PRIMING TDCS DOES NOT FACILITATE SUBSEQUENT TDCS EFFECTS ON FATIGABILITY AND MOTOR PERFORMANCE IN YOUNG AND OLDER ADULTS. 138
5.1. ABSTRACT 138
5.2. INTRODUCTION 139
5.3. METHODS 141
5.3.1. Experimental set-up 142
5.3.2. Electrical Recordings 144
5.3.3. Experimental protocol 144
5.3.4. Electrical stimulation of the brachial plexus 147

5.3.5. Transcranial magnetic stimulation 147
5.3.6. Transcranial direct current stimulation 148
5.3.7. Simple Movement Time Task 149
5.3.8. Data analysis 151
5.3.9. Statistical analysis 152
5.4. RESULTS 152
5.4.1. Baseline measures 153
5.4.2. Time to Task Failure 155
5.4.3. MVCs 155
5.4.4. EMG_{rms} during 5% EMG contraction 157
5.4.5. Corticospinal excitability measures 158
5.4.6. M_{max} 163
5.4.7. Simple movement time task 163
5.4.7.1. Baseline measures 163
5.4.7.2. Post fatigue measures 164
5.5. DISCUSSION 167
5.5.1. Effects of primed anodal tDCS on corticospinal excitability post fatiguing exercise 167
5.5.2. Effects of primed anodal tDCS on $GABA_B$ mediated inhibition post fatiguing exercise 170
5.5.3. Fatigability and motor skill performance 171
5.5.4. Conclusion 173
6. GENERAL DISCUSSION 175
6.1. *Age related difference in corticospinal excitability measures and motor skill performance with fatigue* 175
6.2. *Impact of muscle and exercise type on age related changes in corticospinal excitability with fatigue* 177
6.3. *SP versus LICI* 179
6.4. *TDCS as a potential clinical intervention for reduced fatigability* 180
7. CONCLUDING REMARKS 183
8. BIBLIOGRAPHY 185

Publications arising from thesis

Otieno LA, Semmler JG, and Sidhu SK (2021). Single joint fatiguing exercise decreases long but not short-interval intracortical inhibition in older adults. *Exp Brain Res* 239: 47-58.

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Additional publications

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ABSTRACT

The impact of fatigue on corticospinal excitability and GABAergic inhibitory activity has been relatively well studied in young adults. However, this is yet to be established in older adults. Furthermore, the role of fatigue on motor skill performance remains largely understudied with a majority of the studies ranging between the 1970s-90s in young adults. The purpose of this thesis was to identify the age-related differences in corticospinal mechanisms and motor performance with isometric single joint fatiguing exercise. This was achieved by applying single and paired-pulse transcranial magnetic stimulation (TMS) prior to, during, and after exercise, as well as performance of a speed-accuracy movement task in both age groups. The first experimental study (chapter two) evaluated the effect of fatigue induced by a fifteen-minute sustained submaximal isometric contraction (15% of maximum electromyography [EMG]) of the first dorsal interosseous (FDI) muscle on corticospinal excitability, short- (SICI) and long- (LICI) interval intracortical inhibition in young and older adults. While no change in SICI was identified in both age groups, an age-related reduction in amount of LICI was seen suggesting a compensatory decline in $GABA_B$ mediated inhibition in older adults. Nevertheless, a varying magnitude of fatigue was observed between young and old which required further investigation. The second experimental study (chapter three) implemented a larger muscle group (elbow flexor muscles) and a submaximal isometric contraction held at 30% of their maximum force to task failure to achieve a similar amount of fatigue in both age groups. Contrary to chapter two, an identical decline in $GABA_B$ mediated inhibition was observed in both age groups when a similar amount of fatigue was induced, indicating that fatigue related changes in GABA modulation may be task and muscle dependent. Using a similar exercise model, the third experimental study (chapter four) investigated age-related differences in performance of a speed-accuracy task with fatigue. While there was an attenuation in motor skill performance with increasing task difficulty, there

were no differences across age groups. Given the noted impact of fatigue on corticospinal excitability and motor performance in chapters two, three and four, investigation of transcranial direct current stimulation (tDCS) as a possible intervention was assessed as the final step. TDCS can be used to manipulate fatigability either as a priming tool or in conjunction with a fatiguing task. In the final study (chapter five), the impact of cathodal primed anodal tDCS on corticospinal excitability, fatigability, and motor skill performance was assessed in both age groups with an unexpected suppression in corticospinal excitability and greater attenuation in $GABA_B$ observed following anodal tDCS. However, cathodal priming as standalone had no direct impact on corticospinal excitability, GABA modulation, fatigability, or motor skill performance.

This thesis provides novel evidence of an age-related retention in the ability to modulate corticospinal excitability and motor performance when a similar amount of fatigue is induced in young and older adults.

DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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1. LITERATURE REVIEW

Fatigue imposes a significant effect on our ability to learn and perform everyday tasks such as independent household work (Bonner et al. 2010; Close et al. 2020; Hofman et al. 2007), childcare (Cooklin et al. 2012), long-distance driving (May and Baldwin 2009), or exercise (Belza 1994). Once fatigued, a decline in performance is usually evident and rest is required to recover before resuming these activities. With ageing, comes a predisposition to a varying magnitude and duration of fatigue due to age-related changes within the central and peripheral nervous system (Hunter et al. 2016b). While older adults do portray less fatigability during isometric tasks compared to the young (Hunter et al. 2005; Yoon et al. 2012), this advantage is lost during dynamic activities where older adults experience increased fatigability and delayed recovery compared to the young (Callahan and Kent-Braun 2011; Dalton et al. 2012; Yoon et al. 2013). This can impact their ability to perform day to day tasks.

Although fatigue can be induced in a variety of ways, this thesis will focus on the effects of exercise-induced fatigue on the neuromuscular system (neuromuscular fatigue). For a long time, it was believed that neuromuscular fatigue was attributed mainly to peripheral changes within the muscle (Fitts 1994). However, there is evidence to show that factors within the central nervous system (CNS) also contribute significantly to fatigue, a phenomenon known as central fatigue (Gandevia 2001). It is the progressive decline in maximal voluntary activation of a muscle/muscle group during exercise. This can be attributed to fatigue related changes in input to the motoneuron pool from descending motor pathways such as the corticospinal tract and/or spinal reflex circuits (Gandevia 2001).

This chapter aims to provide a comprehensive review of the literature investigating the effects of fatigue on corticospinal excitability and motor performance in young versus healthy older adults. The focus will be on changes in excitatory and inhibitory processes within the primary motor cortex as a consequence of fatigue, measured by non-invasive brain stimulation

techniques such as transcranial magnetic stimulation (TMS) as well as fatigue's impact on motor skill performance. Furthermore, the gaps in knowledge addressed by specific research questions within the thesis will also be identified.

1.1. MOTOR CONTROL

1.1.1. Human motor cortex

The neocortex is comprised of several areas within the frontal agranular cortex responsible for motor control. These include the premotor area, supplementary motor area and primary motor cortex. Pre-motor and supplementary motor cortices are located rostral to the primary motor cortex (M1) while M1/Brodmann's area 4 is located in the rear end of the frontal lobe, immediately in front of the central sulcus and next to the somatosensory cortex. M1 assists in voluntary control of skeletal muscle movement (Penfield 1950).

The idea of an area responsible for motor function within the cerebral cortex existed for many centuries. Robert Boyle proposed this in the early 1600s following his case report on a patient with a depressed cranial fracture caused by a horse riding accident (Rengachary and Ashan 2007). However, this concept was not realised until John Hughlins Jackson observed that convulsions in epileptic patients travelled between neighbouring muscles suggesting that M1 had an orderly representation of the body mapped on its surface (Jackson 1873). Further physiological evidence was provided when Ferrier performed electrical stimulation experiments on animals such as cats and dogs, and showed similar results (Ferrier 1874). Nonetheless, it was not until Albert Leyton and Charles Sherrington first detailed the map of the primate motor cortex using electrical stimulation that the motor cortex was characterised (Leyton and Sherrington 1917). Wilder Penfield further expanded on these findings by applying direct electrical stimulation to the cerebral cortex of epileptic patients undergoing surgery. This resulted in the construction of a topographical map known as the homunculus, for the sensory cortex (Penfield and Boldrey 1937) and later the motor cortex (Penfield 1950).

The cellular makeup of the cortex is characterised by a complex yet flexible nature (Brodmann and Garey 2006). It is made up of six horizontal layers present in the cerebral cortex with layer I closest to the outer surface and layer VI preceding the white matter (Cajal 1911). The presence or absence of different cell types is mainly what differentiates each layer. This is because the cerebral cortex is made up of two types of cells, projection neurons (pyramidal cells) and interneurons (stellate cells). These cells determine which layers receive information and which layers send out information to other regions. For instance, the size of layer IV, which primarily expresses stellate cells, is significantly diminished in the primary motor cortex, but quite extensive in the primary visual cortex because it is mainly associated with receiving sensory information from the thalamus and is considered an output region of the cortex. In humans, pyramidal cells are located significantly within layers III, V and VI, and are driven by glutamate as their neurotransmitter (Kandel et al. 2000). In the primary motor cortex, these large pyramidal cells originate from layer V and terminate directly onto the motor neurons located in the ventral horn of spinal cord. They provide the most direct pathway for voluntary movement (Kandel et al. 2000). Traditionally, it was believed that neurons forming direct pathways to the spinal motor neurons originated only from the primary motor cortex while surrounding motor regions transmitted their output to the motor cortex for movement execution. However, more information found that premotor areas also have direct projections to the spinal motor neurons (Dum and Strick 1991); but likely have a higher threshold of activation to evoke movement (Dum and Strick 2002). Nevertheless, while multiple regions within the neocortex project directly onto the spinal motor neurons, voluntary movement is primarily executed by the primary motor cortex.

1.1.2. Transcranial Magnetic Stimulation

Initial investigations into the brain areas responsible for movement were restricted to the application of electrical current directly on the exposed cortex. However, due to the

invasive nature of these techniques, the practicality of these studies remained limited. As a result, Merton and Morton (1980) developed a method that allowed direct activation of the brain in awake and intact human subjects by applying high voltage electrical currents onto the scalp. Application of these currents over M1 resulted in twitch-like movements of the contralateral hand and leg. However, the high resistance of the scalp and skull tissue to electrical stimulation resulted in very little current penetrating to the brain. Therefore, the superficial excitable tissues were activated generating contraction of scalp muscles and activation of pain receptors leading to subject discomfort and pain.

Barker et al (1985) were able to provide a solution to this problem by applying strong magnetic pulses on M1 via a flat coil (100 mm in diameter) passed from a high voltage capacitor discharge system. This resulted in twitch like movements in the contralateral hand and leg with minimal subject pain and discomfort. The highly resistant scalp and skull does not impede magnetic pulses and therefore allows them to easily penetrate the brain and subsequently generate an electrical current in the underlying neuronal tissue. The induced current results in activation of the corticospinal cells and generation of recordable responses from the muscles via electromyography (EMG). This technique is referred to as TMS and is now implemented worldwide in many clinical and research settings. Indeed, it has significantly aided in the understanding of motor control in humans.

1.1.2.1. Physiology underlying TMS

Animal studies have shown that direct application of an electrical stimulus onto the motor cortex produces a series of high frequency discharges within the corticospinal neurons (Patton and Amassian 1954). The first wave, referred to as the D-wave, occurs due to direct activation of the corticospinal neurons whereas the latter waves (I-waves), which appear approximately 1.5 ms later, are said to originate from interneuron activity causing trans-

synaptic depolarisation of the same corticospinal neurons (Kernell and Chien-Ping 1967; Phillips 1956).

The D/I wave hypothesis has also been explored further in humans. Day and colleagues indirectly showed that corticospinal neuron activation occurs according to the D/I-wave system by looking at single motor unit responses following cortical stimulation (Day et al. 1989). Moreover, studies have been able to compare differences in corticospinal responses between transcranial electrical stimulation (TES) and TMS by measuring corticospinal volleys directly from electrodes implanted in the cervical epidural space of patients undergoing treatment of intractable pain (Di Lazzaro et al. 1998a; Nakamura et al. 1997). When TES was applied, the first volley generated was a D-wave, similar to the responses recorded in animals (Kernell and Chien-Ping 1967; Patton and Amassian 1954; Phillips 1956). However, when TMS was applied, the onset of the first wave was approximately 1.5 ms later than that elicited by TES indicating the first I-wave within the volley (I_1). Furthermore, as TMS intensity increased, latter I-waves became more apparent and D-waves begin to appear at moderate to high intensities. This suggests that corticospinal neurons within M1 are preferentially activated trans-synaptically by TMS with direct activation only occurring at high intensities.

The differences in D/I wave recruitment have been attributed to the direction of current flow in each technique. In TES, current is transmitted radially from its source and penetrates into the brain; while in TMS, current flows parallel to the surface of the brain (Rothwell et al. 1991). Therefore, neurons that are oriented perpendicularly to the surface of the brain, such as the pyramidal cells of the corticospinal tract, have a higher threshold to activation via TMS compared to TES (Rothwell et al. 1999), while those oriented parallel to the brain surface such as the interneuron projections to the corticospinal cells have a lower threshold for activation via TMS (Day et al. 1989). This has been demonstrated by studies that have altered the direction of TMS-induced current by manipulating coil orientation (Werhahn et al. 1999). When current

was directed anteriorly (parallel to the brain's surface), responses from single motor unit recordings and EMG were delayed by approximately 1.5 ms (Werhahn et al. 1999). However, when the current was directed latero-medially (perpendicular to the brain's surface), onset latencies of responses were similar to those generated by TES.

1.1.2.2. Application of TMS

When applied over the primary motor cortex, TMS evokes a response in the peripheral musculature known as a motor evoked potential (MEP; Barker et al. 1985). The amplitude of the MEP indirectly reflects the excitability of the corticospinal pathway and spinal motor neurons activated by the pulse at the time of stimulation. TMS can be used to assess M1 excitability in one of two main ways: single or paired pulse TMS.

1.1.2.3. Single pulse TMS

Single pulse TMS can be used to assess corticospinal excitability in four main ways. The first is by mapping the cortical representation of a target muscle (Wassermann et al. 1992). This is done by systematically applying stimulations at a constant intensity along neighbouring scalp sites closest to the most optimum site of stimulation (cortical hotspot) until no MEP response is observed. A functionally relevant cortical map of the target muscle is then produced via graphical representations (Bashir et al. 2013; Davies 2020; Wilson et al. 1993). The second method is by gradually increasing stimulus intensity over the cortical hotspot. The resulting MEP amplitudes can then be used to produce a sigmoidal curve when plotted against the relevant TMS stimulus intensities. This curve represents the corticospinal input-output properties (Devanne et al. 1997). The third method is via observing changes in the MEP amplitude in response to interventions or pathology. The MEP amplitude reflects neural excitability of the elements innervating the target muscle and acts as an indirect measure of the number of corticospinal neurons activated by TMS when normalised to maximal motor response i.e. excitability of the corticospinal tract (Paulus et al. 2008). The final method of

application is known as the motor threshold. This is the lowest TMS intensity that can be used to produce a reliable MEP response within the target muscle. This method reflects excitability of the corticospinal neurons as well as the corticocortical interneurons projecting onto the corticospinal neurons (Paulus et al. 2008; Rothwell et al. 1991). However, it is important to note that an MEP response is not only dependent on synaptic excitability at the supraspinal level (intrinsic of the cortex) but also spinal (motoneurons) and the neuromuscular junction (Devanne et al. 1997). Motor threshold can be measured in two ways: the resting motor threshold (RMT) and active motor threshold (AMT). RMT is measured during complete relaxation of the muscle at a minimum intensity where MEP amplitude is more than 50 μV in 5-10 consecutive stimulations (Carroll et al. 2001; Rossini et al. 1994). AMT is measured during a low intensity isometric contraction (5-10% of maximum muscle force, MVC) to keep the muscle tonically activated. AMT is similarly defined as the minimum intensity at which MEP amplitude is ≥ 200-300 μV (Rothwell et al. 1999) in 5-10 consecutive stimulations. Due to a decrease in threshold to activation of cortical and spinal excitability, AMT stimulation intensity is expected to be lower than that required for RMT (Garry and Thomson 2009).

1.1.2.4. Paired pulse TMS

Paired pulse TMS is used to determine the effect of corticocortical excitatory and inhibitory projections onto the corticospinal neurons. This stimulation paradigm involves the use of two magnetic stimuli separated by a short duration. The first stimulus (conditioning pulse) activates the interneurons within M1 while the second stimulus (test pulse) activates the corticospinal neurons responsible for generating the MEP response. When the conditioning pulse precedes the test pulse, its effects temporarily summate at the corticospinal cell resulting in changes in the test MEP amplitude (Kujirai et al. 1993; Valls-Sole et al. 1992). Activation of the inhibitory circuits will reduce test MEP amplitude while activation of excitatory circuits will increase test MEP amplitude.

Whether the circuit activated is inhibitory or excitatory is dependent on the conditioning and test stimulation intensity as well as the time interval between the stimuli (interstimulus interval or ISI). For example, when a conditioning pulse intensity set below RMT (subthreshold) is applied 3-5 ms prior to the test stimulus above RMT (suprathreshold) (Kujirai et al. 1993), inhibition of the test MEP is observed which is thought to be mediated by γ-aminobutyric acid A ($GABA_A$) inhibitory interneurons. This is referred to as short interval cortical inhibition (SICI) (*See Fig 1.1*). On the other hand, when a suprathreshold conditioning stimulus is applied 100-150 ms prior to the test MEP, inhibition of the test MEP is observed due to activity of the $GABA_B$ inhibitory interneurons (Valls-Sole et al. 1992). This is known as long interval cortical inhibition (LICI) (*see Fig 1.1*). However, when a subthreshold conditioning pulse is applied 10-20 ms prior to the test pulse, facilitation of the test MEP is observed which is thought to be mediated by glutamergic interneuron activation (Paulus et al. 2008). This is referred to as intracortical facilitation (ICF). This thesis will focus primarily on paired pulse inhibitory circuits (SICI and LICI).

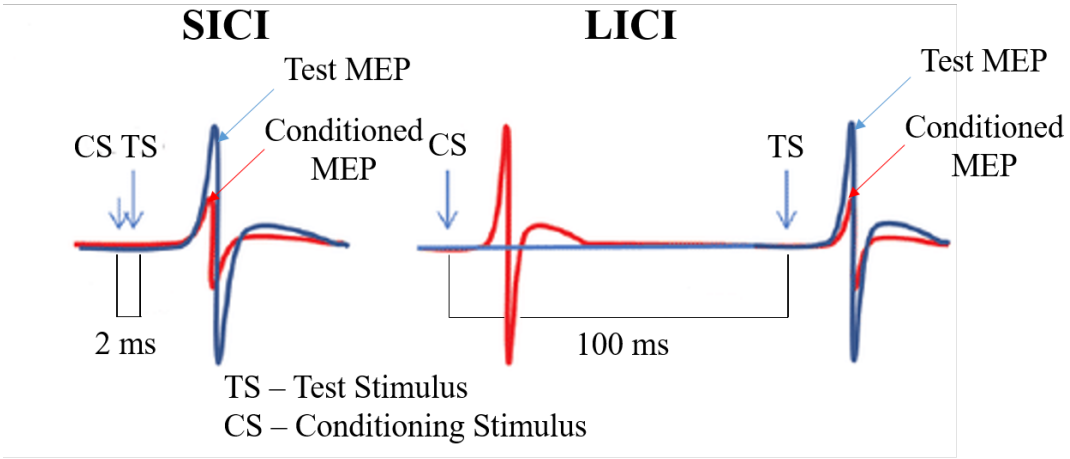

Figure 1.1: *Schematic showing paired-pulse inhibitory measures SICI and LICI.*

1.1.3. Intracortical inhibition and γ-aminobutyric acid (GABA)

Intracortical inhibition (ICI) refers to the suppression of neuronal activity by cortical GABA inhibitory interneurons (Krnjevic 1997). GABA is a major inhibitory neurotransmitter

generated via the alpha-decarboxylation of glutamate by glutamic acid decarboxylase (GAD) and metabolised to succinate with the help of GABA-transaminase (GABA-T) and succinic semialdehyde dehydrogenase (SSADH) (Petroff 2002). It mediates its actions via 2 classes of receptors; ionotropic $GABA_A$ receptor mediated or metabotropic $GABA_B$ receptor mediated (Connors et al. 1988). A third receptor class known as $GABA_c$ has also been described (Cutting et al. 1991) however research shows that $GABA_c$ receptors are a specialised subtype of $GABA_A$ and are now referred to as $GABA_{Ac}$ according to the International Union of Pharmacology (Barnard et al. 1998).

$GABA_A$ and $GABA_B$ receptors demonstrate different structural compositions. $GABA_A$ is a ligand gated ionotropic chloride channel with five transmembrane domains clustered around a central ion pore. These receptors, located postsynaptically, prompt the influx of Cl- ions after the binding of GABA. This causes hyperpolarisation of the affected cell resulting in inhibitory post-synaptic potentials (IPSPs) generation. Furthermore the ionotropic nature of $GABA_A$ allows them to rapidly modulate membrane excitability causing the inhibitory potentials generated to be fast and short lived (Farrant and Nusser 2005). Conversely $GABA_B$ receptors are metabotropic G-coupled receptors and heterodimeric in nature. These receptors are located both presynaptically and postsynaptically as well as on extrasynaptic membranes (Benarroch 2012). $GABA_B$ receptors influence IPSP generation via increased activation of potassium channels postsynaptically, reduced activation of voltage-dependent calcium channels presynaptically and inhibition of adenylyl cyclases (Benarroch 2012). Furthermore, due to their metabotropic nature, $GABA_B$ receptor mediated activity results in prolonged IPSP generation causing a much more delayed change in the polarisation of affected cells.

Interestingly, animal studies have shown that GABAergic activity may affect the onset of fatigue. Abdelmalki et al (1997) demonstrated that administering baclofen ($GABA_B$ agonist) one hour prior to exercise results in an increase in run-time to exhaustion in trained and

untrained rats suggesting that the pharmacological administration of GABAergic agonists can delay the onset of fatigue. Chen et al (2016) also demonstrated that administration of crystallised GABA on male NIH mice results in an increase in loaded-swimming time compared to control. These studies show that GABAergic cells play a crucial role in the onset and development of exercise induced fatigue, however the role of inhibition on exercise capacity and fatigue in humans is not as well understood.

1.2. CENTRAL FATIGUE, TMS AND CORTICOSPINAL EXCITABILITY

1.2.1. Central fatigue measures via voluntary activation

As discussed briefly in section 1.1, central fatigue refers to the progressive decline in maximal voluntary activation (VA) of a muscle/muscle group during exercise. Measurement of VA via twitch interpolation is one of the most traditional methods used to quantify central fatigue because changes in VA reflect alterations in voluntary drive from the brain to the muscle during maximal effort (Bigland-Ritchie et al. 1986; Gandevia 2001; Merton 1954). Here, a superimposed twitch elicited by an electrical stimulus delivered to the muscle or nerve during a contraction is assessed and compared to the twitch evoked at rest. The increment in force observed in response to the stimulation given during contraction reflects the force reserve still present during muscle activity, which may indicate that the stimulated axons were discharging at sub-tetanic rates (Herbert and Gandevia 1999; Merton 1954). To quantify VA, the amplitude of the superimposed twitch is expressed as a fraction of that elicited from the resting muscle (Thomas et al. 1989), given that the twitch elicited at rest represents maximal activation of all motor units.

Changes in VA have been reported in the context of fatiguing exercise to represent the development of central fatigue (Gandevia 2001; Gandevia et al. 1996). This was first identified by Merton (1954) who reported that the increment in force produced by a supramaximal stimulus to the ulnar nerve supplying the adductor pollicis decreased linearly as voluntary force

increased. Merton also stated that no additional force was evoked at maximal voluntary effort. However, this experimental set-up could not easily detect subtle force increments as low as 5% during maximal contractions, whereas modern systems can detect increments as low as 1% (D'Amico et al. 2020; Kennedy et al. 2015; Khan et al. 2011; Prasartwuth et al. 2005; Todd et al. 2003a; Todd et al. 2004). For example, using a more refined system with similar methodology, Gandevia and McKenzie (1988) reported that a difference of about 5% in the superimposed twitch may still be present during maximum contractions suggesting that submaximal activation occurs during maximal force production.

Other neurostimulation techniques such as TMS have also been used to quantify VA. Several studies have shown that VA, measured using TMS, declines during single-joint fatiguing exercise of both upper and lower limb muscles (Hoffman et al. 2009; Mileva et al. 2012; Smith et al. 2007; Sogaard et al. 2006). This is demonstrated by an increase in the size of the superimposed twitch indicating that inadequate descending drive from the brain to the muscle contributes to a decline in maximal performance (i.e., supraspinal fatigue). A reduction in descending drive at or above the motor cortex (Sidhu et al. 2009a; Sidhu et al. 2009b; Smith et al. 2007; Todd et al. 2003a) accounts for up to one quarter of the decline in maximal force during fatiguing exercise. However, VA measurement via TMS should also be approached with caution given the known technical challenges. One such challenge is the inability to obtain a resting twitch measurement in a relaxed muscle with TMS. This is because corticospinal excitability increases substantially with contraction strength (Di Lazzaro et al. 1998a; Rothwell et al. 1991). Therefore, the same magnetic stimulus evokes less cortical output and recruits fewer motor units during rest. As a result, an alternative method has been implemented where the resting twitch size is estimated based on the extrapolation of the linear relationship between size of the superimposed twitch and voluntary force (Todd et al. 2004). Furthermore, the unavoidable activation of the antagonist motor neuron pool at higher TMS intensities presents

a challenge given that this directly affects the size of the superimposed twitch (Todd et al. 2016). A similar problem occurs with the inadvertent stimulation of the cortical representation of other synergist muscles following TMS (Todd et al. 2016). Nevertheless, TMS remains a useful tool in the investigation of changes in corticospinal excitability with fatigue.

1.2.2. Corticospinal excitability during single-joint fatiguing contractions

TMS has been used to investigate changes in excitability of the corticospinal tract during fatiguing contractions (Vollestad 1997). Since central fatigue is known to limit single joint and whole-body exercise, it is important to understand the influence of exercise induced fatigue on excitability of the corticospinal pathway where transmission of descending drive from higher brain centres to skeletal muscles to evoke contractions predominantly occurs (i.e. the motor cortex and descending motoneurons) (Brouwer and Ashby 1990). Decreased excitability of this pathway can be attributed to a decline in excitability of motor cortical neurons and spinal motoneurons thereby requiring increased synaptic input from the motor cortex and/or spinal motor neurons to maintain muscle activation. Fatigue studies involving single-joint exercise have revealed that during intermittent maximum voluntary contractions (MVCs), the size of the MEP grows in response to TMS, even when corrected for changes within the muscle, suggesting an increase in the excitability of the corticospinal pathway (Benwell et al. 2007b; Mileva et al. 2012; Taylor et al. 2000; Taylor et al. 1996). A similar growth is observed during submaximal sustained voluntary contractions as low as 5-20% MVCs (Smith et al. 2007; Sogaard et al. 2006; Williams et al. 2014; Yoon et al. 2013; Yoon et al. 2012). However, a more gradual increase in MEP amplitude is observed during fatigue contrary to the steep initial increase seen in MVCs (Sogaard et al. 2006). Although the increase in MEP response may be indicative of an increase in excitatory output from the motor cortex, this conclusion cannot be made without comparative data from the spinal cord. This is because it is not possible to distinguish between changes in excitability of the motor cortex and spinal

motoneurons via an MEP alone. One way to account for this is through electrical stimulation of the lumbar spinal segments (Skarabot et al. 2019) or cervicomedullary junction (CMEP) (Petersen et al. 2002; Taylor 2006; Taylor and Gandevia 2004; Ugawa et al. 1991). CMEP responses represent changes in excitability of the motoneuron pool as corticospinal axons are activated directly without the involvement of cortical circuitry (Taylor 2006). Multiple studies have investigated the changes in CMEP *during* sustained fatiguing contractions in both upper (elbow flexors) and lower limb muscles (plantar flexors) (Butler et al. 2003; Hoffman et al. 2009; McNeil et al. 2009; Taylor et al. 1996), and have concluded that the growth in MEP amplitude observed during fatigue is attributed to changes predominantly within the motor cortex since no corresponding increase in CMEP was observed (Butler et al. 2003; Hoffman et al. 2009; McNeil et al. 2009; Taylor et al. 1996).

Changes in MEP response post exercise have also been characterised via TMS. Brasil-Neto et al (1993) provided preliminary data to show that the MEP was attenuated following repetitive wrist flexion and extension contractions sustained to task failure (i.e. post exercise depression) when measured at rest. In the same study, TES was also applied immediately after exercise to directly activate the spinal motoneurons and no change in the size of this response was observed. This suggests a reduction in responsiveness of the cortical cells but no change in motoneuron excitability. Since then, multiple studies have reported a similar depression in MEP following both sustained isometric short duration MVCs and isometric submaximal contractions lasting up to 30 minutes (Gandevia et al. 1999; McKay et al. 1996). Interestingly, McNeil et al (2011a) identified a similar degree of attenuation in CMEP responses elicited during a 10 min sustained 25% EMG contraction. Contrary to the findings made by Brasil-Neto and colleagues, this suggests that a reduction in motoneuron excitability (possibly related to repetitive motoneuron discharge during a sustained contraction) may be responsible for the depression in MEP observed rather than suppression in motor cortical excitability. However,

these measurements were collected during activity, not at rest. On the other hand, a transient increase in MEP to magnetic stimulation has also been reported immediately after exercise – a phenomenon referred to as post contraction facilitation (Brasil-Neto et al. 1993; Samii et al. 1997). While the mechanisms underlying this remain unclear, a similar facilitation was not observed in MEPs elicited by TES (Brasil-Neto et al. 1993) suggesting that post exercise facilitation may be intracortical in nature.

1.2.3. Intracortical Inhibition (SICI, LICI and SP) during Fatigue

TMS has also been instrumental in the investigation of intracortical inhibition (ICI) via single pulse measures such as the silent period (SP) and paired pulse paradigms like SICI and LICI during fatiguing exercise. When single pulse TMS is delivered during a voluntary contraction, a suppression in the EMG signal is observed following the elicited MEP response (known as the SP). The earlier part of the SP (< 50 ms) is thought to be mediated by spinal mechanisms such as recurrent inhibition of spinal motoneurons and after-hyperpolarisation (Inghilleri et al. 1993); while the latter part (> 100 ms) is thought to be mediated by $GABA_B$ mechanisms and has been shown to last up to 200 ms. Following transmastoid stimulation (stimulation at the cervicomedullary junction) or TMS, SP duration increases when measured during fatiguing contractions involving upper limb muscles indicating an increase in inhibition (Taylor et al. 1996). Nonetheless, the longest EMG silence observed following transmastoid stimulation was ~60 ms shorter than the duration of the TMS evoked SP with fatiguing exercise (Taylor et al. 1996). This suggests that any modulation in spinal inhibition during fatigue ceased before commencement of voluntary output from the motor cortex and is therefore unlikely to contribute to the fatigue related increase in TMS evoked SP duration observed. This instead can be attributed to increased $GABA_B$-ergic activity (Benwell et al. 2007b; McKay et al. 1996; Mileva et al. 2012; Taylor et al. 2000) based on pharmacological evidence (McDonnell et al. 2006). However, evidence now shows that the spinal portion of SP accounts

for more than half of the measurement rather than the initially hypothesised 50 ms (Yacyshyn et al. 2016). Nevertheless, during maximal and submaximal contractions, both TMS evoked SP duration and MEP size increase, suggesting a contradictory increase in the excitability of both corticospinal neurons and inhibitory interneurons (Benwell et al. 2007b; Sogaard et al. 2006; Taylor et al. 1996).

To investigate this paradox, paired pulse TMS techniques were implemented to determine changes in GABA mediated inhibitory mechanisms. The two most common paradigms are SICI (Benwell et al. 2006a; Kujirai et al. 1993; Valls-Sole et al. 1992) and LICI (Benwell et al. 2007b; McDonnell et al. 2006; McNeil et al. 2011b; Valls-Sole et al. 1992). Contrary to SP, fatiguing studies show a decrease in both SICI and LICI as well as a concomitant increase in MEP size. This suggests an increase in corticospinal excitability and decrease in intracortical inhibition during fatiguing single-joint exercise. However, this has also been challenged by work that reported an increase in LICI following intermittent 2 min sustained MVC fatiguing contractions and a 10 min sustained submaximal contraction held at 25% EMG in young adults (McNeil et al. 2011b; McNeil et al. 2009). Methodological disparities could be the major contributor for this inconsistency. For example, differences in exercise model implemented i.e., whilst Benwell and colleagues implemented intermittent 7 s brief MVCs for 10 min to fatigue the muscle, McNeil and colleagues implemented 2 min sustained MVCs. Furthermore, Benwell and colleagues measured LICI after a brief MVC (at rest), whereas McNeil and colleagues collected paired pulse measurements during sustained 2 min MVC, which were shown to be influenced by changes in spinal cord excitability when measured during muscle activity (McNeil et al. 2011b; McNeil et al. 2009).

1.3. AGEING, INTRACORTICAL INHIBITION AND FATIGUE

Ageing is accompanied by significant changes in the anatomy and physiology of the CNS and/or muscle that can affect functionality and contribute to the differences in fatigability

between young and old adults. CNS changes such as reduction in size of the cortex (Raz et al. 2007), and attenuated cortical (Dickstein et al. 2007; Oliviero et al. 2006) and motoneuronal excitability (Lexell 1997; Sale and Semmler 2005; Scaglioni et al. 2002) may contribute to age-related differences in fatigability. The following sections will highlight some of the neuromuscular changes that occur with ageing as well as a summary of TMS studies that have investigated fatigability, corticospinal excitability and intracortical inhibition in older adults. In addition, current gaps in knowledge will also be identified.

1.3.1. Age-related changes in the central nervous system

1.3.1.1. The brain

Research into the age-related changes within the brain has produced some conflicting results over the years (Raz et al. 2005). This most likely stems from differences in methodology, subject cohort characteristics and study design (cross-sectional versus longitudinal). However, there appears to be a consensus regarding regional sensitivities in the brain to ageing effects with differences seen across varying cortical areas (DeCarli et al. 2005; Resnick et al. 2003), subcortical regions (Raz et al. 2005; Walhovd et al. 2011) and white and grey matter (Ge et al. 2002; Salat et al. 2004; Salat et al. 2005). For example, Walhovd et al (2011) found variations in the rate of volumetric change in white and grey matter amongst different regions at different time points in the lifespan. Some structures such as the cerebral cortex show a consistent linear reduction from a young age while other structures like the hippocampus and cerebral white matter demonstrate an initial volume increase in the younger years, a plateau in the middle years and a rapid decline in the later years (Walhovd et al. 2011). The physiological processes underpinning these trends are not completely understood. However, it has been suggested that changes seen during the early years of life indicate maturation while those seen in the later years indicate ageing (Ge et al. 2002; Raz et al. 2005). Indeed, there have been reports suggesting some effects of ageing on motor control are linked

to a reduction in cortical thickness observed in the precentral gyrus (M1) (Salat et al. 2004) and degradation in areas of the corticospinal tract (Salat et al. 2005).

Ageing has also been shown to affect neural activity patterns in older adults. This has been captured using positron emission tomography (PET) or functional magnetic resonance imaging (fMRI) whilst participants performed specific tasks. Activation changes typically show less lateralised areas of activation with a noticeable bilateral activation in the prefrontal areas (Cabeza 2001). This specific pattern was referred to as hemispheric asymmetry reduction in older adults (Cabeza 2002). This can be explained by either compensation or dedifferentiation. The compensation model suggests that the increased pattern of neural activation acts as a form of plasticity to compensate for the functional and structural deficits that occur with ageing (Cabeza et al. 1997). On the other hand, the dedifferentiation model suggests that this increase in the pattern of neural activation results from a breakdown of neural processes with age leading to inefficient and non-specific activation of brain areas in older adults (Logan et al. 2002). Bilateral activity has also been reported in the primary sensory and motor areas during unilateral motor tasks (Mattay et al. 2002). However, studies investigating the link between neural activation levels and motor control have shown that increased levels of physical activity in older subjects may be beneficial to performance, thus supporting the compensatory hypothesis (Seidler et al. 2010).

Ageing is also known to be associated with changes in neurochemistry. Animal models show a reduction in glutamate, the major excitatory neurotransmitter within the CNS, with ageing (Segovia et al. 2001), which has further been confirmed in humans via magnetic resonance spectroscopy (MRS) (Kaiser et al. 2005). There is also some evidence from post-mortem tissue analysis to suggest that GABA concentrations are reduced within multiple brain regions as a consequence of ageing (Spokes et al. 1980). MRS studies identified a decline in GABA concentration within the frontal and parietal regions of the brain in older adults (Gao

et al. 2013; Porges et al. 2017). However, no age-related difference in GABA concentration in the bilateral primary motor cortex, bilateral dorsal premotor cortex and presupplementary motor area has been identified.

1.3.1.2. The motor unit

Age related changes occur not only within the CNS, but also in the periphery. For example, with ageing comes a loss of alpha motor neurons attributed to spinal motor neuron apoptosis and distal axon retraction (Campbell et al. 1973; Hepple and Rice 2016). This results in denervation of the muscle fibres (Roubenoff and Hughes 2000) leading to muscle fibre atrophy (a process referred to as sarcopenia) or reinnervation of the fibres by surviving motor neurons via axonal sprouting (Lang et al. 2010). In the case of reinnervation, the muscle fibre will adjust its properties such as contraction speed and fatigability to that of the surviving motor unit. This process of denervation and reinnervation accelerates with advanced age and leads to fewer surviving motor units (Campbell et al. 1973; Fling et al. 2009) resulting in functional consequences for older men and women starting as early as 50-60 years old (Reid and Fielding 2012). Evidence suggests that this atrophy occurs to a greater extent in fibres expressing myosin heavy chain (MHC) II isoforms resulting in greater expression of MHC I isoforms (Hunter et al. 1999; Lexell et al. 1988). The type II muscle fibres are also referred to as the fast twitch muscle fibres due to their higher myosin ATPase (ATP-splitting) activity. The higher the ATPase activity, the more rapidly ATP is split and the faster the rate at which energy is made available. Therefore, higher expression of type II muscle fibres would result in higher contraction speeds and peak power output. On the other hand, the type I fibres have lower myosin ATPase activity resulting in slower twitches (slower contraction speed and less power output). Type I fibres also rely heavily on oxidative phosphorylation to synthesise ATP (resulting in higher ATP yields) making them more resistant to fatigue whereas type II fibres rely heavily on glycolytic phosphorylation which yields a much lower ATP concentration

making them less resistant to fatigue. Therefore the age-related increase in the proportion of type I fibres likely results in more fatigue resistant muscles during isometric exercise with less contraction speed, strength and peak power output (Trappe et al. 2003), an observation that remains prevalent in older adults.

1.3.2. Effects of fatiguing exercise in older adults

Despite the deterioration of the neuromuscular system with ageing, older adults are characterised by increased tolerance for exercise. For example, in both maximal and submaximal isometric fatiguing contractions, younger adults fatigue faster than older adults even when matched for strength (Hunter et al. 2005; Kent-Braun 2009). In addition, older adults take longer to approach task failure than their younger counterparts, during a 20% sustained isometric contraction (Yoon et al. 2013; Yoon et al. 2012), and intermittent sustained maximal (22 s) voluntary contractions of the elbow flexor muscles separated by 10 s intervals (Hunter et al. 2008). Furthermore, younger adults experienced a more significant decline in MVC post fatiguing contraction compared to the older adults. This advantage in fatigability amongst older adults does not appear to be muscle specific as similar observations have been made in the lower limb muscles. For example, Callahan and Kent Braun (2011) reported greater fatigue in younger adults compared to older adults when performing intermittent 5 s MVCs of the knee extensor muscles in 5 s intervals for a total of 4 min. This resistance to fatigue has been attributed to the slower contractile properties of the muscle in older adults mediated by the increase in proportion of type I fibres (slow contracting and slow to fatigue) (Hunter et al. 1999; Hunter et al. 2008). Yoon et al (2012) also confirmed this age-related difference in proportionality by identifying an association between peak relaxation rates of the muscle and time to task failure. Peak relaxation rates were determined during each MVC by calculating the steepest fall in torque during the EMG silent period following TMS (Todd et al. 2007). Results showed that peak relaxation rates were faster in younger adults than older adults both

before and after the fatiguing contraction, providing further evidence that older adults have a greater proportion of the slower type I fibers (Hunter et al. 1999).

However, the age-related fatigue resistance seems to be dependent on exercise type. During high velocity dynamic contractions, older adults exhibit greater reductions in maximum force compared to the young in the lower limbs. Callahan and Kent-Braun (2011) showed a more significant decline in maximum voluntary dynamic contraction force (MVDC) in older adults compared to young adults following a performance of 120 MVDCs (performed once every 2 s at $270^{o}.s^{-1}$ through a 70^{o} range of motion [ROM]) in the knee extensor muscles. A similar observation was recorded by Dalton et al (2012) when participants were instructed to voluntarily move as fast as they could at a fixed resistance of 20% MVC through a 90^{o} ROM at an unconstrained velocity. This increase in fatigability with dynamic exercise can also be attributed to the age-related loss of type II fibres, which are responsible for high force velocity contractions thus giving young adults an advantage. Furthermore, metabolic demand of the muscle is high during repeated dynamic contractions which leads to accumulation of inorganic phosphate and hydrogen ions (Krustrup et al. 2003; Sundberg et al. 2018a). This results in a decrease in contractile force and actin-myosin head detachment rates as documented in vitro (Debold et al. 2008). Given that reduced myosin head detachment rates have been observed in the older unfatigued muscle due to the differences in fibre type composition (Hook et al. 2001), any further decrease in detachment rates would exacerbate their ability to produce power and force at high velocities. Therefore, while slowed contractile properties of older muscles may improve their resistance to fatigue during isometric contractions, the slower kinetics also result in greater fatigue during high velocity contractions.

Interestingly, the age-related difference in fatigability is not observed during slow or intermediate contractions (Callahan and Kent-Braun 2011; Dalton et al. 2012). Yoon et al (2013) observed no difference in fatigability between young and old when both age groups

were instructed to perform dynamic contractions of the elbow flexor muscles at a fixed resistance of 20% MVC through a 90º range of motion at 1 cycle every 3 s until task failure. Subjects were required to select their preferred speed of contraction and strategy during the dynamic task. This suggests that older adults with slower contractile properties may show greater fatigue resistance when contraction velocity is adjusted for individual torque-velocity characteristics during slow to moderate dynamic exercises. No doubt differences in muscle fibre type composition play a significant role in fatigability between young and older adults. Therefore, it is important to take exercise model differences into consideration when investigating age-related differences in fatigability.

With regards to central fatigue measures, a greater decline in VA is observed in older adults compared to younger adults during low-intensity sustained contractions (Hunter et al. 2008) suggesting greater decline in central drive to the muscle with ageing (Rozand et al. 2020). Furthermore, although MEP size increases with fatigue in older adults, the amount of increase is attenuated when compared to that of young adults (Hunter et al. 2008) which suggests less net excitation of the corticospinal tract and motoneuron responsiveness with fatigue in older adults (Hunter et al. 2008). A slight increase in intracortical inhibition mediated by fatigue is also demonstrated as SP increases in older adults. However, this increase is greater in younger adults compared to their older counterparts (Hunter et al. 2008; Yoon et al. 2012), which indicates an age-related impairment in modulation of inhibitory processes (Oliviero et al. 2006; Peinemann et al. 2001). Older adults also have a shorter SP duration compared to young adults (Oliviero et al. 2006; Sale and Semmler 2005) suggesting a difference in $GABA_B$ mediated inhibitory processes. However, SP is not the best indication of intracortical inhibitory measures since it is thought to be influenced by spinal mechanisms (*see section 1.2.3*).

1.3.3. Intracortical inhibition in older adults

Multiple discrepancies exist within the literature regarding age-related differences in ICI between young and old adults. SICI has been the most commonly investigated intracortical inhibitory paradigm in older adults. Some studies have reported reduced SICI with ageing (Peinemann et al. 2001) while others have reported increased SICI (McGinley et al. 2010), as well as no difference between age groups (Bhandari et al. 2016; Cirillo et al. 2010; Cirillo et al. 2011; Oliviero et al. 2006; Rogasch et al. 2009; Smith et al. 2009). Although the exact mechanisms underlying these discrepancies remain unknown, methodological factors such as differences in ISIs, conditioning intensities and test pulse are thought to contribute. Of these factors, the conditioning stimulus (CS) intensity is one of the most important considerations (Orth et al. 2003). Evidence suggests that varying CS intensities may recruit different populations of low threshold interneurons (Oliviero et al. 2006; Peinemann et al. 2001; Peurala et al. 2008; Wassermann 2002). However, the best technique for assessing SICI has been the application of multiple, sequentially increasing conditioning intensities producing what we refer to as a SICI recruitment curve. This allows for a more comprehensive assessment of SICI characteristics when assessing different populations with unknown intracortical inhibitory recruitment profiles (Rosenkranz et al. 2007a). Some studies have implemented this technique where three intensities, 70%, 80% and 90% AMT (Cirillo et al. 2010; Rogasch et al. 2009; Sale et al. 2016) or seven (Smith et al. 2009) ranging from 60%-120% AMT in 10% increments have been used to determine the most effective conditioning intensity. All studies reported no age-related changes in inhibition at any point of the recruitment curves. However, this finding may also be attributed to TMS coil orientation. Many studies implement the more conventional posterior-anterior (PA) coil orientation. However, there is evidence to suggest that when using the more sensitive anterior-posterior (AP) coil orientation, a pronounced increase in SICI is observed in older adults compared to the young (Sale et al. 2016). This is because the neural

elements that are susceptible to SICI preferentially excite the corticospinal pathway in the AP orientation (Di Lazzaro et al. 2012; Zoghi et al. 2003). Nonetheless, further investigation into the impact of coil orientation during paired pulse TMS measures remains outside the scope of this thesis.

Differences in test TMS parameters are also an important consideration for the discrepancies observed in the literature. Modulation of inhibition has been demonstrated through alterations in both test MEP amplitude and test TMS intensity (Sanger et al. 2001). These observations resulted in a debate regarding the most appropriate method of setting the test MEP size, relative to threshold or as a target amplitude. Within the literature, the process of targeting a specific MEP amplitude (usually 1 mV) has been the more popular option. In any case, when test parameters were controlled for (test MEP set to absolute values, percentage of AMT or normalised to maximum compound muscle action potential (M_{max})), no age-related differences in SICI modulation were observed (Opie and Semmler 2014a). This suggests that differences in test TMS parameters between studies may not be a key contributor to the reported inter-study variability.

To the authors knowledge, age-related differences in LICI have only been assessed in two studies (McGinley et al. 2010; Opie and Semmler 2014a). McGinley et al (2010) reported an increase in the magnitude of LICI in older adults compared to the young. In this study, test pulse intensity was set at an intensity producing an MEP of 0.5-1 mV, with conditioning and test stimuli separated by an ISI of 100 ms. Although maximal inhibition has been routinely produced at an ISI of 100 ms, limited research suggests that this ISI may not be sufficient to allow recovery of spinal excitability following the suprathreshold conditioning stimuli (McNeil et al. 2011b; McNeil et al. 2009). Since age-related changes in excitatory and inhibitory processes appear to occur predominantly within the spinal cord (Earles et al. 2001; Kido et al. 2004), the observations made by McGinley and colleagues may have been influenced by spinal

excitability changes between young and older adults. On the other hand, Opie et al (2014a) reported a decrease in LICI with ageing when using an ISI of 150 ms to control for possible spinal cord excitability influence.

One final consideration for the discrepancies mentioned above is the different muscle groups utilised in each study. While some studies investigated age-related differences in ICI within the FDI muscle (Cirillo et al. 2011; Oliviero et al. 2006; Opie and Semmler 2014b; Peinemann et al. 2001; Smith et al. 2009), other studies have examined the abductor pollicis brevis muscle (Cirillo et al. 2010; Rogasch et al. 2009) and larger forearm muscles (McGinley et al. 2010). It is therefore likely that the differing muscle groups studied may have contributed to the inconsistencies observed given that an optimum stimulation configuration for one muscle may not be the same for another. Nonetheless, evidence on the impact of fatigue on ICI in older adults remains limited.

A recent study from our laboratory investigated this using single pulse combined TMS with electroencephalography (TMS-EEG) (Opie et al. 2020). TMS-EEG is a non-invasive technique that allows for the measurement of cortical network properties such as cortical oscillatory frequencies and cortical connectivity at a high temporal resolution, where measurement of the TMS-evoked response occurs within the millisecond range (Rogasch and Fitzgerald 2013). Here, TMS elicits a highly reproducible evoked potential in EEG recordings known as TMS-evoked EEG potentials (TEPs) (Casarotto et al. 2010). They contain a series of peaks and troughs that last up to 300 ms with TEP amplitude being greatest at the site of stimulation (Bonato et al. 2006). The electrophysiology behind these fluctuations is difficult to interpret directly because EEG recordings represent a summation of both excitatory postsynaptic potentials (EPSPs) and inhibitory postsynaptic potentials (IPSPs) (Kirschstein and Kohling 2009). However, the amplitude of the different peaks and troughs in TEPs appear to reflect information regarding the excitability of the underlying cortical network. For example,

administering $GABA_A$ agonists such as benzodiazepine increases the amplitude of the N45 peak suggesting that IPSP activity of the $GABA_A$ mediated inhibitory interneurons mediates the N45 peak (Ferrarelli et al. 2010) while administration of $GABA_B$ agonists such as baclofen has been shown to increase the amplitude of the later N100 peak suggesting that this peak reflects $GABA_B$ mediated inhibitory activity (Premoli et al. 2014). Following performance of intermittent 30 s MVCs for a total of 15 minutes, older adults showed a greater reduction in N45 peak activity post fatigue while younger adults showed a greater reduction in N100 peak activity post fatigue (Opie et al. 2020). These findings suggest a greater decrease in $GABA_A$ inhibitory activity following fatigue with ageing. Nevertheless, the impact of fatigue on inhibitory brain circuits studied with paired pulse paradigms such as SICI and LICI in older adults remains undetermined. This will form the primary aim of chapters two and three of this thesis.

1.4. MOTOR PERFORMANCE, AGEING AND FATIGUE

Motor skills play a crucial role in all phases of life. All age groups perform fundamental motor tasks such as walking and grasping, or specific tasks like driving or writing. As previously discussed, the ageing process is usually accompanied by impairments in sensorimotor, cognitive, and peripheral function (*see section 1.3.1*). As a result, older adults perform complex tasks at a slower rate and in some cases, less accurately compared to younger adults (Allum et al. 2002; Enoka et al. 2003; Olafsdottir et al. 2007). This means that the ability to perform day to day motor tasks declines with ageing which will likely reduce the older population's quality of life and diminish their ability to live and work independently. These disabling outcomes may be further exacerbated by the difference in fatigability observed with ageing. The following sections will highlight some of the cortical mechanisms underlying motor performance in older adults and how it is influenced by fatigue.

1.4.1. Plasticity, Motor Performance and Ageing

One factor thought to contribute to the attenuation in performance seen in older adults is an age-related reduction in plasticity of the CNS. Learning of simple motor tasks induces long term potentiation (LTP)-like mechanisms within M1 which mediate cortical plasticity. LTP refers to a continual strengthening between synapses due to repeated patterns of activity or stimulation. The age-related decline in M1 plasticity has been investigated using TMS where a decrease in the ability to induce use-dependent (Rogasch et al. 2009; Sawaki et al. 2003) and stimulation-induced (Bashir et al. 2014; Todd et al. 2010b) plasticity has been identified in older adults. For example, Muller-Dahlhaus et al (2008) reported a decreased magnitude in motor cortex plasticity induction in older adults compared to the young following paired associative stimulation (PAS) treatments. PAS is a widely used non-invasive stimulation method that induces lasting changes in excitability of the human cortex. It employs repetitive pairing of a peripheral electrical stimulus (usually applied to the median nerve), which precedes a single pulse TMS applied to the hand areas of the motor cortex (Stefan et al. 2000). The facilitatory effect of PAS on corticomotor excitability measured with TMS-evoked MEP is thought to rely on LTP-like mechanisms as this effect can be blocked by the administration of NMDA receptor antagonists (Stefan et al. 2002). Therefore, an attenuated increase in MEP size following PAS treatments shows a reduction in the LTP-like increase of cortical excitability in older adults (Muller-Dahlhaus et al. 2008; Tecchio et al. 2008). However, this finding is not consistent since other studies have also reported no effect of age on PAS-induced plasticity (Dickins et al. 2017; Opie et al. 2019; Opie et al. 2017a). Another common method implemented is TBS; a basic element of repetitive TMS containing a three-pulse burst at 50 Hz given every 200 ms, which is thought to mimic cross-frequency coupling between natural theta and gamma range rhythms (Suppa et al. 2016). Continuous stimulation for 40s results in an LTD-like effects (cTBS) while intermittent stimulation for 190 s (iTBS) results in an LTP-like

effect (Huang et al. 2005). Interestingly, both iTBS and cTBS exhibit varied outcomes in age-related M1 plasticity. Whilst four studies have demonstrated no difference in M1 plasticity with advancing age using iTBS (Di Lazzaro et al. 2008; Dickins et al. 2015b; Opie et al. 2017c; Young-Bernier et al. 2014), one study implementing the use of cTBS identified a progressive decline in magnitude and duration of MEP depression in older adults, signalling a decline in M1 plasticity (Freitas et al. 2011). Given that iTBS and cTBS differentially affect the corticospinal volley, with cTBS influencing early I-waves and iTBS influencing latter I-waves (Di Lazzaro and Rothwell 2014), it is plausible that the age-related shift in plasticity could be linked to modulation of the inhibitory circuits responsible for early but not late I-wave. However this is unlikely given that evidence shows a delay in the temporal characteristics of late not early I-waves in older adults (Opie et al. 2018). Other non-invasive brain stimulation techniques have also been implemented such as low frequency rTMS (Bashir et al. 2014; Todd et al. 2010b), iTMS (Opie et al. 2018) and transcranial direct current stimulation (tDCS) (Fujiyama et al. 2014; Ghasemian-Shirvan et al. 2020; Heise et al. 2014; Mooney et al. 2019) with similar conflicting findings reported.

With regards to use dependent plasticity, studies have shown no change in MEP amplitude following a simple thumb abduction training in older adults, whereas young adults demonstrated an increase in MEP amplitude (Rogasch et al. 2009; Sawaki et al. 2003). On the other hand, multiple studies report no age-related change in M1 plasticity with young and older adults displaying similarities in MEP modulation (Cirillo et al. 2010; Cirillo et al. 2011; Dickins et al. 2015b; Goodwill et al. 2015; Hinder et al. 2013a; Hinder et al. 2013b; Hinder et al. 2011; Mooney et al. 2019). Factors such as muscle type, method used to quantify M1 plasticity and age-range of the subjects remain evident contributors to this discrepancy. However other factors must also be considered. For example, opposing results were found in two studies that implemented the same task (300 ballistic thumb abductions) and measurement

outcomes (MEP and SICI) with Rogasch and colleagues reporting no change in MEP amplitude in older adults (Rogasch et al. 2009) while Cirillo and colleagues reported similar MEP facilitation in both hands of young and older adults (Cirillo et al. 2010). Therefore given the congruent experimental design between studies, other population-based factors such as habitual hand activity (Rosenkranz et al. 2007b), physical activity levels (Cirillo et al. 2009), attentional focus (McNevin et al. 2000), sleep patterns (Huber et al. 2008) and emotional state of the participants (Tormos et al. 1997) might come into play. Differences in type of task performed must also be taken into consideration. Several studies have also aimed to identify age-related differences in M1 plasticity by implementing visuomotor tracking tasks to increase task complexity (Berghuis et al. 2016; Cirillo et al. 2011; Goodwill et al. 2015; Mooney et al. 2019). The rationale for this shift was based on the finding that more complex tasks result in increased attentional demand and require greater activation and connectivity between the memory, visual and motor systems for accurate performance. As a result, enhanced excitability of the corticospinal tract was expected leading to greater M1 plasticity. However contrary to this, no age-related difference in M1 plasticity was identified (Berghuis et al. 2016; Cirillo et al. 2011; Goodwill et al. 2013; Mooney et al. 2019). Furthermore, when simple ballistic versus complex (sequential finger opposition) training tasks were implemented in the same study (Dickins et al. 2015a), task dependent MEP facilitation was observed following the simple but not complex task performance in both young and older adults emphasising the impact of task selection during M1 plasticity measures .

The induction of LTP-like mechanisms is also characterised by a complimentary decrease in GABAergic inhibition (Jones 1993; Ziemann et al. 2001). While several studies have reported a reduction in SICI after performing both simple and complex tasks in young subjects (Gallasch et al. 2009; Garry et al. 2004; Liepert et al. 1998; Perez et al. 2004), inconsistencies currently exist regarding changes in SICI during motor skill performance in

older adults. For example, Hinder et al (2011) reported a similar decline in SICI following performance of a ballistic index finger abduction task in both age groups with no age-related differences. In an earlier study, Cirillo et al (2011) showed no change in SICI in both populations following performance of a visuomotor tracking task after training. Nonetheless, this discrepancy might be attributed to the constant MEP size used before and after training by Cirillo and colleagues. To investigate this further, Cirillo et al (2011) adjusted MEP size to account for the change after training. A reduction in SICI was later observed in both young and old with no significant age differences (Cirillo et al. 2011). In contrast, a recent systematic review showed that SICI was not affected in either age group following ballistic motor training (Berghuis et al. 2017). However, an age-related compensatory decrease in inhibition (~20%) was observed when performing visuomotor tasks (no difference in young adults). Nevertheless, these outcomes suggest that older adults indeed maintain the capacity to modulate GABA-mediated intracortical inhibition when performing complex motor tasks.

1.4.2. Motor Performance and Fatigue

Investigation into the effect of fatigue on motor performance remains limited with a majority of studies performed in the 1970s-1990s (For comprehensive review see: Taylor and Ivry 2012). Contradictory results have been identified with some studies reporting a detrimental effect of fatigue on motor performance (Carron and Ferchuk 1971; Thomas et al. 1975) while other studies report no effect of fatigue on motor performance (Alderman 1965; Cotten et al. 1972; Spano and Burke 1976). However more recent work suggests otherwise. During performance of a simple grip-lift task, fatigue disrupted the complex sensorimotor coordination required to lift and hold an object (Todd et al. 2010a). However, an improvement in coupling between grip and lift force was observed during the trials which suggested that subjects were able to retain their ability to learn sensorimotor tasks (Emge et al. 2013; Todd et al. 2010a). Interestingly this theory was recently challenged by Branscheidt and colleagues who showed

that not only did fatigue result in lower levels of performance of a sequential pinch force task immediately after exercise, this detrimental effect persisted for almost two more additional days of training, suggesting that fatigue had a significant impact on learning as well (Branscheidt et al. 2019). However, this effect was only observed when the sequential motor task was paired with a significant cognitive load which may explain the discrepancy observed. Although the impact of fatigue on motor performance in younger adults has been briefly explored, fatigue's impact on performance in older adults remains undetermined. Chapter four of this thesis will investigate this further.

1.5. NEUROMODULATION, MOTOR PERFORMANCE AND FATIGUE

1.5.1. Transcranial direct current stimulation

Application of direct electric currents to modify brain activity/function has been a heavily studied technique for more than 200 years. In 43-48 AD, Scribonius Largus observed that delivering a strong direct electrical current over the scalp of a patient with headache using live torpedo fish resulted in a short-lived stupor with pain relief. Similar findings were shown by the Greek physician Claudius Galen (131-401 AD) who discovered that the pain relief effects were due to numbness, lethargy and the narcotic effects induced by the electric fish (Kellaway 1946). This eventually led to the electrical stimulation of the first exposed human cortex by Galvani, who showed that cortical stimulations evoked facial muscle contractions in deceased humans (Zago et al. 2008).

Soon after Galvani's discovery, direct current stimulation was promptly applied in clinical medicine, specifically psychiatric cases with systematic studies in patients beginning in the 1960s. Redfearn et al (1964) reported that anodal stimulation of the scalp improved the mood of more than half of their twenty six depressed patients while cathodal stimulation reduced manic symptoms (Carney 1969). On the other hand, anodal stimulation of 32 normal subjects using direct currents (50- 500 μA) resulted in an increase in alertness, mood and motor

activity while cathodal stimulation resulted in quietness and apathy (Lippold and Redfearn 1964). Subsequent invasive animal studies revealed some of the underlying neural mechanisms behind the behavioural changes mentioned above (Bindman et al. 1964). Delivery of weak direct currents through intracerebral or epidural electrodes in anesthetized rats induced prolonged changes in cortical activity via an enhancement or attenuation of cortical excitability (Bindman et al. 1964). These long-lasting changes were shown to be mediated by intracellular cAMP (Hattori et al. 1990) and calcium modifications (Islam et al. 1995) similar to the well characterised phenomena of LTP and LTD.

As a result, non-invasive methods such as tDCS have since been introduced in numerous research fields as a way of altering neural activity and behaviour. In fact, studies have shown that 50% of transcranially applied current is able to penetrate the scalp (Dymond et al. 1975; Rush and Driscoll 1968) and induce intracerebral current flow that is sufficiently large enough to alter neuronal activity and behaviour. TDCS is a non-invasive neuromodulation technique involving the direct application of electrical currents (1-2 mA) on the scalp delivered through sponge electrodes (25-35 cm^2) (Williams et al. 2013). Unlike TMS, tDCS does not directly stimulate axons causing them to discharge but instead acutely modulates the resting membrane potential of the underlying neuronal tissue; cathodal stimulation modulates via hyperpolarisation of neurons which decreases cerebral excitability, while anodal stimulation causes depolarisation which increases excitability (Nitsche et al. 2003a; Rothwell 2010; Stagg and Nitsche 2011). These changes have been shown by numerous TMS studies via an increase in MEP size (increased excitability) following anodal tDCS and a corresponding decrease in MEP size (decreased excitability) following cathodal tDCS (Nitsche et al. 2003a; Nitsche and Paulus 2000; Nitsche et al. 2007; Priori et al. 1998; Rothwell 2010). Furthermore, these excitability changes are known to last beyond the duration of stimulation. For example, if the current flow is applied for 10-30 min, after-effects last for several hours after stimulation in

both animals (Bindman et al. 1964; Bindman et al. 1962) and humans (Nitsche and Paulus 2001). This phenomenon is thought to be NMDA mediated given that anodal tDCS-driven post-synaptic membrane depolarisation results in NMDA-receptor mediated augmentation of synaptic strength, presumably by mimicking LTP-like mechanisms (Liebetanz et al. 2002). On the other hand, a combination of both post-synaptic hyperpolarizing pulses with low frequency pre-synaptic stimulation mediated by cathodal tDCS may induce prolonged depression (mimicking LTD-like mechanisms) (Liebetanz et al. 2002). Due to its capabilities in modifying cortical excitability and eliciting long lasting changes, tDCS has become a major tool in the investigation of motor performance and fatigue.

1.5.2. TDCS, motor performance and ageing

Neuromodulation has been influential in the investigation of skill acquisition with numerous studies showing its ability to enhance skill acquisition when applied during task performance. For example, Stagg et al (2011) observed faster reaction times during an 11-digit explicit finger sequence learning task when anodal tDCS was applied during performance of the task. However, when applied prior to training of the task, anodal stimulation led to delayed reaction times when compared to the sham stimulation (Stagg et al. 2011). Improvements in performance were also reported following performance of a sequential visual isometric pinch task (SVIPT) using the hand muscles (Reis et al. 2009) and a pinch-force task using the lower leg muscles (Tanaka et al. 2009). However, unlike anodal stimulation, cathodal stimulation doesn't seem to have the similar effects on motor performance. In fact, multiple studies have shown that cathodal stimulation does not affect task performance regardless of time of application (prior or during the task) (Galea and Celnik 2009; Nitsche et al. 2003b; Reis et al. 2009; Tanaka et al. 2009). This supposition was investigated further using magnetic resonance spectroscopy (Stagg et al. 2009). Here, anodal stimulation was shown to decrease GABA within M1 suggesting a decrease in inhibitory activity (a necessary requirement for the

induction of LTP-like mechanisms during motor performance) while cathodal stimulation resulted in decreased glutamate levels (Stagg et al. 2009) which have not been reported to change during task performance. A decline in glutamate levels translates to a decline in NMDA receptor activity which would in turn minimise the induction of LTP like mechanisms during motor performance. Nonetheless, some evidence has surfaced to suggest that cathodal stimulation may still result in decreased motor performance (Stagg et al. 2011). Although cathodal stimulation resulted in no difference in performance of a simple reaction time task, an increase in reaction time (decrease in performance) was observed during performance of a sequential learning task. The factors underlying the discrepancy remain unclear however one possibility could be the difference in demand of M1 due to the varying motor tasks. If implicit tasks used in previous studies are less demanding of M1 then a decrease in cortical excitability may have no behavioural consequences. On the other hand, if the sequential learning task was more demanding of M1 (Stagg et al. 2011), then a decline in excitability induced by cathodal stimulation may lead to a decrease in functional outcomes due to insufficient redundancy within the system.

In ageing studies, there is also good evidence to show that neuromodulation enhances motor performance and retention in older adults (Goodwill et al. 2013; Heise et al. 2014; Panouilleres et al. 2015; Parikh and Cole 2014). For example, in a double-blind cross over study led by Hummel and colleagues, an improvement in performance of the Jebsen Taylor Hand Function Test (JTT) test was reported following a single session of anodal tDCS compared to sham (Hummel et al. 2010). This improvement outlasted the stimulation period by at least 30 min and was more evident in tasks that engaged dexterous and fine motor skills, compared to gross proximal arm functions. Anodal tDCS application has also been shown to improve skill acquisition in older adults during training of a motor task relative to younger adults (Zimerman et al. 2013). These findings may be attributed to the possibility that anodal

tDCS influences the ability of the aged cortex to undergo plastic modifications by modulating GABA-ergic neurotransmission to promote unmasking of excitatory connections as well as an indirect enhancement of NMDA-dependent processes supporting LTP. This improvement in plastic modifications observed in older adults is demonstrated further by studies investigating possible cross-limb transfer during skill acquisition. Goodwill et al (2015) not only showed an improvement in performance of a visuomotor tracking task in the trained limb in both young and older adults following anodal tDCS application over the ipsilateral M1 relative to sham, but an improvement in the untrained limb was also identified in older adults following anodal tDCS. This demonstrated greater cross-limb transfer of performance following neuromodulation which was otherwise absent with motor practice alone.

Despite the abundance of research on the effects of tDCS on plasticity, recent years have revealed large inter-individual variability in excitability measures drawn from tDCS application (Fujiyama et al. 2014; Puri et al. 2015). It has been shown that ~50% of participants do not respond to anodal tDCS application (Puri et al. 2015). One possible explanation for the large interindividual variability is history of synaptic activity prior to stimulation. The human motor system is regulated by homeostatic metaplasticity mechanisms to ensure stability of neural function (Muller et al. 2007; Murakami et al. 2012; Siebner et al. 2004) thereby making it possible for excitatory stimuli to decrease excitability when applied with/directly after another excitatory stimulus (Huang et al. 2008; Thirugnanasambandam et al. 2011). Based on the Bienenstock-Cooper-Munro theory of homeostatic metaplasticity, plasticity at a synapse can result in either LTP or LTD (bidirectional) (Bienenstock et al. 1982). The threshold for LTP versus LTD induction varies according to the history of postsynaptic activity. If previous activity of the postsynaptic synapse was low then the synaptic modification threshold is lowered thus favouring LTP over LTD. Alternatively, if previous activity of the postsynaptic synapse was enhanced then the synaptic modification threshold will be increased resulting in

occurrence of LTD over LTP (Bienenstock et al. 1982). Therefore, an interesting strategy to decrease the threshold for induction of LTP-like plasticity by lowering neuronal activity within M1 prior to commencing task performance has been developed. Christova et al (2015) found greater improvement in task performance when cathodal stimulation (priming) was applied prior to anodal stimulation than when sham stimulation preceded anodal stimulation. The additional application of cathodal stimulation benefits motor performance by possibly lowering the synaptic modification threshold favouring LTP induction. Similar findings were observed in older adults where cathodal priming resulted in greater task improvement compared to sham stimulation (Fujiyama et al. 2017). Corticospinal excitability measures also revealed greater and more reliable changes across participants for both age groups. By reducing the threshold for subsequent anodal tDCS, cathodal priming resulted in a greater increase in corticospinal excitability and improved skill acquisition during training. Furthermore, the significantly lower number of non-responders following priming provided further evidence that it may be used as a tool to reduce inter-individual variability in response to tDCS.

From these findings we can see that neuromodulation has the potential to improve motor performance through enhancement of LTP-like processes within the motor cortex. Furthermore, induction of homeostatic metaplasticity mechanisms reduces inter-individual variability and results in greater improvement of task performance. In any case, its impact on fatigability in young and older adults is yet to be determined. Chapter five of this thesis investigates this question.

1.5.3. TDCS and fatigue

The capacity of neuromodulation to modulate both corticospinal excitability and motor performance indicates that it has the potential to influence fatigue mechanisms. As mentioned in *section 1.5.1*, anodal tDCS increases cortical excitability by enhancing depolarisation of neurons and reducing GABA concentration which translates to a decline in GABAergic

inhibitory activity. Increased excitability and decreased GABAergic inhibition are two known mechanisms that are influenced by fatigue. When applied prior to a submaximal sustained isometric contraction (35% MVC) of the elbow flexor muscles, anodal tDCS resulted in prolonged time to task failure in a group of young adults compared to sham (Abdelmoula et al. 2016; Cogiamanian et al. 2007). Additional data confirmed an increase in corticospinal excitability following anodal tDCS application, suggesting a possible relationship between enhanced facilitation of corticospinal neurons and fatigability. However, this was later disputed by Abdelmoula et al (2016) who found no correlation between increased corticospinal excitability and improved fatigability thereby bringing this conclusion into question. Anodal tDCS application also lengthened time to task failure when delivered during a submaximal sustained contraction at 20% MVC of the elbow flexor muscles (Williams et al. 2013) with no observable change in excitability. Angius and colleagues found a similar improvement in the knee extensors when anodal tDCS delivery for 10 minutes resulted in an increase in time to exhaustion (Angius et al. 2016), suggesting that the effects of tDCS are not muscle specific. However, only one study to date has investigated the effects of anodal tDCS on fatigability in older adults. Oki et al (2016) identified an improvement in time to task failure following 20 min anodal tDCS application during a sustained submaximal contraction of the elbow flexors at 20% MVC. This indicates that the ability of anodal tDCS to modify supraspinal mechanisms underlying fatigue may not be age specific. Nevertheless, no corticospinal excitability measures were recorded, and no measures were collected from a young healthy cohort (control) for comparison, forming the primary basis for chapter five.

Nonetheless, inconsistencies exist within the current literature regarding the effects of tDCS on fatigability with three studies reporting a lack of effect of anodal tDCS on fatigability (Kan et al. 2013; Muthalib et al. 2013; Radel et al. 2017). The lack of consensus on preferable methodological practices within the field such as double versus single blinded studies, timing

of stimulation, sample size, and electrode configuration may be the primary contributor for these disparities.

Caution must be taken when assessing single blinded studies given that investigator bias may play a significant role in analysis of results (Abdelmoula et al. 2016; Angius et al. 2016; Cogiamanian et al. 2007). Knowledge of the treatment applied can unconsciously affect subjective measures such as TTF making it imperative to ensure that both experimenter and participant are blinded. In any case, three out of the eight studies mentioned above were double-blinded studies (Oki et al. 2016; Radel et al. 2017; Williams et al. 2013) with only one study reporting a lack of improvement in TTF (Radel et al. 2017). This suggests that other factors might be contributing to the inconsistencies. Timing of stimulation (before or during fatiguing contraction) is another consideration. Out of the eight studies mentioned above, three studies applied tDCS during contraction (Oki et al. 2016; Radel et al. 2017; Williams et al. 2013), with one study reporting no improvement in time to task failure (Radel et al. 2017). All other studies applied tDCS prior to exercise making a direct comparison between studies difficult. Furthermore, with regards to Radel and colleagues, although tDCS was applied during contraction, stimulation began prior to the onset of contraction which would explain the lack of effect observed. Consensus is required regarding the appropriate timing of stimulation. Electrode placement montage (monocephalic; one electrode over cerebrum and one electrode over shoulder: Bicephalic; both electrodes over cerebrum) is another important consideration. No consensus currently exists regarding the most effective electrode placement. Both montages show significant improvements in TTF (Abdelmoula et al. 2016; Angius et al. 2016; Cogiamanian et al. 2007; Oki et al. 2016; Williams et al. 2013), thus requiring further investigation. Additional factors include sample size and high versus low-definition focal stimulation. Radel and colleagues also attempted to control for these factors by implementing a double blinded study with a large sample size and use of high-definition focal stimulation

(Radel et al. 2017). No difference in time to task failure was observed. Furthermore, unlike Cogiamanian and colleagues, subsequent investigation via TMS revealed that tDCS treatment had no effect on cortical excitability within M1 (Radel et al. 2017).

Nevertheless, these inconsistencies may also be attributed to the history/level of synaptic activity prior to stimulation (*see section 1.5.2*) leading to significant inter individual variability i.e., ~50% of participants do not respond to tDCS (Puri et al. 2015). Therefore, induction of homeostatic metaplastic mechanisms through priming tDCS can be used as a potential tool to account for history of synaptic activation and reduce inter-individual variability to further investigate tDCS's potential as a clinical intervention. This forms the basis of the study presented in chapter five.

1.6. SUMMARY AND AIMS

It has been well established that both excitatory and inhibitory processes within M1 are largely affected by fatigue through the use of non-invasive brain stimulation technique (TMS). However, despite notable changes within the CNS, fatigue related differences in excitability and inhibitory modulation as a consequence of ageing are yet to be established. The effects of fatigue on motor task performance also remain significant in our day-to-day life, particularly in older adults where increased fatigability may impact their quality of life and ability to live and work independently. Therefore, understanding how fatigue affects motor performance as well as the corticospinal mechanisms underlying fatigue in older adults is essential.

The following chapters describe experiments that investigate the age-related differences in corticospinal excitability during and post exercise induced fatigue, and its subsequent effect on motor performance. Chapters two and three investigated the impact of fatigue on corticospinal excitability and GABA mediated inhibitory processes in young and older adults. Chapter four aims to expand on the impact of exercise-induced fatigue on motor skill performance (simple movement time task) in young and older adults. Finally, chapter five

examines the use of tDCS as a tool to enhance corticospinal excitability, via the induction of homeostatic metaplastic mechanisms, in an effort to improve fatigability. The subsequent effect of neuromodulation (applied during fatiguing task) on motor skill performance was also assessed to identify the possible use of tDCS as an intervention to counteract the negative effects of fatigue on motor performance in the ageing population.

CHAPTER II

SINGLE JOINT FATIGUING EXERCISE DECREASES LONG BUT NOT SHORT- INTERVAL INTRACORTICAL INHIBITION IN OLDER ADULTS

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Overall percentage (%)	60%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	15/11/2021

Co-Author Contributions

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

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

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

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

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2. SINGLE JOINT FATIGUING EXERCISE DECREASES LONG BUT NOT SHORT- INTERVAL INTRACORTICAL INHIBITION IN OLDER ADULTS

2.1. ABSTRACT

Ageing is accompanied by neuromuscular changes which may alter fatigue in older adults. These changes may include changes in corticospinal excitatory and inhibitory processes. Previous research has suggested that single joint fatiguing exercise decreases SICI and LICI in young adults. However, this is yet to be established in older adults. In 19 young (23 ± 4 years) and 18 older (69 ± 5 years) adults, SICI (2 ms ISI) and LICI (100 ms ISI) were measured in a resting FDI muscle using TMS before and after a 15-minute sustained submaximal contraction at 25% of their maximum EMG. Subsequent ten 2-minute contractions held at 25% EMG were also performed to sustain fatigue for a total of 30 minutes while SICI and LICI were taken immediately after each contraction. There was no change in SICI post fatiguing exercise compared to baseline in both young and older adults ($P = 0.4$). Although there was no change in LICI post fatiguing exercise in younger adults ($P = 1.0$), LICI was attenuated in older adults immediately post fatiguing exercise and remained attenuated post fatigue (PF)1 and PF2 ($P<0.05$). Contrary to previous studies, the lack of change in SICI and LICI in young adults following a sustained submaximal EMG contraction suggests that GABA modulation may be dependent on the type of fatiguing task performed. The reduction in LICI in older adults post fatiguing exercise suggests an age-related decrease in $GABA_B$ mediated activity with sustained submaximal fatiguing exercise.

Key Words: Ageing, Cortical excitability, Fatigue, Intracortical inhibition, TMS.

2.2. INTRODUCTION

Neuromuscular fatigue is defined as an exercise-induced reduction in the ability of a muscle or muscle group to produce muscle force (Taylor et al. 2000). Older adults are predisposed to differing magnitude and duration of fatigue due to age-related changes within the neuromuscular system. For instance, age-related slowing of the muscle mediated by a shift in muscle fibre type to a greater proportion of type I fibres (Andersen 2003; Lexell et al. 1983) and reduced calcium regulation (Hunter et al. 1999) increases fatigue resistance in older adults compared to young adults during isometric contractions (Bilodeau et al. 2001b; Chung et al. 2007; Hunter et al. 2008; Yoon et al. 2013; Yoon et al. 2012). On the other hand, this advantage is absent and both age groups tend to present with a similar degree of fatigue during low to moderate speed dynamic contractions, whilst older adults fatigue faster during high-speed dynamic contractions (Callahan and Kent-Braun 2011; Dalton et al. 2012; Yoon et al. 2013).

TMS studies have shown that cortical modifications may mediate some of the fatigability differences between young versus older adults during isometric contractions. For instance, reduced modulation of corticospinal excitability and intracortical inhibition – evidenced by a smaller increase in the MEP and SP duration following fatiguing exercise is observed in older adults when compared to the young adults (Yoon et al. 2012). SP (an index of intracortical inhibition) is the latency of suppression in the EMG signal during a voluntary contraction following a single pulse TMS. Since it is thought to be influenced by both spinal (<50 ms) and intracortical $GABA_B$ mediated inhibitory mechanisms (>100 ms) (Inghilleri et al. 1993), SP is typically not considered the most appropriate marker of intracortical inhibition. As a result, paired pulse TMS paradigms such as LICI (Benwell et al. 2007c; McNeil et al. 2011b; Valls-Sole et al. 1992) and SICI (Benwell et al. 2006b; Kujirai et al. 1993; Valls-Sole et al. 1992) are increasingly used. LICI, a reflection of $GABA_B$ mediated inhibition, is measured with the application of a suprathreshold conditioning stimulus that attenuates the

amplitude of a suprathreshold TMS evoked test response at an interstimulus interval (ISI) of 50-200 ms (McDonnell et al. 2006); while SICI, a reflection of $GABA_A$ mediated inhibition, is measured with the application of a subthreshold conditioning stimulus applied at an ISI of 1-5 ms (Kujirai et al. 1993). Contrary to SP, both SICI and LICI decline when measured in a resting muscle in the presence of fatigue in young adults; suggesting a decrease in $GABA_A$ and $GABA_B$ mediated inhibition (Benwell et al. 2007c; Benwell et al. 2006b; Maruyama et al. 2006). Using single-pulse TEP measured TMS-EEG (Casarotto et al. 2010), we have recently demonstrated age-related differences in GABA modulation in the presence of fatigue (Opie et al. 2020). The TEP contains a series of peaks and troughs that last up to 300 ms (Bonato et al. 2006), the amplitudes of which reflect the excitability of the underlying cortical network. For example, pharmacological studies have shown that the N45 peak reflect $GABA_A$ mediated activity (Ferrarelli et al. 2010), while the N100 peak reflect $GABA_B$ mediated activity (Premoli et al. 2014). Our findings demonstrated a greater reduction in the N45 peak following fatiguing exercise in older adults suggesting a greater reduction in $GABA_A$ inhibitory activity; while younger adults displayed a greater reduction in the N100 peak, suggesting a greater reduction in $GABA_B$ mediated inhibitory activity compared to older adults post fatiguing exercise (Opie et al. 2020). Although there is some pharmacological and behavioural evidence of a relationship between TMS-EEG and TMS-EMG measures of inhibition (Bender et al. 2005; Bonnard et al. 2009; Premoli et al. 2014), it should also be acknowledged that it is not entirely clear as to whether paired pulse MEPs and TEPs are mediated by the same mechanisms (Biabani et al. 2019a). In any case, the age-related effect of fatiguing exercise on SICI and LICI measured with TMS-EMG remains undetermined in older adults.

The aim of the current study is to determine age-related differences in SICI and LICI with sustained isometric fatiguing contractions. The current study used single joint fatiguing exercise of a hand muscle for a number of scientific and functional reasons, including the fact

that there is a relatively large representation of the hand muscle in the motor cortex and that SICI and LICI can be reliably measured from the hand muscle in both young and older adults (Wassermann et al. 1992). On a functional level, hand muscles play a significant role in day to day activities including gardening, cleaning, holding shopping bags and are pivotal to independent living in older adults. We used a sustained contraction held at a constant submaximal EMG to fatigue the muscle because it allowed for the measurement of the development of fatigue during the contraction per se (via reduction in absolute muscle force and with no interruptions from performance of MVC). SICI and LICI were assessed at rest (Benwell et al. 2007c; Benwell et al. 2006b) in order to obtain a sufficient amount of inhibition at baseline; since magnitude of inhibition is attenuated during activity (Ridding et al. 1995). We hypothesised that LICI and SICI would decline in both young and older adults as a consequence of fatiguing exercise. In addition, we hypothesised that younger adults would display a greater decline in LICI compared to older adults, while a greater decline in SICI would be observed in older adults compared to the young with fatiguing exercise.

2.3. METHODS

We recruited nineteen young and eighteen older healthy participants from the university community, community centres and social media for participation in this study (Table 2.1). Any ongoing use of psychoactive medication (e.g. sedative, antipsychotics and antidepressants) or history of neurological and/or psychiatric disease excluded participants from the study. Physical activity assessment (work index, sport index, leisure-time index) was performed in both groups via an activity questionnaire (adapted from Baecke et al. 1982). The Edinburgh Handedness inventory was used to evaluate hand preference which confirmed that all participants were right-handed (Table 2.1). All procedures were performed in accordance with the ethical standards of the University of Adelaide Human Research Ethics Committee,

which complies with the 1964 Helsinki Declaration. Written, informed consent was provided by each participant prior to participation.

2.3.1. Experimental set-up and EMG recordings

Participants were seated upright with their right arm fixed onto a horizontal surface and index finger abducted against a force transducer (MLP 100 Transducer Techniques, Temecula California, USA) as previously described (Otieno et al. 2019). The elbow was flexed at approximately 90^{o}, with the forearm and wrist restrained in a custom-designed manipulandum. Responses evoked from the right first dorsal interosseous (FDI) muscle were recorded using surface EMG with two Ag-AgCl electrodes placed over the muscle in a belly-tendon montage (8 mm diameter; 16 mm between electrodes; 3M Red Dot, Canada). Two grounding straps were also attached around the forearm. EMG was amplified (1000 times) and band-pass filtered (20 Hz high pass, 1 kHz low pass) using CED 1902 hardware (Cambridge Electronics Design, Cambridge, UK) before being digitized at 2 kHz with a 1401 interface (Cambridge Electronics Design, Cambridge, UK) and stored offline for analysis.

2.3.2. Experimental protocol

The study comprised of two sessions (SICI, LICI) with two age groups (young, older adults) and repeated measures to monitor the effects of muscular fatigue over time. Fig 2.1 shows the experimental protocol carried out in each session. Prior to the fatiguing task, participants received twenty TMS pulses (ten single pulses, ten paired pulses, in five blocks of four stimulations) and two peripheral nerve stimulations (PNS) at rest. Maximum force and EMG were determined by calculating the average value of three, 3–5 s MVCs (separated by 30 s) performed by participants via abduction of the index finger. Participants then performed a submaximal contraction at 25% of their maximum EMG for a total of 15 min. A submaximal EMG, rather than force contraction, was selected to document the development of fatigue during the exercise per se, which is typically seen as a reduction in force magnitude during the

contraction (Hunter et al. 2016a; McNeil et al. 2011a). A verbal cue was provided which indicated when participants should begin exercise or rest. Participants were instructed to refrain from using other hand muscles during the exercise. Visual feedback of EMG output (smoothed using a 500 ms time constant) was displayed on a computer screen in front of the participant and verbal encouragement was provided throughout the protocol. Immediately post-exercise, participants performed two brief 5 s MVCs with a 10 s rest period in between contractions. Following this, a set of stimulations consisting of six single TMS, six paired-pulse TMS and two PNS was delivered. The order of all single and paired pulse TMS were randomised, with the two PNS always delivered at the end. This was to ensure that the muscle contraction from the nerve stimulation did not impact the MEP response.

In order to sustain fatigue, participants were instructed to perform a series of contractions that consisted of a two min submaximal contraction at 25% EMG followed by 2 MVCs and a set of stimulations (as detailed above post exercise; *also see Fig. 2.1*). This was repeated ten times (sustained fatigue: SF1-SF10). A 10 min recovery period was then provided before a final set of twenty TMS pulses (ten single, ten double, in five blocks of four stimulations) and two PNS pulses. To conclude the session, participants were instructed to perform a final 2 min submaximal contraction at 25% EMG followed by 2 MVCs (Fig 2.1). The difference in number of stimulations given between post fatiguing contractions time points (6 single and 6 paired TMS) versus baseline and recovery time points (10 single and 10 paired TMS) is due to the fact that fatigue recovery is known to be fast (Carroll et al. 2017; Gandevia 2001; Kennedy et al. 2014), and a higher number of stimulations at the post fatigue time points would have required too much time to measure. The protocol was designed to allow for 1-minute rest period in between each of the SF1 – SF10 contractions, and in this timeframe, only six single TMS, six paired-pulse TMS and two PNS could be feasibility delivered.

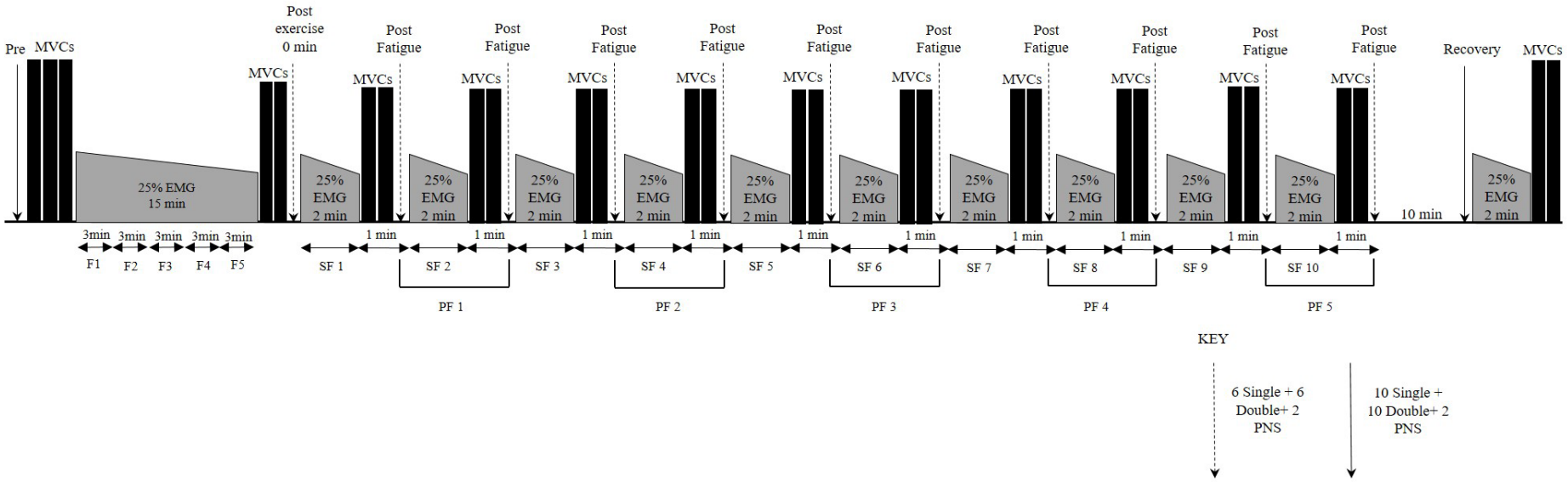

Figure 2.1. ***Experimental protocol schematic***. ***A) Baseline:*** *Before fatiguing exercise, participants received 10 single and 10 paired pulse TMS as well as 2 peripheral nerve stimulations (PNS) at rest (solid arrow). Force during 3 MVCs was measured during a 3- to 5-s index finger abduction before exercise. Participants then performed a sustained submaximal contraction at 25% of the maximum* EMG_{rms} *of the FDI muscle via index finger abduction for a total of 15 min. Mean force measurements were calculated every 3 min during the fatiguing contraction (F1-F5). Immediately after the fatiguing exercise, participants performed two brief (5 s) MVCs with a 10 s rest period in between, followed immediately by 6 single and 6 paired pulse TMS as well as 2 PNS at rest (dotted arrow).* ***B) Fatigue:*** *Participants were then instructed to perform a 2 min contraction at 25% EMG in order to sustain fatigue (SF) followed by 2 brief (5 s) MVCs with a 10-s rest period in between. 6 single and 6 paired pulse TMSs were then given at rest. These contractions (SF1- SF10) and measurements were repeated ten times. Measurements taken during two consecutive SF contractions were pooled for post fatigue (PF) measurements (PF1-PF5).* ***C)*** *A 10-minute recovery period was then provided followed by 10 single TMS, 10 paired pulse TMS and 2 PNS. Participants then performed one more 2-minute contraction at 25% EMG immediately followed by 2 brief (5 s) MVCs with a 10-s rest period in between*

2.3.3. *Peripheral nerve stimulation*

Bipolar surface electrodes with conducting gel were placed at the wrist with the cathode positioned distal to the forearm. The location that produced the largest M-wave response in FDI at a current of approximately 12 mA (1 ms duration) using a constant-current stimulator (DS7A, Digitimer, UK) was marked as the site of stimulation. Stimulation intensity was gradually increased in increments of 5 mA until no further change in M-wave was observed to determine the maximum compound muscle action potential (M_{max}). M_{max} intensity was then set at 120% of the intensity required to produce the largest M-wave response at rest (Table 2.1).

2.3.4. *Transcranial magnetic stimulation*

TMS was applied to the left primary motor cortex using a figure-of-eight coil (external wing diameter 9 cm) with two monophasic Magstim 200^2 magnetic stimulators connected through a Bistim (Magstim, Dyfed, UK). The coil was positioned at an angle of 45° to the sagittal plane, tangentially to the scalp, with the handle pointing laterally and backwards, creating an anteriorly directed current flow in the brain. The region that evoked the largest response in the relaxed FDI muscle at a fixed stimulator intensity (60-65% maximum stimulator output) was marked as the site of stimulation. This location was marked on the scalp using a pen, and the coil position was checked continually throughout the experiment. TMS was delivered at a rate of 0.2 Hz with a 10% variance in time between trials (Otieno et al. 2019).

RMT was defined as the lowest stimulus intensity that produced a response amplitude ≥ 50 μv in at least five out of ten trials in resting FDI muscle (Carroll et al. 2001). AMT was defined as the lowest stimulus intensity to produce a visible MEP response relative to background EMG in at least five out of ten trials during a 5% MVC finger abduction contraction (Ortu et al. 2008).

2.3.5. Intracortical Inhibition

SICI and LICI were assessed in a resting muscle as previously documented with fatigue (Benwell et al. 2007c; Benwell et al. 2006b; Maruyama et al. 2006; Vucic et al. 2011). In SICI, subthreshold conditioning intensity (i.e. 70%, 80% and 90% AMT) that evoked closest to 50% inhibition of the unconditioned test MEP response (set to 1 mV) at an ISI of 2 ms (Ortu et al. 2008) was selected for experimentation (Table 2.1). In LICI, suprathreshold conditioning intensity (110%, 120%, 130% and 140% RMT) that evoked closest to 50% inhibition of the unconditioned test MEP response (set to 1 mV) at an ISI of 100 ms (Benwell et al. 2007c) was selected for experimentation (Table 2.1). SICI and LICI were measured in two separate sessions, separated by at least 48 hours. Eleven young and eleven older adults participated in both sessions. The rest of the participants were not able to participate in both sessions due to withdrawals from the study because of personal circumstances (1 young and 2 older participants) and inability to obtain inhibition at baseline (3 young and 3 older participants).

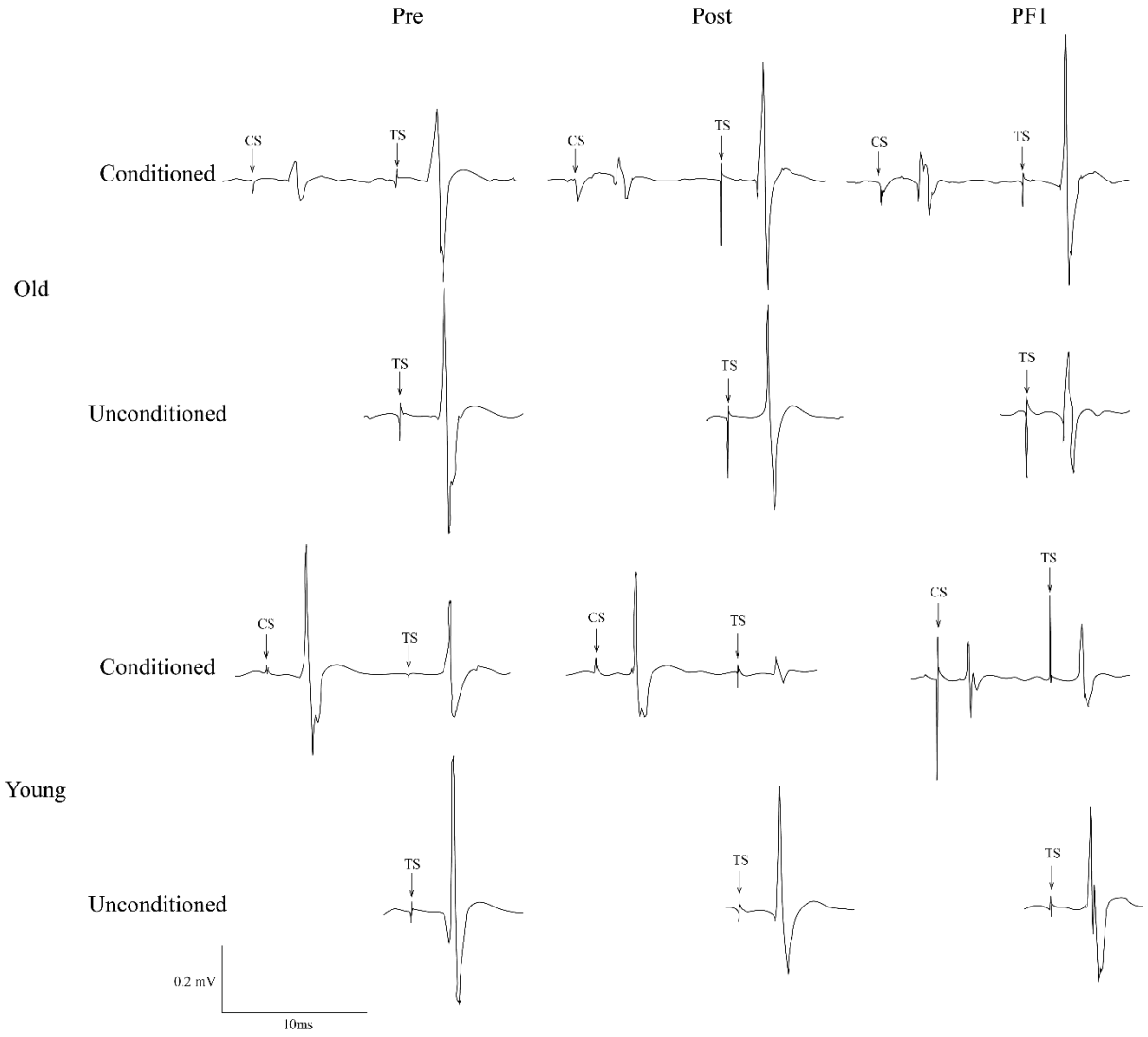

Figure 2.2: *Representative raw traces of conditioned and unconditioned MEP responses at pre, post and post fatigue (PF1) in both young and older adults during the LICI session. CS represents conditioning stimulus and TS represents test stimulus. There was a reduction in unconditioned MEP post fatigue in both age groups, an increase in conditioned MEP size post fatigue in the older adult and an attenuation in conditioned MEP size in the young adults.*

2.3.6. Data analysis

All analyses were completed offline using Spike 2 software and custom-written scripts. Traces showing background voluntary EMG activity exceeding 10 μV in amplitude 100 ms prior to stimulation were removed from analysis (< 0.1% trials), to ensure the muscle was in a complete rested state while measurements were taken (Otieno et al. 2019). Voluntary EMG

(measured as root mean squared EMG; EMG_{rms}) during each 25% EMG was calculated during the plateau in force of the 25% EMG contractions (~15 min or ~2 min for the 25% EMG contractions). Force amplitude during each 25% EMG contraction and MVC was also calculated during the plateau in force of the MVCs and 25% EMG contractions (~5 s for MVC and ~15 min or ~2 min for the 25% EMG contractions). MEP and M_{max} amplitudes from each trial were measured as peak-to-peak in mV. Unconditioned MEPs were expressed as a percentage of M_{max} to account for muscle-dependant changes (Samii et al. 1997; Todd et al. 2007). SICI and LICI were calculated as the ratio (expressed as a percentage) between the peak-to peak amplitudes of the conditioned and unconditioned MEPs (conditioned/unconditioned × 100) (Opie and Semmler 2014a). Therefore, an increase in SICI and LICI ratio in the study reflects less inhibition and vice versa (Fig. 2.2). Since there were age-related differences in SICI and MEP measures at baseline, all variables were expressed as a percentage of baseline. EMG_{rms} and force during the 15 min 25% EMG fatiguing exercise were binned and averaged into five time points (Fatigue 1–5; F1-F5). Force measured after the 15 min 25% EMG contraction (MVC); and during and after each of the 2 min 25% EMG contractions was represented as individual time points (Post, sustained Fatigue 1 – 10; SF1-SF10). MEP and M_{max} measurements collected during the 1 min rest intervals after each of the 2 min 25% EMG sustained fatigue contractions were pooled across two sets and averaged into five bins (Post Fatigue 1 – 5; PF1-PF5; *see Fig. 2.1*).

2.3.7. Statistical analysis

Linear mixed model with repeated measures (sensitive to analyses with varying data within each time point) was used to compare the effect of time and age on the magnitude of MVC, force, EMG_{rms}, SICI, LICI, MEP and M_{max} independently. Significant main effects were further investigated via Bonferroni's post hoc tests corrected for multiple comparisons. For all variables, assumption of normality was violated as demonstrated with Shapiro-Wilk test

(*P<0.05*). As a result, all data sets were log transformed prior to statistical analysis. Statistical significance was set at $P<0.05$, and we interpreted this as the level at which a significant main effect or interaction was observed in the statistical analysis. All data in figures are presented as means (exponentiated for all log transformed data) and 95% confidence interval of the mean. All data in text is presented as estimated mean differences (EMD) (exponentiated for all log transformed data) and 95% confidence interval for the estimate, providing a non-standardised measure of effect size. All data in tables is presented as means ± SE.

2.4. RESULTS

2.4.1. Baseline Measures

All participants completed the experiment in full and without any adverse reactions. No differences in RMT and AMT were found between groups (Table 2.1). Older adults had less SICI and greater MEP (%M_{max}) compared to the young at baseline (Table 2.1). Older adults also demonstrated lower M_{max} and MVC at baseline compared to the young during the LICI session (Table 2.1). Finally, older adults had a greater Leisure-time index compared to young adults in the SICI session (Table 2.1).

Table 2.1: Participant and baseline corticospinal characteristics.

	SICI		LICI	
	Young	Old	Young	Old
N	15	15	15	14
Females	5	9	7	5
Age (yrs)	22.4 ± 0.4	68.3 ± 1.4*	22.8 ± 0.9	69.0 ± 1.4*
Weight (Kgs)	72.3 ± 3.1	75.26 ± 4.7	70.4 ± 3.8	79.5 ± 3.7*
Height (cm)	172.8 ± 2.6	167.9 ± 2.8	172 ± 2.9	167.1 ± 3.3
Handedness	0.8 ± 0.05	0.8 ± 0.1	0.7 ± 0.06	0.8 ± 0.1
Work index	2.4 ± 0.1	2.6 ± 0.1	2.4 ± 0.1	2.6 ± 0.1
Sport index	2.6 ± 0.3	2.6 ± 0.2	2.5 ± 0.3	2.5 ± 0.2
Leisure-time index	2.9 ± 0.2	3.5 ± 0.2*	2.9 ± 0.2	3.4 ± 0.2
MVC (N)	33.0 ± 10.4	29.3 ± 8.5	35.5 ± 11.2	27.1 ± 8.4*
M_{max} Intensity (mA)	40.8 ± 2.5	41.0 ± 3	42.2 ± 1.3	41.0 ± 3.4

M_{max} (mV)	12.6 ± 3.2	10.5 ± 3.4	15.4 ± 0.9	9.1 ± 0.9*
CS (% MSO)	40.2 ± 14.5	41.5 ± 12.0	70.5 ± 2.1	65.1 ± 2.7
TS (% MSO)	68.7 ± 14.1	68.7 ± 11.8	72.9 ± 2.1	68.1 ± 3.4
RMT (% MSO)			56.9 ± 1.5	51.7 ± 2.4
AMT (% MSO)	44.2 ± 7.1	46.0 ± 7.0		
EMG_{rms} during 25% EMG contraction (mV)	1.9 ± 0.3	2.3 ± 0.3	1.6 ± 0.7	2.9 ± 0.6

Values are shown as mean ± SE. *$P<0.05$ when compared to the young participants. MSO, Motor Stimulator Output; CS, Conditioning Stimulus; TS – Test Stimulus

2.4.2. SICI Session

SICI did not change across time ($F_{7,793}$ = 1.4, *P = 0.2*) and no interaction between time and age ($F_{7,773}$ = 1, *P = 0.4*) was observed. However, conditioned MEP size (% unconditioned MEP) was greater in younger adults compared to older adults post fatigue (age effect: $F_{1,283}$ = 27.1, *P<0.01*) with an EMD of 119% (95% CI [107, 132], *P<0.05;* Fig 2.3A). While there was no interaction between time and age ($F_{7,729}$ = 1.2, *P = 0.29*), unconditioned MEP was reduced post fatigue and gradually recovered across time compared to baseline in both populations (time effect: $F_{7,738}$ = 15.1, *P<0.05*) with EMD ranging from 277% (95% CI [199, 384], *P<0.05*) post fatigue to 147% (95% CI [105, 205], *P<0.05;* Fig 2.3B) at PF5. Unconditioned MEP was also greater in older adults compared to young adults (age effect: $F_{1,364.5}$ = 11.7, *P<0.05*) with EMD of 82% (95% CI [72,92], *P<0.05;* Fig 2.3B). M_{max} did not change across time ($F_{12,625}$ = 0.8, *P = 0.63*) and no interaction between time and age ($F_{12,669}$ = 1.4, *P = 0.12*) was observed for M_{max}. M_{max} was however greater in the young compared to old (age effect: $F_{1,139}$ = 4.9, *P<0.05)* with an EMD of 119% (95% CI [102, 139], *P<0.05*).

MVC changed across time ($F_{12,423}$ = 11.7, *P<0.001)* with a significant decline in maximum force observed immediately post exercise (EMD, 132% (95% CI [118, 146], *P<0.05*)) to recovery (EMD, 158% (95% CI [133, 188], *P<0.05*)). However, older adults showed less decrement in their MVC relative to baseline (age effect: $F_{1,55}$ = 62.3, *P<0.05*) with an EMD of 142% (95% CI [130, 155], *P<0.05;* Fig 2.3C) between young and old. No interaction between time and age was observed for MVC ($F_{12,389}$ = 1, *P = 0.4*; Fig 2.3C*)*. Force during 25% EMG (Fig 2.3D) contraction also changed across time ($F_{15,380}$ = 5.6, *P<0.05;* Fig 2.3D) with a significant decline in force from F2 (EMD, 139% (95% CI [117, 166], *P<0.05)*) to recovery (EMD, 180% (95% CI [115, 283], *P<0.05*)). However, no main effect of age ($F_{1,88}$ = 1.2, *P = 0.27*), or interaction between time and age ($F_{15,388}$ = 1.4, *P = 0.13*) was observed on

force during 25% EMG. EMG_{rms} during the 25% EMG contraction did not change across time ($F_{15,404} = 0.5$, $P = 0.9$) nor was there any effect of age ($F_{1,208} = 3$, $P = 0.08$). No interaction between time and age ($F_{15,417} = 0.2$, $P = 1.0$) was observed either.

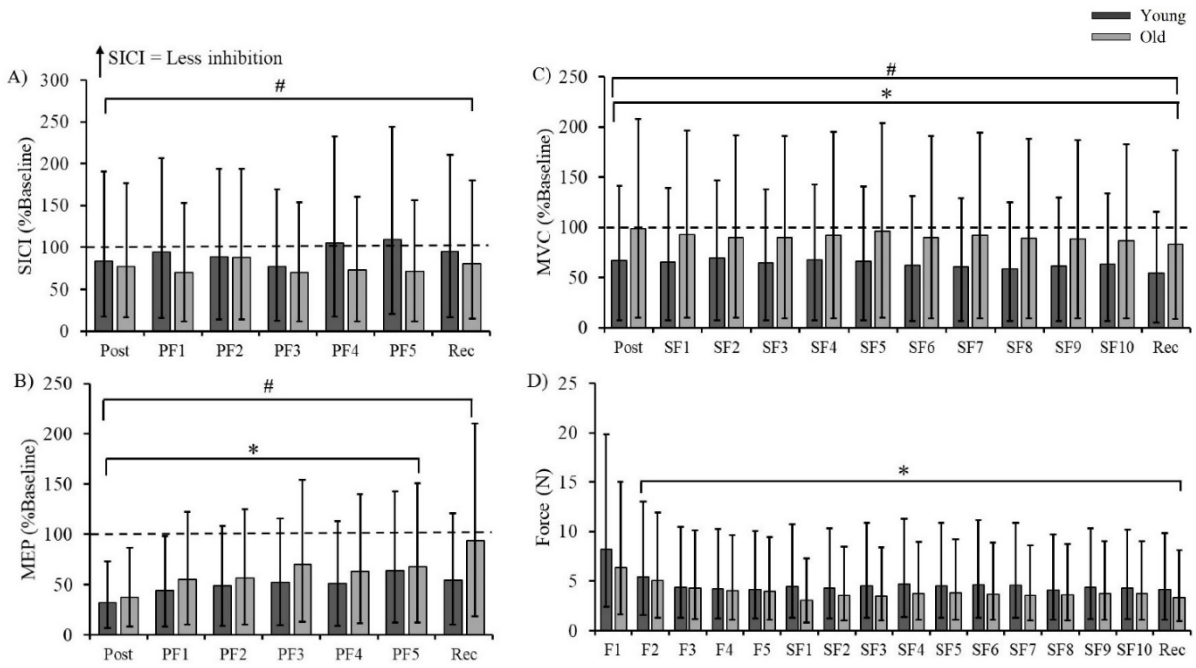

Figure 2.3: SICI session. *Effect of age and fatigue on exponentiated data. SICI (A), unconditioned MEP amplitude (B), MVC force (C) and force during 25% EMG (D) normalised to baseline in young (black bars and dark circles) and older (grey bars and clear circles) adults. #P < 0.05 young vs. older adults; *P < 0.05 compared to baseline. PF, Post Fatigue; SF, Sustained Fatigue.*

Table 2.2: Conditioned and Unconditioned responses during SICI session.

	Conditioned (%Test)		Unconditioned (%Mmax)	
	Young	Old	Young	Old
Pre	35.9 ± 6.0	55.5 ± 6.0*	12.0 ± 1.4	14.0 ± 1.6
Post	48.6 ± 7.6	64.0 ± 7.6	3.6 ± 1.5	6.8 ± 1.8
PF1	51.2 ± 5.5	47.6 ± 5.6	6.0 ± 1.3	9.7 ± 1.5
PF2	52.4 ± 5.5	60.8 ± 5.6	6.5 ± 1.3	11.8 ± 1.6
PF3	43.0 ± 5.5	52.0 ± 5.6	8.0 ± 1.3	11.2 ± 1.6
PF4	57.0 ± 5.5	53.4 ± 5.5	7.7 ± 1.3	11.6 ± 1.6
PF5	56.0 ± 6.4	54.0 ± 5.5	7.9 ± 1.4	15.9 ± 1.6
Recovery	45.8 ± 6.1	54.0 ± 6.2	7.8 ± 1.4	16.6 ± 1.7

Values are shown as mean ± SE. *$P<0.05$ at baseline when compared to younger adults. PF, Post Fatigue

2.4.3. LICI Session

LICI was reduced across time ($F_{3,425}$ = 11.8, *P<0.05*) with a significant age effect ($F_{1,160}$ = 6.5, *P<0.05*) and interaction between time and age ($F_{3,398}$ = 3.1, *P<0.05;* Fig 2.4A). An increase in conditioned MEP (% unconditioned MEP) was evident immediately post exercise (EMD, 252% (95% CI [148, 427], *P<0.05*)) to PF2 (EMD, 215% (95% CI [123, 378], *P<0.05*)) in the older adults relative to baseline, demonstrating a decrease in inhibition (*see Fig. 2.2* and 2.4A). However, no changes were seen in the younger adults across time. LICI was also reduced to a greater extent in older adults compared to the younger adults at PF1 (EMD, 203% (95% CI [138, 299], *P<0.05)*). Unconditioned MEP modulated across time ($F_{7,789}$ = 6.3, *P<0.05*; Fig 2.4B), with an attenuation observed immediately post fatigue that gradually recovered across the PF time points; with EMD ranging from 211% (95% CI [143, 312], *P<0.05*) at immediately post to 166% (95% CI [105, 260], P<0.01) at PF3). However, there was no main effect of age ($F_{1,364}$ = 11.7; *P=0.4*) or interaction between time and age ($F_{7,765}$ = 1.4, *P = 0.18*) on unconditioned MEP (Fig 2.4B). M_{max} did not change across time ($F_{12,616}$ = 0.9, *P = 0.54*) and no interaction was seen between time and age ($F_{12,662}$ = 0.9, *P = 0.46*). Younger adults however displayed higher M_{max} values throughout the exercise compared to older adults (age effect: $F_{1,247}$ = 118.5, *P<0.05*) with EMD at 244% (95% CI [208, 288], *P<0.05*).

MVC changed across time ($F_{12,333}$ = 7.0, *P<0.05*) with a main effect of age ($F_{1,64}$ = 61.8, *P<0.05*; Fig 2.4C) and interaction between time and age ($F_{12,325}$ = 2.1, *P<0.05*; Fig 2.3C). Magnitude of force declined in the young (*P<0.05*) with EMD ranging from 145% (95% CI [123, 170], *P<0.05*) immediately post to 149% (95% CI [116, 191], *P<0.05*) at recovery. However, older adults showed no decrement in their MVC relative to baseline throughout the protocol (P = 1.0). Force during 25% EMG contraction changed across time ($F_{15,366}$ = 6.9,

P<0.05) with an interaction between time and age ($F_{15,363}$ = 2.5, *P<0.05*) observed. However, no main effect of age ($F_{1,75}$ = 2.4, *P = 0.12*) was evident *(*Fig 2.4D*)*. A decline in force compared to F1 was seen in the young adults throughout the protocol (*P<0.05*) with EMD ranging from 179% (95% CI [138, 232], *P<0.05*) immediately post to 205% (95% CI [103, 413], *P<0.05*) at recovery. On the other hand, older adults displayed a decline in force ranging from F3 (EMD, 145% (95% CI [101, 208], *P<0.05*)) to SF4 (EMD, 188% (95% CI [104, 338], *P<0.05*)) when compared to F1 (Fig 2.4D). EMG_{rms} during the 25% EMG contraction did not change across time ($F_{15,403}$ = 0.8; *P* = 0.65) nor was there any age effect observed ($F_{1,337}$ = 3; *P* = 0.83). No interaction between time and age ($F_{15,406}$ = 0.5, *P = 0.92*) on EMG_{rms} was observed either.

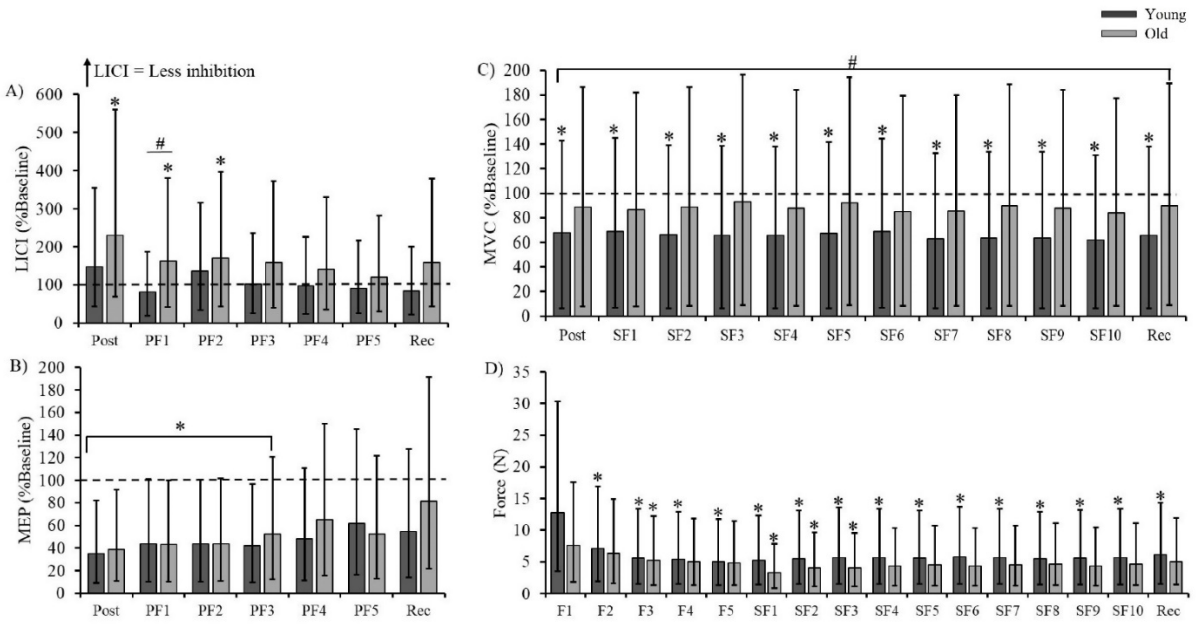

Figure 2.4: LICI session. *Effect of age and fatigue on exponentiated data. LICI (A), unconditioned MEP amplitude (B), MVC force (C) and force during 25% EMG (D) normalised to baseline in young (black bars and dark circles) and older (grey bars and clear circles) adults. #P < 0.05 young vs. older adults; *P < 0.05 compared to baseline. PF, Post Fatigue; SF, Sustained Fatigue.*

Table 2.3: Conditioned and Unconditioned responses during LICI session.

	Conditioned (%Test)		Unconditioned (%Mmax)	
	Young	Old	Young	Old
Pre	32.5 ± 27.1	21.3 ± 28.9	9.5 ± 0.6	18.4 ± 2.5*
Post	61.3 ± 35.0	52.4 ± 36.7	3.7 ± 0.3	8.2 ± 1.5
PF1	52.8 ± 24.8	41.2 ± 26.1	5.0 ± 0.3	7.7 ± 0.8
PF2	72.3 ± 24.8	43.2 ± 26.3	4.4 ± 0.3	9.5 ± 1.0
PF3	132.7 ± 24.8	39.6 ± 26.1	5.1 ± 0.3	9.0 ± 0.8
PF4	101.3 ± 24.8	37.6 ± 26.0	5.5 ± 0.3	14.2 ± 1.4
PF5	106.3 ± 30.3	35.0 ± 26.4	5.1 ± 0.3	13.1 ± 1.2
Recovery	35.0 ± 27.1	51.6 ± 28.8	5.6 ± 0.4	18.8 ± 3.3

Values are shown as mean ± SE. *$P<0.05$ at baseline when compared to younger adults. PF, Post Fatigue

2.5. DISCUSSION

2.5.1. Main findings

This aim of the current study was to determine whether there were age-related changes in GABA modulation following a sustained submaximal isometric contraction. While young adults were characterised by no change in SICI (Fig 2.3A) or LICI (Fig 2.4A), we provide evidence of an attenuation in LICI and no change in SICI with fatiguing exercise in older adults. The outcomes of the study suggest an age-related decrease in $GABA_B$ mediated inhibition in the presence of fatigue; but no change in $GABA_A$ mediated inhibition with fatigue in both young and old adults.

2.5.2. Fatigability in young and older adults

Neuromuscular fatigue has two origins – peripheral (distal to neuromuscular junction, e.g. due to impairment in the contraction coupling process) (Spriet et al. 1987) and central (proximal to neuromuscular junction) (Gandevia 2001). Central fatigue occurs when motor unit recruitment is insufficient and/or motor unit firing rates remain suboptimal due to variations in spinal reflex circuits and/or descending motor pathways (Sidhu et al. 2013a). During a sustained submaximal force contraction, the active muscle fibres progressively fatigue leading to the recruitment of additional motor units to sustain the required force. This is usually seen as an increase in EMG during a fatiguing task (Fuglevand et al. 1993; Riley et al. 2008). However, in the current study, motoneuron output was 'clamped' by maintaining a constant 25% EMG output during the sustained contraction (Hunter et al. 2016a; McNeil et al. 2011a). The implementation of an EMG contraction, rather than a force contraction to induce fatigue allowed us to document the development of fatigue during the course of the contraction by measuring the reduction in the magnitude of absolute force held by the participant, without having to interrupt the exercise to perform an MVC. As expected, force generating capacity of

the muscle during the 25% EMG contraction was substantially reduced in both young and older adults during both sessions, illustrating the development of fatigue (Hunter et al. 2016a). Although MVC force of the hand muscle was attenuated throughout the exercise protocol in young adults, older adults did not experience a similar decrease in maximum muscle force, albeit only during the LICI session, suggesting that the reduction in force during the 25% EMG contraction was insufficient to induce a change in maximum muscle force during this session. The absence of a reduction in MVC force in the LICI session in older adults may be attributed to participant variability, as four older participants were not matched between the two sessions. Indeed, the eleven older adults who were matched between the two sessions showed a decrease in MVC with the fatiguing exercise in both sessions. The fact that force was attenuated during the 25% EMG contraction but not during MVCs in some older adults may also be partially related to the differences in muscle fiber recruitment during the 25% EMG contraction versus MVC. For instance, during low level contractions, type I fibres (slow twitch, smaller in size) are largely recruited; whereas during MVCs, both type I and type II fibres (fast twitch, bigger in size) are recruited (Vollestad and Blom 1985). The discrepancy in fatigue measured between the two contraction types may be further colluded by the fact that the older adults are characterised by greater fatigue resistance (Callahan and Kent-Braun 2011; Yoon et al. 2013; Yoon et al. 2012), mediated via the loss of the type II muscle fibres and a concomitant increase in type I muscle fibre composition (Andersen 2003; Lexell et al. 1988).

2.5.3. Change in MEP measures of corticospinal excitability

MEPs reflect the responsiveness of the corticospinal pathway (Gandevia et al. 1996). An increase in MEP size measured in a resting muscle during short-duration intermittent fatiguing contractions (Benwell et al. 2007c; Benwell et al. 2006b; Otieno et al. 2019) suggests an increase in corticospinal excitability in the presence of single-joint exercise fatigue. However, similar to the outcomes reported following sustained submaximal contractions

(Brasil-Neto et al. 1993; Sacco et al. 2000; Samii et al. 1997), we demonstrated a decrease in corticospinal excitability in both age groups following a 15 min submaximal EMG contraction and throughout the sustained fatiguing contractions. This post-exercise MEP depression has previously been attributed to intracortical inhibition, since centrally evoked responses by transcranial electrical stimulation (to directly activate spinal motoneurons) remained unchanged after exhaustive weightlifting repetitions of the wrist flexors (Brasil-Neto et al. 1993). Contrary to this, McNeil et al (2011a) have shown a similar degree of suppression in responses evoked by TMS and spinal stimulations during the SP of a 10 min sustained 25% EMG contraction, suggesting that a reduction in motoneuron excitability (possibly related to repetitive motoneuron activation during a sustained contraction), rather than cortical excitability, may be responsible for the impairment in MEP. Our study was designed to investigate the presence of fatigue on intracortical mechanisms measured with respect to a resting muscle. Indeed, given the task and context dependant influence of fatigue on corticospinal responses (Taylor et al. 2016), parallels between studies that measure central responsiveness with short intermittent maximum versus sustained submaximal contractions, as well as during muscle activity (McNeil et al. 2011a; McNeil et al. 2009) versus resting muscle (Benwell et al. 2007c; Benwell et al. 2006b) may not necessarily be drawn. One consideration for the interpretation of the MEP data is the fact that while the absolute MEP amplitude was kept consistent between participants (1 mV), MEPs were larger in older adults when normalised to M_{max} at baseline since older adults are characterised by smaller M_{max} in the FDI (Opie and Semmler 2014a). Therefore, it is possible that the amount of motoneuron activation influenced the baseline magnitude of inhibition, as in the case of SICI. However, it may also be argued otherwise since the increase in the amount of motoneuron activation did not influence baseline LICI. In any case, to account for these baseline differences, and to address

the primary aim of age mediated influence of fatigue on central responsiveness, we normalised all the post fatigue data to the baseline values.

2.5.4. SICI and LICI post fatiguing exercise in a resting muscle

Paired pulse paradigms including SICI and LICI reflect the suppression of later indirect waves (I-waves) such as I_3, which follow the corticospinal short latency direct wave (D wave) (Di Lazzaro et al. 2002; Di Lazzaro et al. 1998b; Reis et al. 2008). SICI is known to be mediated by $GABA_A$ (Di Lazzaro et al. 2000) while LICI is thought to be mediated by $GABA_B$ inhibitory interneurons (McDonnell et al. 2006). When measured at rest, a decrease in SICI and LICI following intermittent fatiguing maximum contractions (Benwell et al. 2007c; Benwell et al. 2006b; Maruyama et al. 2006; Vucic et al. 2011) and after a 2 min maximum fatiguing contraction (Maruyama et al. 2006) has been reported in young healthy individuals, suggesting a decrease in $GABA_A$ and $GABA_B$ mediated inhibition respectively. A similar decline in SICI has been reported with sustained submaximal isometric contraction when measured in an active muscle (15-25% MVC) (Hunter et al. 2016a; Williams et al. 2014). In the current study, we measured inhibition in a resting muscle instead of active muscle immediately after sustained fatiguing contractions and observed no change in both SICI and LICI in the young adults. This lack of change in SICI and LICI may be attributed to the different exercise modalities used between studies (short duration intermittent exercise versus longer duration sustained exercise), suggesting that the modulation of GABA inhibitory processes with fatiguing exercise is likely task dependant. Interestingly, McNeil and colleagues have identified a contradictory increase in $GABA_B$ mediated inhibition during fatiguing maximum and submaximal isometric exercise in young adults (McNeil et al. 2011a; McNeil et al. 2009). However, in addition to the fact that they measured inhibition during an active contraction, they also demonstrated that impaired motoneuron responsiveness played a predominant role in the increase in $GABA_B$ inhibition.

With no change in SICI, older adults showed a decrease in LICI. Contrary to our recent TMS-EEG data showing an age-related decrease in $GABA_A$ mediated inhibitory activity with intermittent maximal fatiguing exercise (Opie et al. 2020), the current outcomes demonstrate an age-related decrease in $GABA_B$ mediated inhibitory activity with sustained submaximal EMG contraction; suggesting a task-dependant $GABA_B$ modulation in the presence of fatigue in older adults. Even though there is pharmacological and behavioural data demonstrating that TME-EEG data is sensitive to changes in intracortical inhibition (Bender et al. 2005; Bonnard et al. 2009; Premoli et al. 2014), there is some recent data that questions the relationship between LICI elicited with paired pulse MEPs and TEPs (Biabani et al. 2019a), which may partly explain the contradictory observations between our previous work using TMS-EEG (Opie et al. 2020) and the current outcomes with TMS-EMG. In addition, even though we are not able to exclude the role of spinal mechanisms in the modulation of $GABA_B$ mediated inhibition in older adults (McNeil et al. 2011a), there is evidence from TMS-EEG data to show that $GABA_B$ modulation (as measured with LICI) is cortically mediated (Opie et al. 2017b). Furthermore, while fatigue was sustained for a prolonged period, its effect on GABA modulation did not persist during maintenance of fatigue in older adults. This implies that GABA modulation may play a role in the initial stages of fatigue but not when fatigue is sustained for a longer period in older adults.

2.5.5. Methodological Considerations

While it may be argued that measurements during the fatiguing task per se or during muscle activity provide greater functional representation (Sidhu et al. 2018; Sidhu et al. 2014), measurement of intracortical inhibition during muscle activity also present challenges. For example, muscle activity attenuates the magnitude of inhibition (Ridding et al. 1995), making it difficult to obtain sufficient magnitude of inhibition at baseline (i.e. ~ 20% -30% during a 25% muscle contraction). Interestingly, we were not able to get sufficient inhibition in ~13%

of the recruited participants even in a resting FDI muscle. These participants were excluded from data collection. We targeted baseline SICI and LICI that was closest to 50% in all participants to ensure that there was neither a peak nor a trough at baseline to allow for fatigue related modulations in either direction. Furthermore, our experimental design of measuring inhibition in a rested muscle allows for better comparison of outcomes with recent studies from our group investigating cortical inhibition using TMS-EEG, whereby measurements have to be implemented at rest to avoid confounding influences of movement artefacts on the EEG recordings (Opie et al. 2020; Otieno et al. 2019). An additional consideration is the that there are sex differences in fatigability whereby females are typically characterised by greater fatigue resistance due to greater oxygen availability during exercise (Ansdell et al. 2019a); and fatigability and SICI varies across the menstrual cycle (Ansdell et al. 2019b). We did not control for the menstrual cycle phase of the young females in the current study, and this forms a limitation of this work. However, given that most previous work has focused on hormonal influences of fatigue in large locomotor muscles, the evidence for sex differences in fatigability during small muscle exercise remains equivocal. Indeed, in the current study, we did not observe any differences in FDI muscle fatigability between males versus females. Finally, there is some evidence to show that averaging less than 20 simultaneous MEP responses results in increased variability (Biabani et al. 2018). Whilst we were limited with the number of stimulations delivered post fatigue due to the quick recovery of fatigue, the low number of stimulations (10 – 12 at each time point including baseline) should be acknowledged.

2.5.6. Conclusion

The current findings suggest that older adults modulate LICI with a submaximal fatiguing contraction by attenuating $GABA_B$ mediated inhibition. Nonetheless, further investigation into the age-related effects of fatigue on LICI and SICI via implementation of exercise models that induce a similar degree of fatigue in both groups forms an important

expansion of the present investigation. Investigating GABA mechanisms in larger muscle groups of the upper and lower limbs may also provide a more functional representation of the age-related modulation of GABA inhibitory processes with fatiguing exercise. This will form the primary aim of chapter three. The discovery of these components may contribute to the development of interventions and training that effectively improve work efficiency, improve exercise tolerance, and preserve function in older adults.

CHAPTER III

SUSTAINED SUBMAXIMAL ISOMETRIC FATIGUING EXERCISE OF THE ELBOW FLEXORS HAS NO AGE-RELATED EFFECT ON $GABA_B$ MEDIATED INHIBITION

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Contribution to the Paper	Experimental Design, Subject Recruitment, Collection and analysis of data, interpretation of data, wrote manuscript		
Overall percentage (%)	55%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	15/11/2021

Co-Author Contributions

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

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

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

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

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3. SUSTAINED SUBMAXIMAL ISOMETRIC FATIGUING EXERCISE OF THE ELBOW FLEXORS HAS NO AGE-RELATED EFFECT ON $GABA_B$ MEDIATED INHIBITION

3.1. ABSTRACT

Age-related changes in the neuromuscular system can result in differences in fatigability between young and older adults. Previous research has shown that single joint isometric fatiguing exercise of small muscle results in an age-related compensatory decrease in $GABA_B$ mediated inhibition. However, this has yet to be established in a larger muscle group. In 15 young (22 ± 4 years) and 15 older (65 ± 5 years) adults, LICI (100 ms ISI) and corticospinal SP were measured in the biceps brachii during a 5% EMG contraction using TMS before, during and after a submaximal contraction (30% MVC force) held intermittently to task failure. Both age groups developed similar magnitude of fatigue (~24% decline in MVC; $P = 0.001$) and ~28% decline in LICI ($P = 0.001$) post fatiguing exercise. No change in SP duration was observed during and immediately following fatigue ($P = 0.909$) but ~ 6% decrease was seen at recovery in both age groups ($P<0.001$). Contrary to previous work in a small muscle, these findings suggest no age-related differences in $GABA_B$ mediated inhibition following single joint isometric fatiguing exercise of the elbow flexors, indicating that $GABA_B$ modulation with ageing may be muscle group dependent. Furthermore, variations in SP duration and LICI modulation during and post fatigue in both groups suggest that these measures are likely mediated by divergent mechanisms.

Key Words: ageing, cortical excitability, fatiguing exercise, intracortical inhibition, physical activity, transcranial magnetic stimulation.

3.2. INTRODUCTION

Neuromuscular fatigue refers to the exercise induced reduction in force produced by a muscle or muscle group (Taylor et al. 2000). It results from an impairment in voluntary drive to the muscle as well as factors that directly impair contractile properties within the muscle fibres (Fitts 1994; Gandevia 2001). Age- related changes within the neuromuscular system are known to alter fatigability in older adults (Hunter et al. 2016b). For example, the age-related loss and reduction in the proportional area of type II (fast) muscle fibers leads to a weaker, but more oxidative and fatigue resistant muscle (Andersen 2003; Hunter et al. 1999; Lexell et al. 1988) resulting in less fatigable older adults during isometric low level contractions (Bilodeau et al. 2001b; Chung et al. 2007; Hunter et al. 2008; Yoon et al. 2013; Yoon et al. 2012). Although changes within the muscular system that are distal to the neuromuscular junction contribute significantly to the aforementioned differences, modulations within the central nervous system also appear to play a key role.

TMS studies have shown that changes within the corticospinal tract may be an important consideration for the age-related differences in fatigability. For example, an attenuated increase in MEP and SP duration in older adults compared to young has been reported during fatiguing exercise (Yoon et al. 2013; Yoon et al. 2012). This suggests that the ability to modulate corticospinal excitability and intracortical inhibition in fatigued older adults may be compromised (Yoon et al. 2012). SP refers to the suppression in EMG signal observed during a voluntary contraction following a single pulse TMS. Due to the known influence of both spinal and intracortical $GABA_B$-mediated inhibitory mechanisms, SP is not considered the most appropriate representation of intracortical inhibition (Inghilleri et al. 1993; Yacyshyn et al. 2016). Other TMS paradigms have been implemented to provide a more accurate assessment of intracortical inhibitory activity. For example, using combined single pulse TMS with electroencephalography (TMS-EEG; measures acquired at the scalp), analysis of the N100

peak showed no difference in $GABA_B$ modulation in older adults following intermittent fatiguing maximal contractions of the FDI muscle while younger adults showed an attenuation in $GABA_B$ mediated inhibition post fatigue (Opie et al. 2020). Paired-pulse paradigms such as LICI (known to reflect $GABA_B$ mediated inhibition (McDonnell et al. 2006; Valls-Sole et al. 1992)) have also been used. However, the spinal cord is also known to influence LICI measures (similar to SP) (McNeil et al. 2011a; McNeil et al. 2009). Nonetheless, contrary to the increase in SP duration (albeit attenuated compared to young adults) post fatigue in older adults (Yoon et al. 2008; Yoon et al. 2013; Yoon et al. 2012), an age-related reduction in LICI following single joint sustained submaximal EMG contraction of the FDI muscle has been observed; suggesting a decrease in $GABA_B$ mediated inhibition in older adults (Otieno et al. 2021). In any case, previous work has focused on a small muscle (i.e., FDI) mass with fatigue. Age-related differences in baseline LICI measures have been identified across muscle groups. For example, Opie and colleagues showed an age-related *decrease* in LICI in older adults when measured in the FDI (Opie and Semmler 2014a); while McGinley and colleagues identified an age-related *increase* in LICI in older adults when measured in forearm muscles (McGinley et al. 2010). Furthermore, whilst a decrease in LICI was shown post fatiguing exercise when measured in the FDI muscle (Benwell et al. 2007b), a contradictory increase in LICI following fatigue was shown in the elbow flexor muscles when measured during activity in young adults (McNeil et al. 2011a; McNeil et al. 2009); suggesting possible differences in $GABA_B$ modulation in larger versus smaller muscle groups. Nevertheless, the role of fatigue on LICI in a relatively larger muscle-group in older adults remains unstudied.

In the current study, we aimed to investigate changes in LICI and SP duration in older adults compared to a young control group with elbow flexion fatiguing exercise. These corticospinal measurements were obtained during a 5% EMG contraction intermittently performed during a single joint submaximal (30% MVC force) contraction of the elbow flexor

muscles to task failure. A constant EMG contraction was used instead of a force contraction since background EMG increases during a constant force contraction is known to influence MEP measurements (Hunter et al. 2016a). A submaximal force contraction was also implemented in the study to ensure similar decline in force with fatigue in both age groups. This is because our previous work demonstrated that a submaximal EMG contraction led to differential amounts of fatigue in young and older adults which may influence the interpretation of the paired-pulse MEP measures (Otieno et al. 2021). Based on our recent findings (Otieno et al. 2021), we hypothesised an age-related compensatory decrease in LICI in older adults with fatiguing exercise, possibly to allow for greater excitation of the motoneurons as fatigue develops. We also hypothesised a lesser increase in SP duration in older adults compared to the young.

3.3. METHODS

Fifteen right-handed young (22.2 ± 0.9 yrs; 8 females) and fifteen older (65.3 ± 0.8 yrs; 8 females) healthy participants were recruited from the university community, community centres and social media for participation in this study (Table 3.1). Assessment of handedness was performed using the Edinburgh Handedness inventory (Oldfield 1971). Participants were excluded based on current use of psychoactive medication (e.g., sedative, antidepressants, and antipsychotics) or history of neurological and/or psychiatric disease. Self-reported subjective physical activity (work index, sport index, leisure-time index) was assessed in all participants, using an activity questionnaire (adapted from Baecke et al. 1982) where participant scores ranged from 1 (sedentary) to 5 (active) for each index. All procedures were approved by the University of Adelaide Human Research Ethics Committee and conducted in accordance with the Declaration of Helsinki. Each participant provided written, informed consent prior to participation.

Table 3.1: Participant characteristics.

	Young	Old
N	15	15
Females	8	8
Weight (kg)	59.9 ± 10.6	74.3 ± 13.1*
Height (cm)	168.2 ± 8.3	167.4 ± 7.8
Handedness	0.7 ± 0.2	0.9 ± 0.1*
Work Index	2.6 ± 0.8	2.7 ± 0.6
Sport index	2.4 ± 0.8	2.5 ± 1.4
Leisure-time index	3.1 ± 0.8	2.9 ± 0.6

Values are shown as mean ± SD. *$P < 0.05$ when compared to the young participants. Handedness ≥ 0.5 indicates right-handed individuals.

3.3.1. Experimental set-up

Force in newtons (force = torque/lever arm) was evaluated using a Biodex Multi-Joint System (Biodex, Inc, Shirley, NY). Each participant sat upright with their right arm slightly abducted and their elbow resting comfortably on a padded support flexed to 90° so that the supinated forearm was horizontal to the ground. The shoulders were restrained by two nylon straps to minimize shoulder movement. Forces detected by the Biodex were recorded online using a Power 1401 A-D converter and Spike 2 software [Cambridge Electronics Design (CED), Cambridge, UK]. The force exerted in the vertical direction was displayed on a 17-in. monitor located 0.9 m in front of the participant.

3.3.2. Electrical Recordings

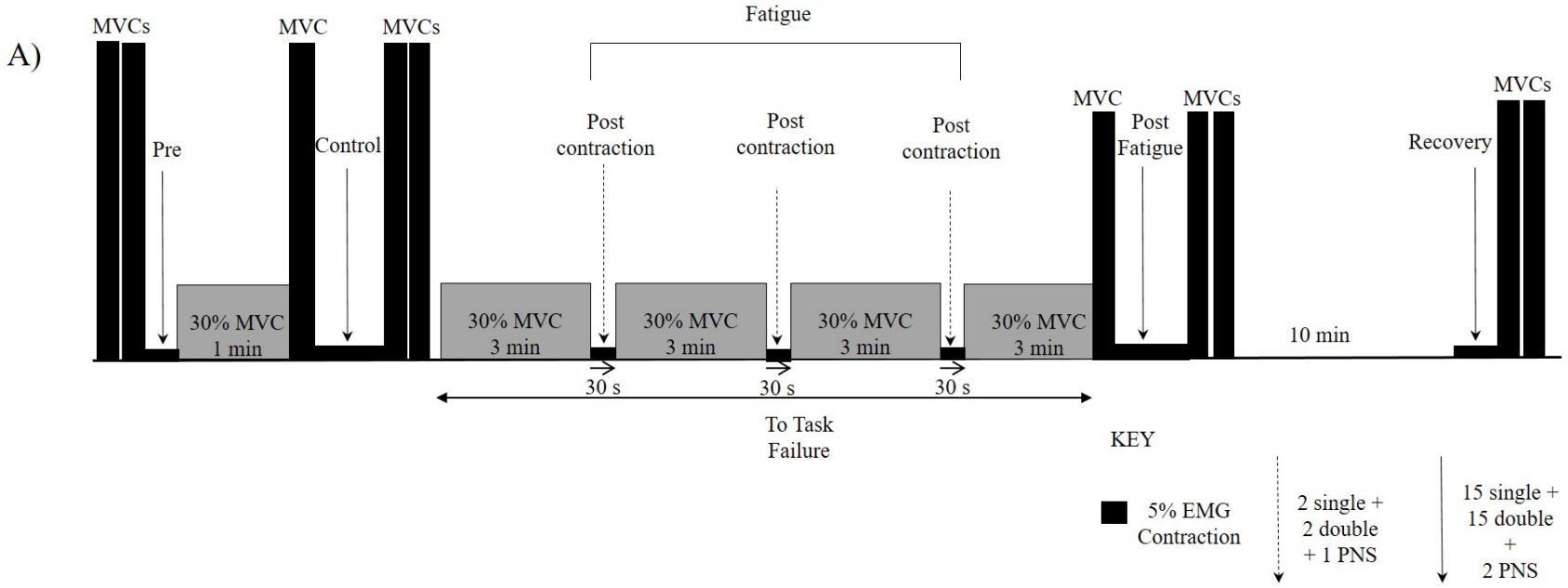
EMG signals were recorded using surface electrodes (Ag-AgCl, 8-mm diameter) that were placed in a monopolar configuration (over the muscle belly and the tendon) of biceps brachii and triceps brachii (Hunter et al. 2008). EMG signals were amplified (×300) and band-

pass filtered (16 –1,000 Hz) [model CED 1902, Cambridge, UK]. Force (1,000 Hz) and EMG (2,000 Hz) signals were sampled with a CED 1401 computer interface and Spike2 software (CED).

3.3.3. Experimental protocol

Figure 3.1 shows the experimental protocol carried out during each session. Maximum force and EMG were calculated by performing two, 3–5 s MVCs of the elbow flexors. If there was a discrepancy of more than 5% between the two MVCs, then participants were instructed to perform additional contractions until less than 5% variability was obtained between two consecutive MVCs. Prior to the fatiguing task, participants received a *set of stimulations* which consisted of fifteen single pulse TMS, fifteen paired-pulse TMS, and two electrical stimulations of the brachial plexus. All stimulations were delivered during 5% of the maximal recorded EMG during MVC in a non-fatigued muscle. EMG_{rms} was provided for feedback instead of raw EMG signal so that it was possible for participants to match the required 5% target and minimise variability which can influence size of MEPs. Participants then performed a control contraction at 30% MVC force (Fig 3.1B) for one minute followed by a short 3 – 5 s MVC and another *set of stimulations*. Two more MVCs were performed with a 10 s break between contraction and a 5 min resting period provided. To fatigue the muscles, participants were instructed to perform a submaximal contraction at 30% MVC force until task failure. A 30 s break was provided every 3 min during the fatiguing contraction where participants received two single pulse TMS stimulations, two paired-pulse TMS and one electrical stimulation of the brachial plexus during a 5% EMG contraction (Brownstein et al. 2020; Hunter et al. 2016a; McNeil et al. 2011b). They were then instructed to immediately resume the submaximal contraction at 30% MVC force. This pattern was continued until task failure (defined as a 5% drop in target force for >3 s in both age groups). A verbal cue provided by the experimenter indicated when participants should commence exercise or rest, and participants were asked to

refrain from using other muscles. Visual feedback of force output (displayed on a computer screen) and verbal encouragement was provided throughout the protocol. Immediately post-exercise, participants performed one brief 5 s MVC and received a *set of stimulations* at 5% EMG contraction. Two more brief 5 s MVCs with a 10 s rest period in between were performed prior to recovery. A 10 min recovery period was then provided followed by a final *set of stimulations* at 5% EMG contraction. To conclude the session, participants were instructed to perform two final MVCs to measure force recovery (Fig 3.1).


A)
MVCs
MVC
MVCs
Fatigue
Pre
Control
Post contraction
Post contraction
Post contraction
MVC
Post Fatigue
MVCs
Recovery
MVCs
30% MVC 1 min
30% MVC 3 min
30% MVC 3 min
30% MVC 3 min
30% MVC 3 min
10 min
30 s
30 s
30 s
To Task Failure
KEY
5% EMG Contraction
2 single + 2 double + 1 PNS
15 single + 15 double + 2 PNS

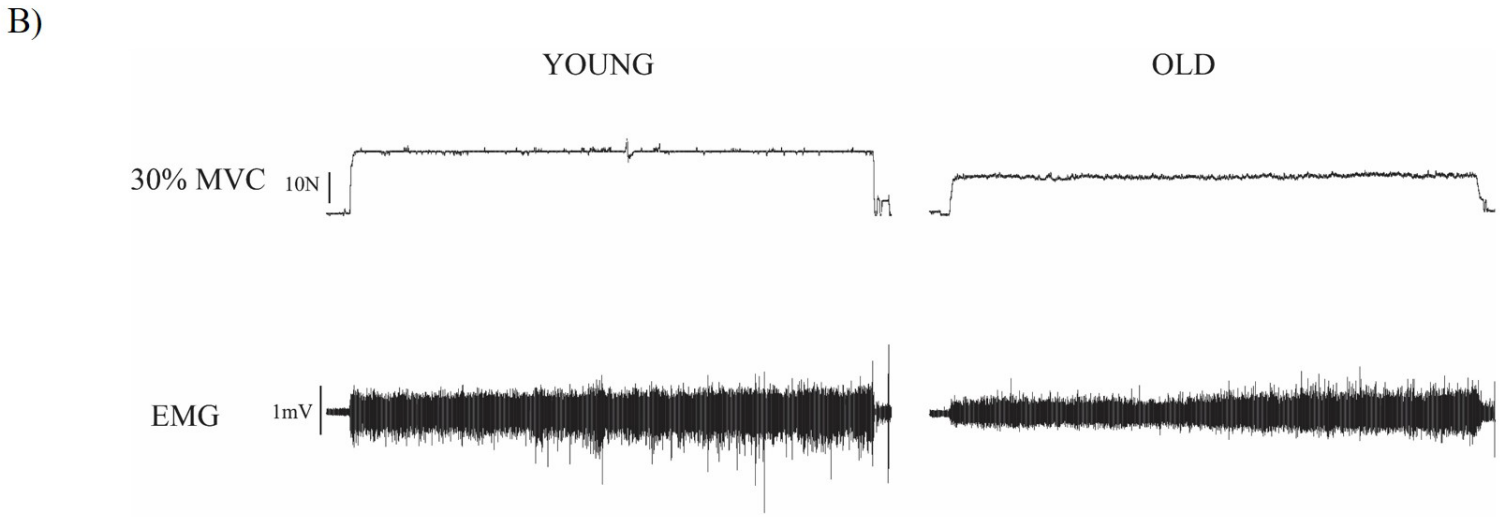

B)
YOUNG
OLD
30% MVC
10N
EMG
1mV

Figure. 3.1. Experimental protocol schematic A) Baseline: *Participants performed two 3-5 s MVCs and received a set of stimulations (solid arrow) during 5% EMG contraction (solid black box) prior to exercise. They were then instructed to perform a minute long control contraction at 30% MVC force followed by one 3-5 s MVC immediately post contraction and a set of stimulations (solid line) during a 5% EMG contraction. Participants then performed two 3-5 s MVCs prior to the fatiguing exercise* ***B) Fatigue:*** *In order to fatigue the muscle, participants were required to perform intermittent 3 min contractions at 30% MVC force followed by a brief 30 s break to receive a brief set of stimulations (dotted arrow) during 5% EMG contraction. This sequence was repeated to task failure (5% drop in force for >3 s). One MVC was then performed at task failure followed by a set of stimulations and two more 3-5 s MVCs. A 10-minute recovery period was then provided, and participants received the final set of stimulations (solid arrow) during 5% EMG contraction. B) Single subject raw representative traces of force and EMG during 3 min 30% MVC force contraction in both young and older adults.* ***C)*** *Single subject raw representative traces of conditioned and unconditioned MEP responses at pre, control, during and post fatigue in both young and older adults. There was an increase in conditioned MEP size post fatigue in both age groups.*

3.3.4. Electrical stimulation of the brachial plexus

The brachial plexus was electrically stimulated to produce a maximal compound muscle action potential (maximum M-wave: M_{max}) of the biceps brachii. A constant-current stimulator (model DS7AH, Digitimer, Welwyn Garden City, Hertforshire, UK) was used to deliver single stimuli (100 μs duration) to the brachial plexus. A cathode was placed in the supraclavicular fossa and an anode on the acromion. The stimulation intensity was determined by increasing the current in increments of 10 mA until the peak-to-peak M-wave amplitude plateaued (M_{max}). To ensure supramaximal activation of the muscles, the stimulation intensity was further increased to 120% of the current needed to evoke largest M-wave response at rest (Mean ± SD: Young, 94 ± 27.2 mA; Old, 108 ± 35.3 mA) – *See Table 3.2*. These measurements were performed prior to any contractions or TMS.

3.3.5. Transcranial magnetic stimulation

TMS was applied to the left primary motor cortex using a figure-of-eight coil (external wing diameter 9 cm) with two monophasic Magstim 200^2 magnetic stimulators connected through a Bistim (Magstim, Dyfed, UK). The coil was placed tangentially to the scalp at an angle of 45° to the sagittal plane, with the handle pointed laterally and backwards, producing an anteriorly directed current flow in the brain. The coil was placed on the scalp over the region that produced the largest response in the active biceps brachii muscle at a fixed stimulator intensity (60–65% maximum stimulator output). This location was marked on the scalp using a pen for reference, and the coil was manually placed on the optimal spot by the experimenter, with coil position continually checked throughout the experiment. TMS was delivered at a rate of 0.2 Hz with 10% variance between trials.

AMT was determined in biceps brachii with the TMS coil placed over the optimal location. AMT was defined as the lowest stimulus intensity that produced a visible MEP

response relative to background EMG in three out of five stimulations during a 5% EMG contraction. LICI was also assessed in an active muscle. Test MEP was set at ~50% of M_{max} (Table 3.2) (Opie and Semmler 2014a; Opie and Semmler 2014b). Suprathreshold conditioning intensity (110%, 120%, 130% and 140% AMT) that a) elicited a SP duration closest to 150 ms (Table 3.2) and b) evoked closest to 50% inhibition of test MEP response at baseline was selected for experimentation (McNeil et al. 2011a; Otieno et al. 2021). Interstimulus interval (ISI) was set at 100 ms.

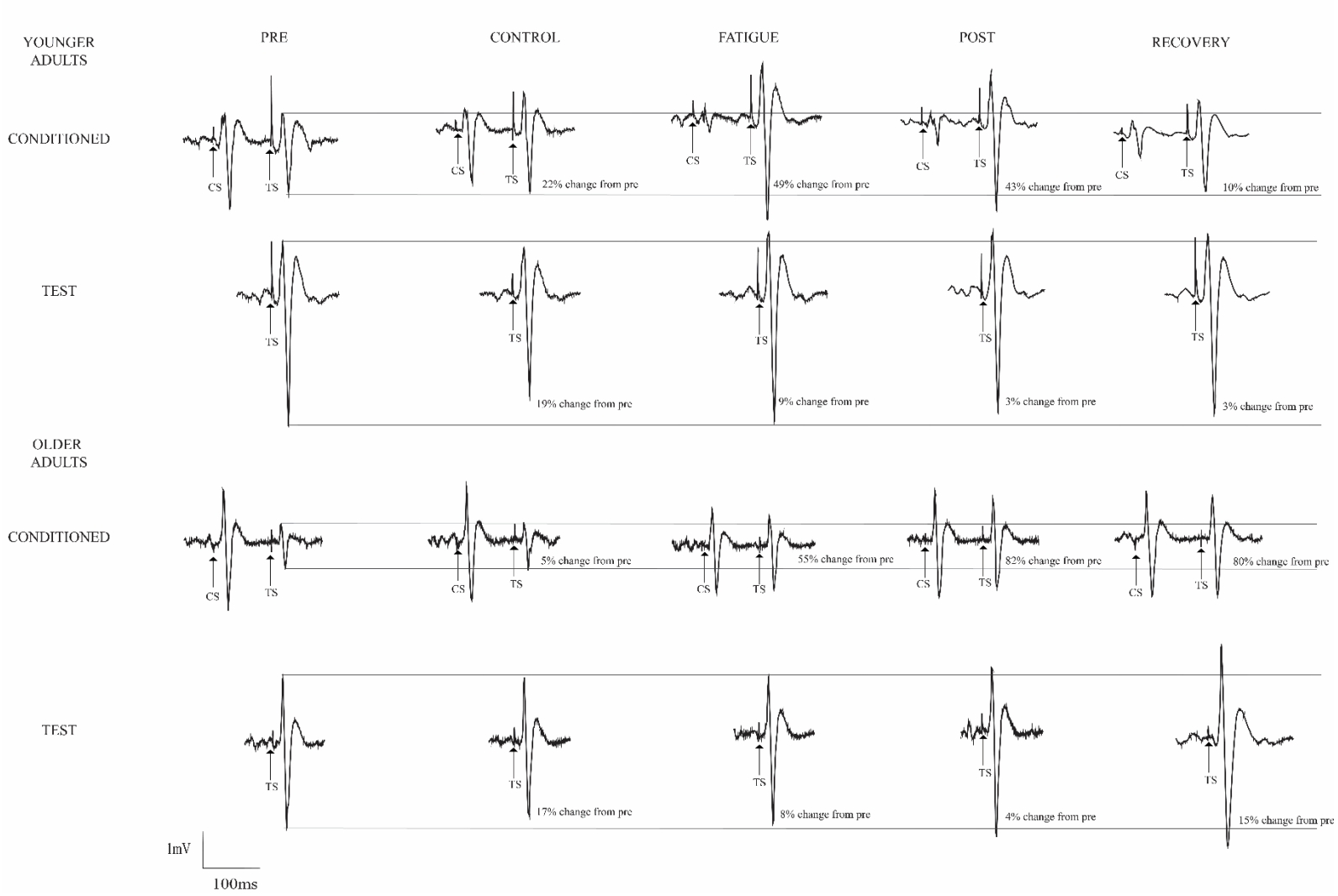


Figure 3.2. *Single subject raw representative traces of conditioned and unconditioned MEP responses at pre, control, during, post fatigue and recovery in both young and older adult*

3.3.6. Data analysis

Peak force amplitude was measured during brief MVCs before and after exercise. Force during each 5% EMG contraction was measured as mean force amplitude. Voluntary EMG during each 5% EMG contraction was measured throughout the protocol as root mean squared EMG (EMG_{rms}) over a duration of 100 ms prior to each stimulation (15 single pulse stimulations per time point). Maximum EMG (EMG_{rms}) was calculated during the plateau in force of the MVCs whereby force was required to plateau for at least 2 s before termination of MVC (Otieno et al. 2021). Voluntary EMG_{rms} during each 30% MVC was also calculated during the plateau in force of each 30% MVC. Both age groups completed nine 3 min contractions to task failure (Mean ± SD; Young, 4.5 ± 1.5; Old, 4.8 ± 1.9; *P = 0.67*), with each contraction represented by time points F1 – F9. MEP and M_{max} amplitudes from each trial were measured as peak-to-peak in mV. Test MEPs were expressed as a percentage of M_{max} to account for any muscle-dependent changes. LICI was calculated as the ratio (expressed as a percentage) between the peak-to peak amplitudes of the conditioned and unconditioned MEPs (conditioned/unconditioned × 100). Therefore, an increase in the magnitude of LICI reflects a reduction in inhibition (Fig 3.2). SP duration was calculated by visual inspection of the raw EMG trace from the point of stimulation until the resumption of pre-stimulus EMG (Brownstein et al. 2020) . All data collected during the 30 s rest intervals after each of the 3 min 30% MVC force contraction were pooled into one data point (Fatigue; *see Fig. 3.1*).

3.3.7. Statistical analysis

Individual linear mixed models with repeated measures (LMM_{RM}) were used to compare the effect of time (pre fatiguing exercise, during fatiguing exercise, post fatiguing exercise; as well as pre fatiguing exercise and recovery) and age on the magnitude of force, EMG_{rms}, LICI, SP, MEP and M_{max} with fatigue. Similarly, LMM_{RM} was used to compare the

effect of the single control contraction and age on the magnitude of force, EMG_{rms}, LICI, SP, MEP and M_{max}. Significant interaction and main effects were investigated via Bonferroni's post hoc tests corrected for multiple comparisons. For all comparisons, normality of the data was confirmed by Shapiro-Wilk test. Paired T-Test was used to compare the effect of age on time to task failure (TTF). All data in figures are presented as means and 95% confidence interval of the mean. All data in text is presented as estimated mean differences (EMD) and 95% confidence interval for the estimate, providing a non-standardised measure of effect size. All data in tables are presented as means ± SD. Statistical significance was set at $P < 0.05$.

3.4. RESULTS

All participants completed the experiment in full and without any adverse reactions. No differences in AMT, M_{max}, 5% EMG (%M_{max}) and MEP (%M_{max}) were found between age groups at baseline (Table 3.2, Fig 3.4A and Fig 3.5B). However, older adults demonstrated increased LICI at baseline (Table 3.2). No difference in TTF was observed between groups (Fig 3.3A).

Table 3.2: Baseline corticospinal characteristics.

	Young	Old
N	15	15
Females	8	8
MVC (N)	103.9 ± 34.8	124.3 ± 40.2*
M_{max} Intensity (mA)	94 ± 27.2	108.0 ± 35.3
M_{max} (mV)	11.7 ± 3.8	10.8 ± 6.5
CS (% MSO)	63.0 ± 7.1	70.0 ± 10.1
TS (% MSO)	80.0 ± 7.7	76.0 ± 10.6
AMT (% MSO)	46.0 ± 5.0	52.0 ± 10.9
BB: Conditioned MEP (%Unconditioned MEP)	54.9 ± 24.9	38.6 ± 18.8*
SP (ms)	143.7 ± 31.6	156.6 ± 45.9
Unconditioned MEP (mV)	4.0 ± 1.7	2.9 ± 1.3*
Maximum EMG_{rms} (mv)	1.2 ± 0.4	0.8 ± 0.4*
5% EMG_{rms} (mV)	0.09 ± 0.03	0.07 ± 0.02*

Values are shown as Mean ± SD. *$P<0.05$ when compared to the young participants. BB, Biceps Brachii; MSO, Maximum Stimulator Output; CS, Conditioning Stimulus; TS, Test Stimulus; SP, Silent Period.

3.4.1. Biceps Brachii

3.4.1.1. Force and EMG

Control Contraction: While there was no interaction between the control contraction and age ($F_{1,90} = 0.41$, $P = 0.522$), the magnitude of maximum force declined in both age groups (contraction effect: $F_{1,93} = 160.30$, $P<0.001$) with an EMD of 10% post control contraction compared to pre (95% CI [9, 12], $P<0.001$; Fig 3.3B). However, there was no main effect of

age ($F_{1,63}$ = 0.06, *P = 0.806*; Fig 3.3B) on maximum force. A single control contraction had no main effect on force during the 5% EMG contraction ($F_{41,41}$ = 3.44, *P = 0.071*), nor was there a main effect of age ($F_{1,56}$ = 0.18, *P = 0.675*) or interaction between the single control contraction and age ($F_{1,36}$ = 0.0001, *P = 0.996*; Fig 3.3C). There was no main effect of a single control contraction ($F_{1,425}$ = 0.08; *P = 0.775*), age ($F_{1,159}$ = 0.73; *P = 0.395*) or interaction between the control contraction and age ($F_{1,383}$ = 0.07, *P = 0.934*) on EMG_{rms} during the 5% EMG contraction (Fig 3.4A).

Fatigue: Young and older adults completed a similar number of 3 min 30% MVCs to task failure (Mean ± SD; Young, 4.5 ± 1.5; Old, 4.8 ± 1.9; *P = 0.67*). Maximum force normalised to pre declined post fatigue in both age groups (time effect: $F_{1,112}$ = 434.38, *P<0.001*) with an EMD at 24% (95% CI [21, 26], *P<0.001*) post fatigue compared to pre. However, there was no main effect of age ($F_{1,70}$ = 0.20, *P = 0.653*) or interaction between time and age ($F_{1,112}$ = 0.53, *P = 0.469*; Fig 3.3B) on MVC force. Force during the 5% EMG contraction declined during fatigue (time effect: $F_{2,93}$ = 13.68, *P<0.001*) with an EMD of 4 N compared to pre (95% CI [2, 6], *P<0.001*) and immediately post exercise with an EMD of 4 N compared to pre (95% CI [2, 7], *P<0.001*; Fig 3.3C) in both age groups. However, no main effect of age ($F_{1,81}$ = 0.05, *P = 0.820*) or interaction between time and age ($F_{2,90}$ = 0.01, *P = 0.994*) was evident (Fig 3.3C). There was no main effect of time ($F_{2,301}$ = 0.18; *P = 0.832*), age ($F_{1,151}$ = 0.36; *P = 0.549*) or interaction between time and age ($F_{2,213}$ = 0.19, *P = 0.827*) on EMG_{rms} during the 5% EMG contraction when normalised to M_{max} (Fig 3.4A). EMG during the 30% MVCs increased with time when normalised to M_{max} (time effect: $F_{8,79}$ = 4.35, *P<0.001*) with an EMD of 0.8% (95% CI [0.3, 1], *P<0.001*), 0.8% (95% CI [0.1, 1], *P = 0.007* and 1.1% (95% CI [0.2, 2], *P = 0.003*; Fig 3.4B) at F3, F4 and F5 respectively. However, no main effect of age ($F_{1,84}$ = 0.28, *P = 0.599*) or interaction between time and age ($F_{8,79}$ = 1.59, *P = 0.140*) was observed.

Recovery: Maximum force at recovery remained attenuated in both age groups (time effect: $F_{1,114} = 113.75$, *P<0.001*) with an EMD at 16% (95% CI [21, 19], *P<0.001*) compared to pre. However, there was no main effect of age ($F_{1,80} = 1.16$, *P = 0.284*) or interaction between time and age ($F_{1,116} = 0.61$, *P = 0.438*; Fig 3.3B) on MVC force. Force during the 5% EMG contraction at recovery was not different from pre (time effect: $F_{1,44} = 1.68$; *P = 0.202*), nor was there a main effect of age ($F_{1,55} = 0.000$; *P = 0.999*) or interaction between time and age ($F_{1,37} = 0.37$; *P = 0.546*: Fig 3.3C). There was no main effect of time ($F_{1,409} = 0.42$; *P = 0.*516), age ($F_{1,153} = 0.008$; *P = 0.930*) or interaction between time and age ($F_{1,404} = 1.54$; *P = 0.215*: Fig 4B) on EMG_{rms} during the 5% EMG contraction (Fig 3.4A).

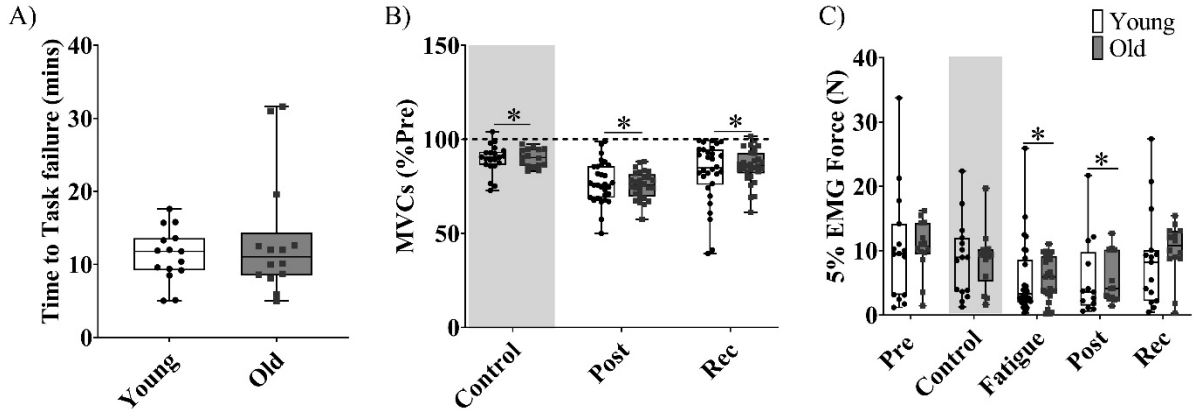


Figure 3.3 *A) Time to task failure (min) B) MVC force (N) and C) 5% EMG force (N) in the biceps brachii in young and older adults. The lower and upper edges of each box show the 25th – 75th percentiles, respectively, whereas the horizontal line within the box shows the median. The whiskers span all data points (dark dots) included within each point. Young: n = 15, 8 females; Old: n = 15, 8 females. *P < 0.05 compared to Pre.*

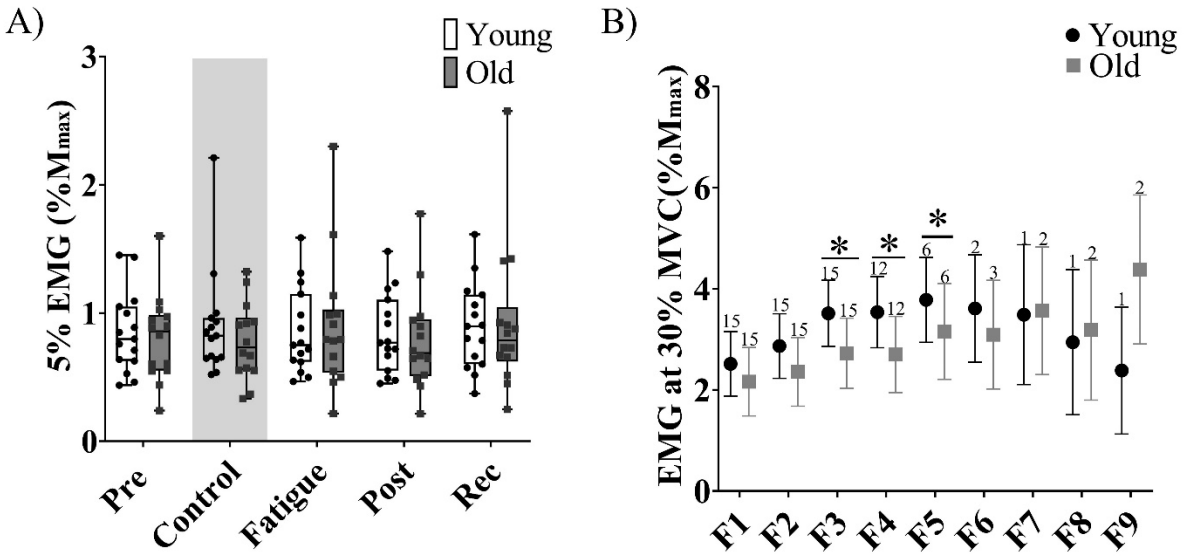


Figure 3.4 *A) 5% EMG (%M_{max}). The lower and upper edges of each box show the 25th – 75th percentiles, respectively, whereas the horizontal line within the box shows the median. The whiskers span all data points (dark dots) included within each point. B) EMG at 30% MVC (%M_{max}) in the biceps brachii in young and older adults. Total number of participants indicated above each data point. Young: n = 15, 8 females; Old: n = 15, 8 females. *P < 0.05 compared to Pre.*

3.4.1.2. Corticospinal excitability measures

Control Contraction: Conditioned MEP (% unconditioned MEP) when normalised to pre increased (contraction effect: $F_{1,277} = 12.33$, *P = 0.001*) with an EMD of 12% post control contraction compared to pre (95% CI [9, 46], *P = 0.001*) demonstrating a decrease in LICI post control contraction (Fig 3.5A). However, there was no difference between the two groups (age effect: $F_{1,180} = 0.03$, *P = 0.866*) or interaction between time and age ($F_{1,228} = 0.64$, *P = 0.425*; Fig 5A) on conditioned MEP (% unconditioned MEP, i.e., LICI). Unconditioned MEP (% M_{max}) was attenuated (contraction effect: $F_{1,398} = 27.28$, *P<0.001*; Fig 3.5B) with an EMD of 4% post control contraction compared to pre (95% CI [2, 5], *P<0.001*). Furthermore, younger adults showed greater unconditioned MEP (% M_{max}) size compared to the older adults (age effect: $F_{1,803} = 12.90$; *P<0.001*) with an EMD of 3% between age groups (95% CI [1, 5], *P<0.001;* Fig 3.5B). However, no interaction between contraction and age ($F_{1,765} = 0.54$, *P = 0.464*) on unconditioned MEP was observed (Fig 3.5B). SP duration did not change with a

single control contraction ($F_{1,277} = 0.93$, *P = 0.337*) and no interaction between contraction and age was observed ($F_{1,219} = 0.44$, *P = 0.509*). However, younger adults had a longer SP duration compared to the older adults (age effect: $F_{1,156} = 4.59$, *P = 0.034*) with an EMD of 7 ms (95% CI [0.6 14], *P = 0.034*; Fig 3.5C) following a single control contraction. There was no main effect of time ($F_{1,109} = 0.46$, *P = 0.499*), age ($F_{1,108} = 2.75$, *P = 0.100*) or interaction between time and age ($F_{1,98} = 0.02$, *P = 0.877*) on M_{max} (Fig 3.5D).

Fatigue: Conditioned MEP (% unconditioned MEP) increased (time effect: $F_{2,285} = 10.96$, *P<0.001*) when normalised to pre with an EMD of 26% during fatigue (95% CI [8, 43], *P = 0.001*) and 28% immediately post exercise (95% CI [11, 46], *P<0.001*) compared to pre in both age groups, demonstrating a decrease in LICI (Fig 3.5A). No significant differences between the two groups (age effect: $F_{1,144} = 0.06$, *P = 0.809*) or interaction between time and age ($F_{2,208} = 0.42$, *P = 0.655*; Fig 5A) were observed for conditioned MEP (% unconditioned MEP i.e., LICI). There was no main effect of time ($F_{2,308} = 2.90$, *P = 0.056*), age ($F_{1,167} = 2.49$; *P = 0.117*) or interaction between time and age ($F_{2,229} = 2.18$, *P = 0.115*) on unconditioned MEP (%M_{max}) (Fig 3.5B). There was no main effect of time ($F_{2,291} = 1.16$, *P = 0.315*), age ($F_{1,114} = 0.28$, *P = 0.598*) or interaction between time and age ($F_{2,333} = 0.10$, *P = 0.909*) on SP duration (Fig 3.5C). There was no main effect of time ($F_{2,154} = 1.53$, *P = 0.220*), age ($F_{1,101} = 2.58$, *P = 0.112*) or interaction between time and age ($F_{2,180} = 0.59$, *P = 0.557*) on M_{max} (Fig 3.5D).

Recovery: There was a main effect of time on conditioned MEP (% unconditioned MEP) ($F_{1,297} = 18.14$; *P<0.001*) and interaction between age and time ($F_{1,237} = 6.09$, *P = 0.014*). However, there was no main effect of age on conditioned MEP (% unconditioned MEP) ($F_{1,167} = 3.19$, *P = 0.076*). Conditioned MEP (% unconditioned MEP) remained elevated at recovery in older adults with an EMD of 25% (95% CI [14, 35], *P<0.001*; Fig 3.5A) indicating a continued attenuation in LICI compared to pre. However, conditioned MEP (% unconditioned

MEP) returned to pre-fatigue levels in younger adults at recovery (*P = 0.253*). There was a main effect of time on unconditioned MEP (%M_{max}) ($F_{1,422}$ = 16.24, *P<0.001*) and interaction between age and time ($F_{1,391}$ = 4.94, *P = 0.027*). Unconditioned MEP (%M_{max}) increased at recovery in older adults with EMD of 10% (95% CI [5, 15], *P<0.001*; Fig 3.5B) compared to pre. However, unconditioned MEP (%M_{max}) recovered in young adults (*P = 0.274*, Fig 3.5B). No main effect of age ($F_{1,152}$ = 0.65, *P = 0.*421) on unconditioned MEP (%Mmax) was observed. SP duration decreased at recovery (time effect: $F_{1,275}$ = 12.84, *P<0.001*) with an EMD of 11 ms (95% CI [5, 16], *P<0.001*; Fig 3.5C) compared to pre in both age groups. Younger adults also displayed a longer SP duration compared to the older adults (age effect: $F_{1,145}$ = 5.07, *P = 0.026*) with an EMD of 8 ms (95% CI [1, 14], *P = 0.026*; Fig 3.5C). However, no interaction between age and time was observed ($F_{1,215}$ = 0.001, *P = 0.982*). There was no main effect of time ($F_{1,108}$ = 0.23, *P = 0.633*), age ($F_{1,110}$ = 1.88, *P = 0.173*) or interaction between age and time ($F_{1,97}$ = 0.01, *P = 0.911*) on M_{max} (Fig 3.5D).

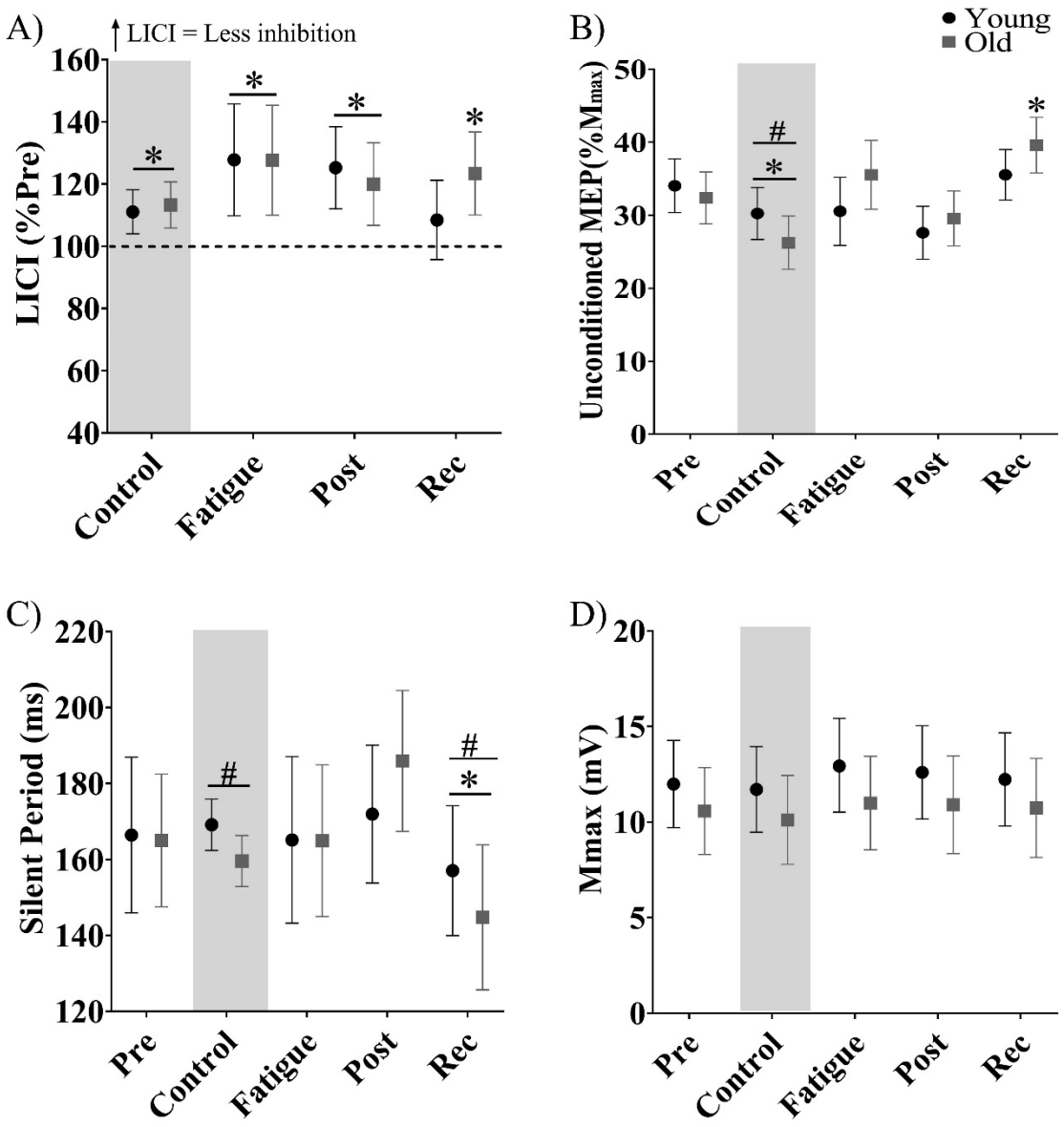


Figure 3.5. *A) LICI (%Baseline), B) unconditioned MEP (%Mmax), C) Silent Period (ms) and D) Mmax (mV) in the biceps brachii in young and older adults. Young: n = 15, 8 female; Old: n = 15, 8 female. *P < 0.05 compared to Pre; #P < 0.05 between young and older adults*

3.4.1.3. Triceps Brachii

Control, Fatigue and Recovery: EMG_{rms} during the 5% EMG contraction did not change with time (*P>=0.18*) when normalised to M_{max} nor was there an interaction between time and age (*P>=0.143*). However, there was a main effect of age (P<0.001) with older adults displaying greater EMG_{rms} compared to the young with an EMD of 0.2% (95% CI [0.2, 0.3], *P<0.001*: Fig 3.6A) in all time points.

There was no main effect of time or interaction between age and time (*P>0.001*) on M_{max}. However, there was a main effect of age on M_{max} (*P<0.001*). Younger adults showed greater M_{max} compared to older adults post control contraction, post fatigue and at recovery with an EMD of 5.9 mV (95% CI [4, 8], *P<0.001*), 6.9 mV (95% CI [5, 9], *P<0.001*) and 6.5 mV (95% CI [4, 8], *P<0.001*: Fig 3.6B) respectively.

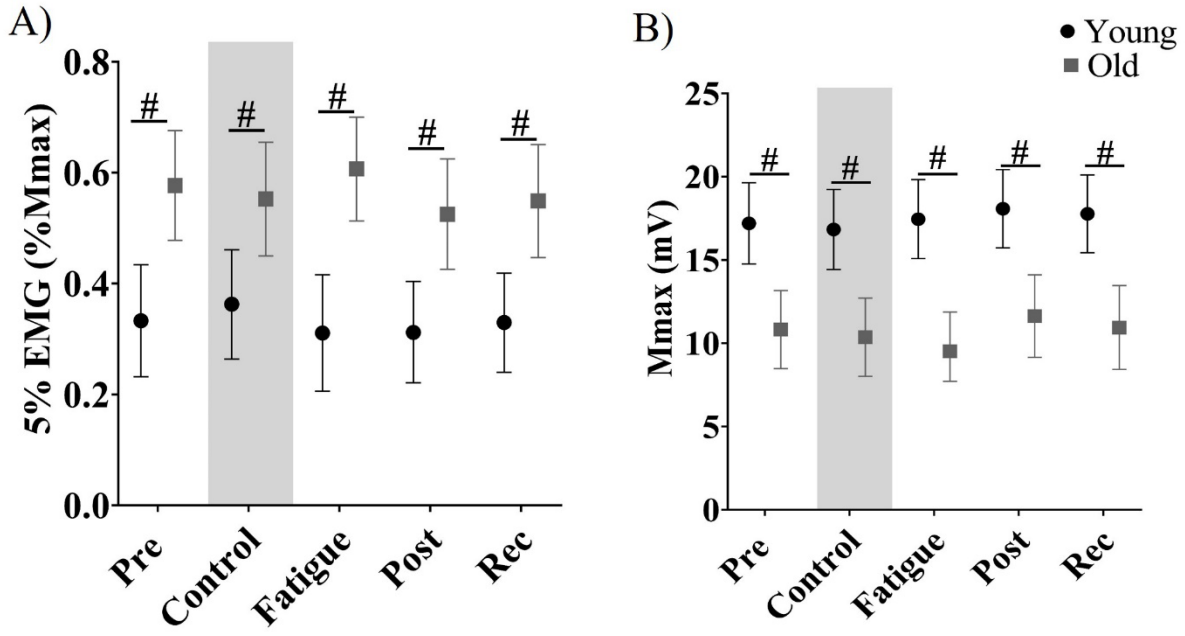


Figure 3.6 *A) 5% EMG (%Mmax) and B) Mmax (mV) in the biceps brachii in young and older adults. Young: n = 15, 8 female; Old: n = 15, 8 female.* $^{\#}P < 0.05$ *between young and older adults.*

3.5. DISCUSSION

3.5.1. Main findings

This study aimed to identify the age-related differences in $GABA_B$ mediated inhibition during a 5% EMG contraction performed intermittently in the course of a single joint submaximal isometric contraction of the elbow flexor muscles to task failure. We provide novel evidence to demonstrate that similar magnitude of fatigue in both young and old resulted in an identical decline in LICI during and post fatiguing exercise; suggesting that there was no age-related decrease in $GABA_B$ mediated inhibition in the biceps brachii.

3.5.2. Fatigability

In contrast to previous work which has identified a significant increase in TTF with ageing (Bilodeau et al. 2001b; Hunter et al. 2005; Hunter et al. 2016b; Yoon et al. 2013; Yoon et al. 2012), no age-related difference in TTF was observed in the current study, despite a similar decline in maximum force in both age groups following the exercise (Fig 3). This discrepancy may be attributed to differences in recovery rates between exercise models i.e. sustained submaximal contraction held to task failure (Bilodeau et al. 2001b; Hunter et al. 2016b; Yoon et al. 2013; Yoon et al. 2012) versus submaximal contraction with intermittent 30 s break, held to task failure in the current study. However, this may also be challenged given that numerous studies implementing isometric submaximal fatiguing contractions identified no age-related difference in recovery rate of isometric force between young and older adults (Allman and Rice 2001; Bilodeau et al. 2001a; Dalton et al. 2010; Kent-Braun et al. 2002; Klein et al. 1988; Lanza et al. 2004), making the reason for this disparity unclear. In addition, the older adults were ~15 kg heavier than the young which likely explains greater strength in the old compared to the young. Nevertheless, a similar magnitude of fatigue in both age groups provides an opportunity for an appropriate comparison of age-related differences in corticospinal and $GABA_B$ mediated inhibitory measures.

Greater activation of the agonist muscles (biceps brachii and brachioradialis) has been previously reported in older adults during a low force elbow flexion contraction whereby older adults have greater EMG_{rms} (% maximum EMG measured during MVC) compared to the young (Yoon et al. 2008; Yoon et al. 2013; Yoon et al. 2012). However, this outcome may at least partially be explained by the attenuation in maximum EMG observed in older adults (*see Table 3.2*). A reduction in maximum EMG may be attributed to age-related changes in corticospinal and muscle properties, for example, reduction in the number of functional motor units and muscle fibres as well as lower intracellular calcium and reduced calcium sensitivity to

activation (Andersen 2003; Campbell et al. 1973; Fielding et al. 2011; Hunter et al. 2016b; Hunter et al. 1999; Lexell et al. 1983; Lexell et al. 1988). Similar to our previous study (Otieno et al. 2021), when normalised to M_{max}, no age-related differences in EMG_{rms} ($\%M_{max}$) of the agonist muscles were seen in the current study during the 5% EMG contraction (Fig 3B). In addition, we observed greater EMG_{rms} ($\%M_{max}$) in the triceps brachii in older adults contrary to the lack of change previously reported (EMG_{rms} normalised to maximum EMG measured during MVC) (Yoon et al. 2008). This suggests greater co-activation of antagonists during agonist muscle contraction in the upper limbs with ageing. Increased co-activation in the elbow flexor muscles has been previously associated with greater accuracy during multi-joint arm movements in young adults (Frey-Law and Avin 2013; Gribble et al. 2003) which could signal a possible compensatory mechanism to facilitate accuracy during the force contractions in older adults. In any case, given that TTF was similar for young and older adults, the age-related differences in antagonist muscle activation is likely not a direct contributor to fatigue.

3.5.3. $GABA_B$ mediated inhibition

An increase in SP duration following and during isometric fatiguing exercise has been reported in young and older adults, suggesting an increase in $GABA_B$ mediated inhibition (Chen et al. 1999) with fatigue (Sogaard et al. 2006; Szubski et al. 2007; Taylor et al. 2000; Taylor et al. 1996; Yoon et al. 2012). However, older adults exhibit less of an increase in SP duration compared to the young, alluding to a reduction in the capacity to modulate $GABA_B$ mediated inhibitory activity (Yoon et al. 2012). Nonetheless, we observed no change in SP post control and fatiguing contractions in both age groups (Fig 4C). While earlier studies predominantly measured SP during a force contraction or MVC (Bilodeau et al. 2001b; Hunter et al. 2008; Sogaard et al. 2006; Szubski et al. 2007; Yoon et al. 2012), we measured SP during a 5% constant EMG contraction which may account for the discrepancy. Therefore, the

previously reported increase in SP (Sogaard et al. 2006; Szubski et al. 2007; Taylor et al. 1996; Yoon et al. 2012) may be partially explained by the increasing MEP (influenced by the rise in EMG) during a force contraction. However, one study identified a similar increase in SP duration when measured during a 20% sustained EMG contraction (Brownstein et al. 2020). Therefore, differences in contraction strength during SP measurements may be the primary cause for this disparity given that SP duration is attenuated with increasing contraction strength (Hammond and Vallence 2007). Interestingly, SP decreased significantly at recovery in both age groups, which is contrary to previous work that demonstrated the resumption of SP to baseline levels (from an initial increase during fatiguing exercise), as early as 30 s post exercise (Brownstein et al. 2020; McNeil et al. 2009; Taylor et al. 1996; Yoon et al. 2012). The reason for the decrease observed is not clear but may be related to the large inter-subject variability in the responses.

The current study also used LICI (Valls-Sole et al. 1992) to further investigate the excitation of $GABA_B$ inhibitory interneurons as evidenced by pharmacological studies (McDonnell et al. 2006). However, contradictions exist within the literature with some studies reporting a decrease in LICI with fatigue (Benwell et al. 2007b) while other studies identified an increase in LICI (McNeil et al. 2011b; McNeil et al. 2009) in young adults. However, contrary to the previously reported age-related compensatory decline in LICI (Otieno et al. 2021), there was a similar attenuation in LICI both during and post fatigue in young and older adults in this study. The implementation of different exercise models could explain this disparity (25% EMG contraction for 15 min of the FDI muscle (Otieno et al. 2021) versus intermittent 30% MVC force contraction of the elbow flexors to task failure in the current study); suggesting that LICI modulation may be task and muscle dependent in young adults. Nevertheless, older adults displayed an attenuation of $GABA_B$ mediated inhibition, congruent to our recent study (Otieno et al. 2021), when measured during a 5% EMG contraction. This

suggests an age-related retention in the ability to modulate $GABA_B$ mediated pathways with fatigue. Interestingly, this finding also disputes previous TMS-EEG data which showed no difference in $GABA_B$ modulation post fatigue in the older adults while the young displayed a contradictory decrease (Opie et al. 2020). Pharmacological evidence shows that TMS-EEG data is sensitive to changes in intracortical inhibition (Premoli et al. 2014) however there is currently insufficient evidence to suggest that LICI measured with paired-pulse MEPs and TEPs are mediated by similar mechanisms (Biabani et al. 2019a). Furthermore, it is suggested that LICI with an ISI of 100 ms reflect both spinal and cortical contributions (McNeil et al. 2011b; McNeil et al. 2009), while single pulse TEP measures predominantly reflect cortical mechanisms (albeit some evidence suggesting TEPs are also influenced by sensory artefacts (Biabani et al. 2019b; Rocchi et al. 2021)) further contributing to the inconsistent findings. Older adults also displayed a continued attenuation in LICI at recovery while younger adults recovered to baseline. Nevertheless, it should be noted the increase in unconditioned MEP observed at recovery in older adults might explain this finding, since a larger unconditioned MEP amplitude attenuates the amount of LICI during muscle activation (Opie and Semmler 2014b).

Baseline measures demonstrate that older adults displayed greater LICI compared to the young; suggesting an age-related increase in $GABA_B$ mediated inhibition contrary to the lack of age effect reported previously (McGinley et al. 2010; Opie and Semmler 2014a). One major contributor for this finding may be differing muscle groups between studies seeing as previous work investigated these changes in the active FDI muscle (Opie and Semmler 2014a) while we focused on the larger elbow flexor muscles, further highlighting the muscle dependent nature of LICI measures. However, it is also important to note that the procedure for setting conditioning TMS intensities in the current study was based on two criteria (SP duration nearest to 150 ms and LICI closest to 50%) rather than a single fixed conditioning intensity. This was

done to reduce baseline variability and avoid “floor’ or ‘ceiling effects that can occur with a single intensity.

3.5.4. Corticospinal excitability

When measured at rest, MEPs (normalised to muscle excitability measured with M_{max}) increase following intermittent maximum fatiguing contractions (Benwell et al. 2007b; Benwell et al. 2006a; Otieno et al. 2019) and decrease following sustained submaximal contractions (Brasil-Neto et al. 1993; Otieno et al. 2021; Sacco et al. 2000; Samii et al. 1997). This demonstrates a task-dependent increase and decrease in corticospinal excitability, respectively. Nevertheless, when measuring MEPs during a constant force fatiguing contraction, the magnitude of background EMG increases (Fuglevand et al. 1993) which influences MEP measurements irrespective of fatigue. This is because additional motoneurons are recruited to maintain force production as the active muscle fibres progressively fatigue. In the current study, the motoneuron output was ‘clamped’ by requiring the participants to maintain a constant 5% EMG contraction (Fig 4B) to ensure that the MEP measurements were a consequence of the development of fatigue rather than influence from background EMG. Similar to previous findings using identical exercise models (Brownstein et al. 2020; Hunter et al. 2016a), we observed no change in MEP amplitude during and post fatigue in both young and older adults, suggesting no age-related differences in MEP modulation. This also aligns with our recent findings (Otieno et al. 2021). Interestingly, unconditioned MEP amplitude increased at recovery in older adults suggesting an age-related decline in the ability to recover corticospinal excitability processes post fatiguing exercise. It is also possible this represents an age-related post-contraction facilitation, a speculation that requires further investigation.

It is not clear why there was an attenuation in MEP post control contraction, especially since there was no corresponding modulation in force or EMG during these contractions. Indeed, while the short one-minute submaximal control contraction in our study induced some

fatigue, the fatiguing exercise of a much longer duration resulted in no change in MEP. As such, it could be postulated that fatigue of increased duration results in disinhibition of corticospinal excitability.

3.5.5. Conclusion

The current findings suggest no age-related difference in $GABA_B$ mediated inhibition when measured during a low-level EMG contraction during and post submaximal fatiguing exercise. Furthermore, while we reported an attenuation in LICI during and post intermittent submaximal fatiguing exercise in young adults, previous work involving intermittent maximal fatiguing contractions show an increase/no change in LICI (Benwell et al. 2007b; McNeil et al. 2011b; McNeil et al. 2009), indicating that modulation of $GABA_B$ mediated activity may be task dependent. Further investigation into the modulation of GABA mediated activity during other types of tasks, for example, dynamic or whole-body exercise will help provide a holistic representation of possible age-related differences in corticospinal excitability with fatiguing exercise. Understanding these components is important in further identifying the age-related differences in neural mechanisms that underlie differences in fatigability between young and old.

CHAPTER IV

SUSTAINED SUBMAXIMAL ISOMETRIC FATIGUING EXERCISE OF THE ELBOW FLEXORS HAS NO AGE-RELATED EFFECT ON MOTOR PERFORMANCE

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Co-Author Contributions

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

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

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

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

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4. SUSTAINED SUBMAXIMAL ISOMETRIC FATIGUING EXERCISE OF THE ELBOW FLEXORS HAS NO AGE-RELATED EFFECT ON MOTOR PERFORMANCE

4.1. ABSTRACT

Previous research has shown that single joint isometric fatiguing exercise has a detrimental effect on motor skill performance in young adults. However, this is yet to be established in the older population. In 15 young (23 ± 0.9 years) and 15 older (65 ± 0.7 years) adults, assessment of speed-accuracy trade-off (Fitts Law) was assessed before and after a submaximal contraction (30% MVC force) held intermittently to task failure via performance on a computer-based movement time task. All participants, regardless of age group, developed similar magnitude of fatigue ($P = 0.001$). Movement time (MT) improved similarly in both young and older adults when task difficulty was low with a contradictory increase observed when task difficulty was high. No difference in performance was recorded following a non-fatiguing contraction versus fatiguing exercise. These findings suggest a beneficial consequence of exercise induced fatigue on task performance when accuracy demands are low as well as a detrimental effect when accuracy demands are high. Furthermore, the lack of difference in task performance between non-fatiguing and fatiguing exercise indicates that activation of the corticospinal pathway irrespective of time and duration of fatigue is sufficient to elicit changes in motor skill performance. However, the lack of difference between age groups points to a retention in the ability to perform simple movement time tasks with ageing in the presence of fatigue.

Key Words: ageing, fatiguing exercise, Fitts Law, motor performance, movement time.

4.2. INTRODUCTION

In chapter three, excitatory and inhibitory processes following single joint fatiguing exercise are modulated similarly in young and older adults when a homogeneous magnitude of fatigue is induced (Otieno et al. 2022). There is evidence to suggest that neuromuscular fatigue and motor skill performance affect excitability and inhibitory processes within M1 in young adults. (Benwell et al. 2007b; Benwell et al. 2006a; Garry et al. 2004; Hunter et al. 2016a; Maruyama et al. 2006; Perez et al. 2004). However, investigation into the interaction between fatigue and motor skill performance remains limited with a majority of the studies performed in the 1970-90s (For comprehensive review see: Taylor and Ivry 2012). These studies have identified contradictory results, with some reporting detrimental effects of fatigue (Carron and Ferchuk 1971; Thomas et al. 1975), while other studies report no significant impact of fatigue on motor skill performance (Alderman 1965; Cotten et al. 1972; Spano and Burke 1976). More recently, Branscheidt and colleagues demonstrated that fatigue induced lower levels of performance of a sequential visual isometric pinch task (SVIPT) immediately after exercise in young healthy adults (Branscheidt et al. 2019). However, very little is known regarding fatigue's impact on motor skill performance in older adults.

With ageing comes deficits in motor performance due to alterations within the neuromuscular system. These deficits include difficulties with coordination (Seidler et al. 2002), increased variability of movement (Contreras-Vidal et al. 1998) and difficulties with balance and gait (Alexander et al. 1992). A pronounced increase in movement duration of about 15 – 30% is also observed in older adults (Buckles 1993) which has been attributed to an age-related shift in movement strategy as older adults place more emphasis on accuracy of movement rather than speed (Goggin and Meeuwsen 1992; Seidler-Dobrin et al. 1998). One task that is frequently used to assess this shift is the Fitts task. The Fitts task is one of the most robust and reproducible paradigms, used to assess behavioural processes underlying speed-

accuracy trade-off (Fitts 1954). It requires rapid movement towards a target of fixed width (W), placed at a given distance (A) either in a reciprocal (continuous movements between two targets) or discrete manner (movements involving complete stops at home and target positions). In both cases, task difficulty is quantified by the index of difficulty (ID) which, based on an extensive study by Fitts himself, is dependent on both W and A (Fitts 1954; Fitts and Peterson 1964). Numerous studies have shown that movement time (MT) increases linearly with an increase in ID (Fitts 1954; Fitts and Peterson 1964; Goggin and Meeuwsen 1992; Jagacinski et al. 1980; Langolf et al. 1976). An increase in MT has also been identified in older adults compared to young adults in a Fitts type task (Bashore et al. 1989; Fozard et al. 1994; Goggin and Meeuwsen 1992; Hines 1979; Pohl et al. 1996; Walker et al. 1997).

Fatigue has been shown to have a detrimental effect on MT in younger adults when measured during tasks like the SVIPT task (Branscheidt et al. 2019; Gates and Dingwell 2008). However, the impact of fatigue on MT in older adults is yet to be investigated. The aim of this study was to identify the impact of a single-joint isometric fatiguing exercise on MT in young and older adults during performance of a speed accuracy trade-off task based on Fitts Law. Given the findings in previous study (Branscheidt et al. 2019), MT was expected to increase (slower movement) in both age groups post fatiguing exercise. Furthermore, one study has reported a greater decline in performance of a one-finger tapping task in older adults compared to the young following isometric single joint fatiguing exercise of the index finger (Singh et al. 2013) suggesting a greater detriment in older adults with fatigue. Therefore, it was also hypothesised that fatigue would result in a greater increase in MT in older adults compared to the young post fatiguing exercise.

4.3. METHODS

Fifteen young (23 ± 0.9 years; 7 females) and fifteen older (65 ± 0.7 years; 7 females) healthy participants were recruited from the university community, community centres and

social media to participate in this study. The assessment of hand preference was carried out using the Edinburgh Handedness inventory (Table 4.1) (Oldfield 1971). Self-reported subjective physical activity (work index, sport index, leisure-time index) was assessed in all participants, using an activity questionnaire (adapted from Baecke et al. 1982) where participant scores ranged from 1 (sedentary) to 5 (active) for each index. The University of Adelaide Human Research Ethics Committee approved all procedures, and each procedure was conducted in accordance with the Declaration of Helsinki. Each participant provided written, informed consent prior to participation.

Table 4.1: Participant and baseline corticospinal characteristics.

	Young	Old
N	15	15
Females	7	8
Weight (Kg)	60.9 ± 2.7	77.5 ± 4.7
Height (cm)	171 ± 2.3	168.4 ± 2.5
Handedness	0.8 ± 0.05	1.0 ± 0.03
Work Index	2.8 ± 0.2	2.8 ± 0.2
Sport index	2.4 ± 0.2	3.0 ± 0.3
Leisure-time index	3.2 ± 0.2	3.0 ± 0.2
MVC (N)	107.5 ± 7.7	116.4 ± 7.4*

Values are shown as Mean ± SD. *$P<0.05$ when compared to the young participants. Handedness ≥ 0.5 indicates right-handed individuals.

4.3.1. Experimental set-up

Each participant was seated upright with their right arm slightly abducted. Their elbow rested comfortably on a padded support, and the elbow joint was flexed to 90° so that the supinated forearm was horizontal to the ground. The shoulders were restrained by two nylon straps to minimize shoulder movement (Fig 4.1). Force (Torque = Force × Lever arm) was evaluated using a Biodex Multi-Joint System (Biodex, Inc, Shirley, NY). Forces were recorded online using a Power 1401 A-D converter and Spike 2 software [Cambridge Electronics Design (CED), Cambridge, UK]. The force exerted in the vertical direction was displayed on a 17-in. monitor positioned 0.9 m in front of the subject. An adjustable rolling table was placed directly under the restrained right arm for performance of the Fitts movement task. Table height was adjusted such that it allowed for a swift swap between performance of the elbow flexion contraction and grasping the mouse for motor task performance (Fig 4.1).

4.3.2. Electrical Recordings

EMG signals were recorded using surface electrodes (Ag-AgCl, 8-mm diameter) that were placed in a monopolar configuration (over the muscle belly and the tendon) of biceps brachii and triceps brachii (Hunter et al. 2008). EMG signals were amplified (×300) and band-pass filtered (16 –1,000 Hz) [model CED 1902, Cambridge, UK]. Force (1,000 Hz) and EMG (2,000 Hz) signals were sampled with a CED 1401 computer interface and Spike2 software (CED). However, EMG data was not analysed for the purposes of this study.

Figure 4.1: Experimental set up: *Elbow flexor force was evaluated using a Biodex Multi-joint system. Participant sat upright with elbow-joint strapped to padded surface for upward elbow flexion. Screen placed directly in front of participant for force feedback. Shoulders and left knee were retrained by nylon straps to minimise shoulder and leg movement. An adjustable rolling table was placed directly under the restrained right arm for performance of the Fitts movement task. Table height was adjusted such that it allowed a swift swap between performance of the elbow flexion contraction and grasping the mouse for motor task performance.*

4.3.3. Fitts Movement Task

Psychopy software (v3.0, Open Science Tools Ltd, Nottingham, UK) was used to set up and present visual stimuli on a monitor placed directly in front of participants. The stimuli included a white square (starting position) and a red square (target). The target only appeared on the bottom right quadrant of the screen (ten trials). ID was set using the Fitts law equation:

$$MT = a + b \log_2(2A/W)$$

The variable $\log_2(2A/W)$ in the equation above provided an ID which was manipulated during experimentation by altering size of the red square (W) and distance from starting point (white square) (Fitts 1954). Table 4.2 presents selected ID values for simple movement time task (SMT) through manipulation of target width and distance.

Table 4.2: ID in SMT task.

	ID	**ID_{range}**	**Distance to target (cm)**	**Target size (cm)**
SMT	2.6	ID_{1-3}	1.8	0.6
	3.3	ID_{3-4}	1.3	0.3
	3.6	ID_{3-4}	5.8	0.9
	3.9	ID_{3-4}	6.8	0.9
	4.1	ID_{4-5}	3.1	0.4
	4.3	ID_{4-5}	2.6	0.3
	4.4	ID_{4-5}	3.8	0.4
	5.4	ID_{5-6}	5.4	0.3
	5.7	ID_{5-6}	6.7	0.3

Using a mouse cursor, participants were instructed to first click on the white square (starting position) and wait for the appearance of the target (red square) – *see Fig. 4.2*. Once

the target appeared participants were required to move towards it as quickly as possible and click again before returning to the starting position. Accurate click on target was confirmed once the target disappeared and starting position (white square) reappeared once more. This sequence was repeated until ten trials were completed. MT was recorded as the amount of time in milliseconds between onset of cursor movement from starting position to the click on the target. To minimise anticipation, the target appeared with a random delay of 0.3 s – 3 s with IDs presented in a randomised fashion.

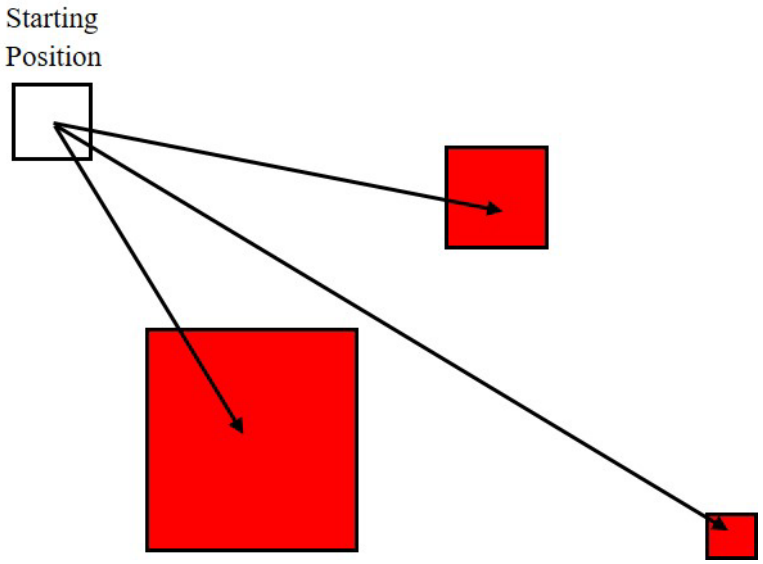

Figure 4.2: *Example of trial with arrow showing direction of movement. Target only appeared on the bottom right quadrant of the screen (three representative trials are shown above however the task included ten trials in total).*

4.3.4. Experimental protocol

Fig. 4.3 shows the experimental protocol carried out during each session. Prior to the fatiguing task, maximum baseline force was calculated by performing two, 3–5 s MVCs of the elbow flexors. Participants then performed a brief familiarisation of the SMT task (practice session – 2 trials) prior to task completion at baseline (i.e., pre fatigue/baseline). A control contraction (submaximal contraction at 30% MVC) was subsequently performed for one minute followed immediately by a brief 5 s MVC (without any rest) and performance of the SMT respectively. This was then followed by two brief 5 s MVCs and a 2 min resting period.

To ensure a similar amount of fatigue in both young and old (Otieno et al. 2022), participants were instructed to hold a 30% MVC until task failure. A 30 s break was provided every 3 min. Participants were instructed to resume the submaximal contraction at 30% MVC immediately for another 3 min. This pattern was continued until task failure (5% decline in force below the 30% target for > 3 s.) A verbal cue provided by the experimenter indicated when participants should commence exercise or perform the SMT task, and participants were asked to refrain from using other muscles during contraction. Visual feedback of force output (displayed on a computer screen) and verbal encouragement were provided throughout the protocol. At task failure, participants were instructed to perform a brief 5 s MVC (immediately without rest) as well as the SMT task (post fatigue). Participants then performed two more brief 5 s MVCs. Finally, a 10 min recovery period was provided, and the session concluded with one final performance of the SMT task and two brief 5 s MVCs (Fig 4.3).

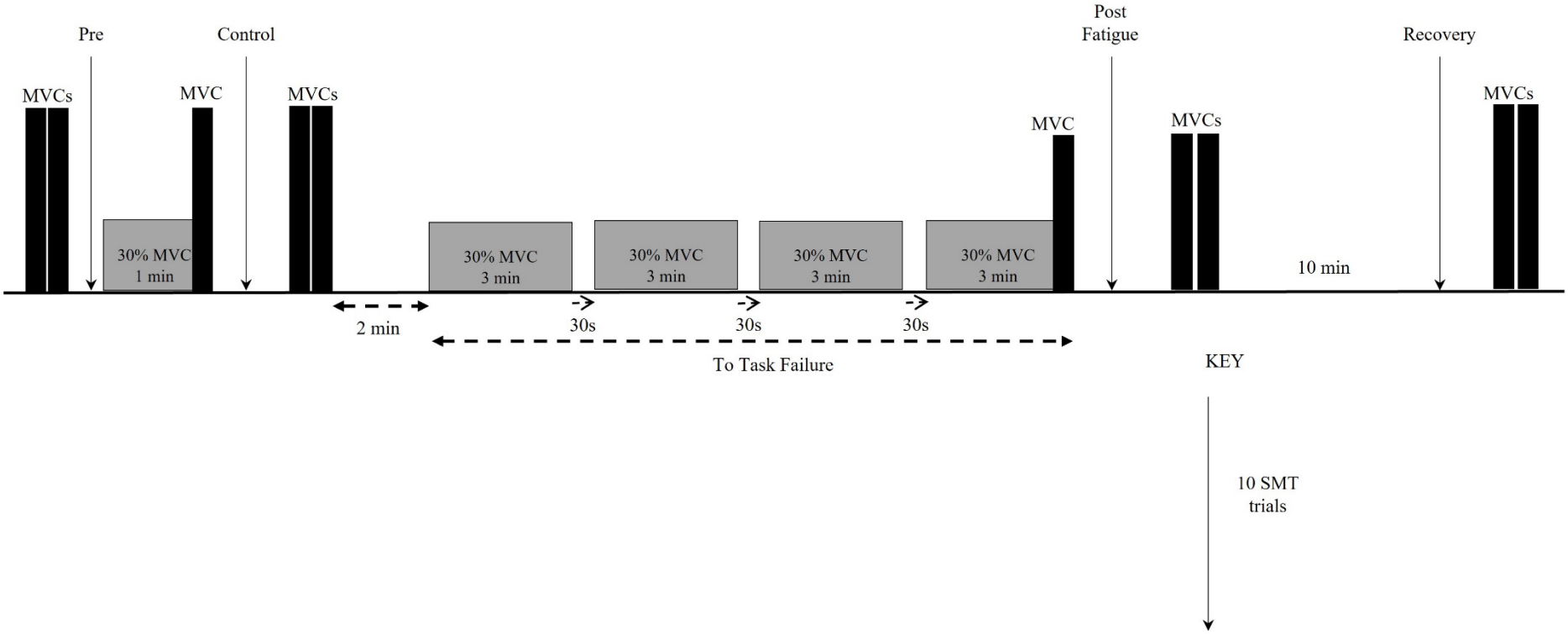

Figure. 4.3. Experimental protocol schematic. A) Baseline; *before fatiguing exercise, maximum force was measured by performing 2 MVCs during a 3- to 5-s elbow flexion. Participants then performed the SMT task (solid arrow). They were then instructed to perform a sustained submaximal 30% MVC of the elbow flexor muscles for 1 minute (control contraction) followed by the SMT task respectively. To fatigue the muscle, participants were required to perform intermittent 3 min contractions at 30% MVC followed by a brief 30 s break. This sequence was repeated to task failure (5% drop in force >3 s). Once task failure was reached participants performed one brief 5 s MVC immediately (no break given), followed by the SMT task. Participants then performed 2 brief (5 s) MVCs with a 10-s rest period in between. To conclude the session, a 10-minute*

recovery period was provided, and participants were instructed to perform the SMT task respectively (solid arrow) once more respectively followed by 2 brief (5 s) MVCs with a 10-s rest period in between.

4.3.5. Data Analysis

Peak force amplitude was measured during brief MVCs before and after exercise. ID was subdivided into four ranges (easiest to most difficult) for analysis; one to three (ID_{1-3}), three to four (ID_{3-4}), four to five (ID_{4-5}) and five to six (ID_{5-6}), at each of the five time points (pre-fatigue, control, fatigue, post, recovery) (*See Table 4.2 above*). MT at each ID was determined using in-built scripts of the Psychopy software as the time between two clicks (onset of cursor movement from starting position to click on target) (Fig 4.3).

4.3.6. Statistical Analysis

Linear mixed model with repeated measures (LMM_{RM}) was used to compare the effect of factors *time* (pre fatigue, post fatigue and recovery), *age* and *ID* on MT and force. Similarly, LMM_{RM} was used to compare the effect of factors *single control contraction*, *age* and *ID* on MT. Paired T-Test was used to compare the effect of age on time to task failure (TTF) and baseline characteristics (Table 4.1). G*Power software was used to calculate Cohen's effect size (d_z) for the significance in time to task failure. All data in text is presented as estimated mean differences (EMD) and 95% confidence interval for the estimate, providing a non-standardised measure of effect size. All data in figures are presented as means ± SE. Statistical significance was set at $P<0.05$.

4.4. RESULTS

4.4.1. Baseline measures

All participants completed the experiment in full and without any adverse reactions. No differences in height, weight, or physical activity scores (work, sport, and leisure-time index) were found between age groups at baseline. However, older adults demonstrated greater maximum force at baseline (Table 4.1). Older adults also required more time to complete the

task at baseline in all IDs and greater time was required by both age groups to complete the task at $ID_{3\text{-}4}$, $ID_{4\text{-}5}$ and $ID_{5\text{-}6}$ compared to $ID_{1\text{-}3}$ (Table 4.3).

Table 4.3: MT at baseline in young and older adults.

	ID	Young (ms)	Old (ms)
SMT	$ID_{1\text{-}3}$	815.0 ± 60.9	1066.4 ± 60.0*
	$ID_{3\text{-}4}$	923.0 ± 38.0$^{\#}$	1172.5 ± 38.0*$^{\#}$
	$ID_{4\text{-}5}$	961.2 ± 42.2$^{\#}$	1291.5 ± 42.2*$^{\#}$
	$ID_{5\text{-}6}$	1132.9 ± 49.4$^{\#}$	1567.1 ± 49.4*$^{\#}$

Values are shown as mean ± SD. *$P<0.05$ when compared to the young participants. $^{\#}P < 0.05$ compared to $ID_{1\text{-}3}$.

4.4.2. Time to Task Failure and Force

TTF: Older adults also required more time to reach task failure compared to the young ($P<0.05$; d_z = 0.09; Fig 4.4A).

Control: There was a main effect of a control contraction ($F_{1,108}$ = 30.56, $P<0.001$), age ($F_{1,64}$ = 10.10, $P = 0.002$) and interaction between control contraction and age ($F_{1,109}$ = 11.31, $P = 0.001$) on MVC when normalised to baseline. MVC declined post control contraction in young adults with EMD of 8.3% (95% CI [6, 11], $P<0.001$; Fig 4.4B). No change in MVC was observed post control contraction in older adults. However, older adults produced more force during the control contraction compared to the young with EMD of 7% between age groups (95% CI [4, 10], $P<0.001$; Fig 4.4B).

Fatigue: There was a main effect of time ($F_{1,107}$ = 334.93, $P<0.001$) but no main effect of age ($F_{1,72}$ = 2.14; $P = 0.148$) or interaction between time and age ($F_{1,110}$ = 2.94; $P = 0.089$)

on MVC when normalised to baseline. MVC declined post fatigue in both age groups with an EMD of 21% (95% CI [19, 24]; *P<0.001*; Fig 4.4B).

Recovery: There was a main effect of time ($F_{1,109} = 171.49$, *P<0.001*) but no main effect of age ($F_{1,70} = 1.33$; *P = 0.253*) or interaction between time and age ($F_{1,112} = 2.43$; *P = 0.122*) on MVC with fatigue. MVC remained attenuated at recovery in both age groups with an EMD of 14% (95% CI [12, 16]; *P<0.001*: Fig 4.4B).

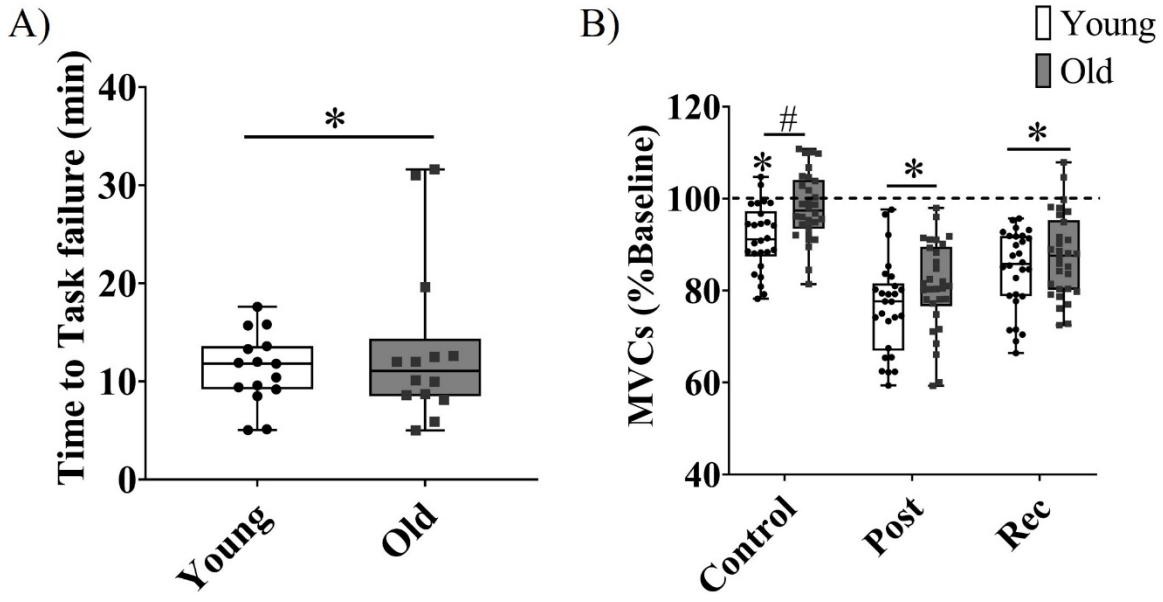

Figure 4.4: *A) Time to task failure (min) in the biceps brachii in young and older adults. *P < 0.05 between young and older adults. B) MVC force (N) in the biceps brachii in young and older adults. *P < 0.05 compared to Pre; #P < 0.05 young versus older adults.*

4.4.3. Simple Movement Time Task

Control: While there was no main effect of a control contraction ($F_{1,362} = 0.02$, *P = 0.877*) or age ($F_{1,242} = 0.64$, *P = 0.425*) on MT, there was a main effect of ID on MT ($F_{3,496} = 44.19$, *P<0.001*). Furthermore, there was no interaction between a control contraction and age ($F_{1,293} = 0.41$, *P = 0.523*), age and ID ($F_{3,476} = 1.03$, *P = 377*) or the control contraction, age, and ID ($F_{3,447} = 1.05$, *P = 0.370*) on MT. However, there was an interaction between the control contraction and ID ($F_{3,413} = 37.65$; *P<0.001*). MT decreased post control contraction with EMD of 20% (95% CI [12, 27], *P<0.001*) for $ID_{1\text{-}3}$ and EMD of 8% (95% CI [4, 12], *P<0.001*; Fig

4.5) for ID_{3-4}. On the other hand, MT increased post control contraction at ID_{5-6} with EMD of 28% (95% CI [22, 34], *P<0.001*; Fig 4.5). Furthermore, greater MT was required to complete the task post control contraction at $ID_{3-4,}$ ID_{4-5} and ID_{5-6} compared to ID_{1-3} with EMD of 12% (95% CI [4, 20], *P<0.01*), 20% (95% CI [12, 57], *P<0.001*) and 48% (95% CI [39, 57], *P<0.001*; Fig 4.5) respectively.

Fatigue: Following fatiguing exercise, there was no main effect of time ($F_{1,331}$ = 0.28, *P = 0.594*) or age ($F_{1,217}$ = 3.55, *P = 0.061*) on MT, there was a main effect of ID ($F_{3,510}$ = 31.36, *P<0.001*). There was no interaction between age and ID ($F_{3,487}$ = 0.09; *P = 0.967*), age and time ($F_{1,264}$ = 3.80, *P = 0.052*) or age, time, and ID ($F_{3,455}$ = 0.10, *P = 0.958*). There was however an interaction between time and ID ($F_{3,420}$ = 25.17, *P<0.001*) on MT. MT decreased in both age groups at ID_{1-3} and ID_{3-4} with an EMD of 16% (95% CI [9, 23], *P<0.001*) and 4% (95% CI [0.8, 9]; *P = 0.019;* Fig 4.5) respectively. On the other hand, MT increased in both age groups at ID_{5-6} with an EMD of 21% (95% CI [15, 26], *P<0.001;* Fig 4.5). Furthermore, greater MT was required to complete the task post fatigue at $ID_{3-4,}$ $ID_{4-5,}$ and ID_{5-6} with EMD of 11% (95% CI [4, 18], *P<0.001*), 19% (95% CI [11, 27] *P<0.001*) and 37% (95% CI [28, 45], *P<0.001*; Fig 4.5) respectively compared to ID_{1-3}.

Recovery: While there was no main effect of time ($F_{1,317}$ = 2.50, *P = 0.115*) or age ($F_{1,207}$ = 0.21, *P = 0.651*) on MT, there was a main effect of ID ($F_{3,520}$ = 20.95, *P<0.001*). Furthermore, there was no interaction between age and time ($F_{1,252}$ = 0.25, *P = 0.619*), age and ID ($F_{3,494}$ = 0.91, *P = 0.436*) or age, time, and ID ($F_{3,461}$ = 0.97, *P = 0.405*) on MT. However, an interaction between time and ID ($F_{3,426}$ = 16.51, *P<0.001*) was observed. MT decreased in both age groups at ID_{1-3} and ID_{3-4} with an EMD of 17% (95% CI [10, 24], *P<0.001*) and 8% (95% CI [4, 12], *P<0.001;* Fig 4.5) respectively. On the other hand, MT increased in both age groups at ID_{5-6} with an EMD of 14% (95% CI [8, 19], *P<0.001;* Fig 4.5). Greater MT was also required to complete the task at recovery at $ID_{3-4,}$ $ID_{4-5,}$ and ID_{5-6} with EMD of 9% (95% CI [2, 17], *P =*

0.005), 19% (95% CI [11, 27], *P<0.001*) and 31% (95% CI [22, 40], *P<0.001*; Fig 4.5) respectively compared to ID_{1-3}.

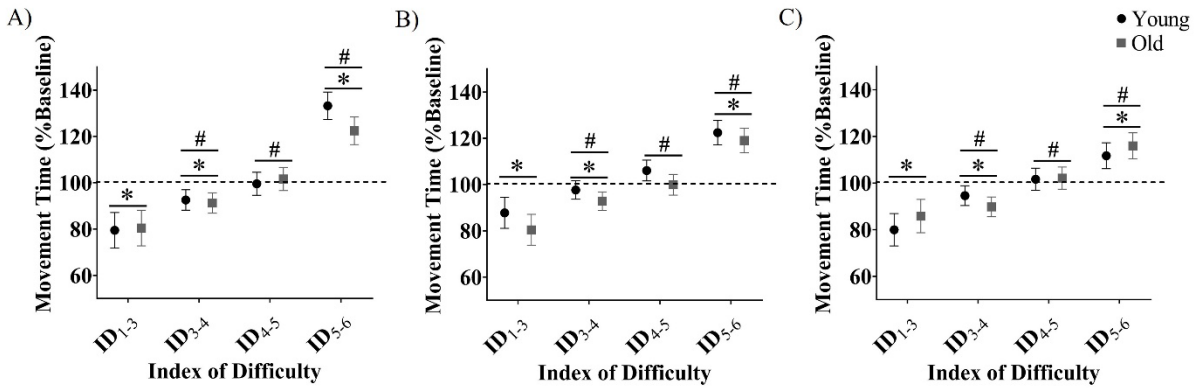

Figure 4.5: *MT normalised to baseline at A) control contraction B) post fatigue and C) recovery in young (black symbols) and older (grey symbols) adults. *P < 0.05 compared to Pre; #P < 0.05 compared to ID_{1-3}.*

4.5. DISCUSSION

4.5.1. Main findings

This study aimed to identify the age-related differences in MT of a simple Fitts type task following a sustained submaximal contraction of the elbow flexor muscles performed intermittently to task failure. A similar magnitude of fatigue in both young and older adults resulted in an identical change in MT at all ID values suggesting no age-related difference in motor performance following fatigue of the biceps brachii.

4.5.2. Short Control Contraction

While younger adults displayed a greater amount of fatigue following the short control contraction compared to the older adults (Fig 4.4), a similar decrease in MT was identified at lower ID levels during SMT task suggesting an improvement in speed of movement when accuracy demands are low. One possible explanation for this finding may be increased activation of the corticospinal pathway. Imaging studies show greater activation of the primary motor areas of the brain following short voluntary exercise (Benwell et al. 2007a; Liu et al.

2003) which can enhance movement during performance of a simple motor task by increasing output to the respective hand muscles. Furthermore, isometric single-joint exercise also increases corticospinal excitability and decreases intracortical inhibition (Hess et al. 1987; Martin et al. 2006; Ridding et al. 1995; Taylor et al. 1997; Thompson et al. 1991; Todd et al. 2003b). Therefore, enhanced corticospinal drive to the muscle and heightened EMG activity could result in improvement of speed of movement when accuracy demands are low. However, increased activation of the visual cortex due to exercise might also explain the current improvement. In a previous fMRI study, a similar bilateral increase in activation of the visual and sensory motor cortex during isometric fatiguing exercise of the hand muscles was identified (Benwell et al. 2007a). It is therefore likely that this increase in visual activation indicates enhanced attentional demand to visual cues during exercise thereby improving identification of the target and resulting in faster movements. Improvement in MT at lower ID levels may also indicate an increase in processing time/planning for initiation of movement prior to movement onset by the higher brain centres (prefrontal/premotor cortex) post exercise. Evidence suggests that delayed movement initiation relative to planning reduces the risk of movement initiation prior to appropriate preparation (Haith et al. 2016) and consequently lessens the number of errors (improving accuracy of movement).

On the other hand, an increase in MT was observed post control contraction at higher ID levels suggesting a decline in speed of movement when accuracy demands are high. Unlike lower ID levels (large target, closer to starting position), greater fine motor control of the intrinsic hand muscles is required for movement at higher IDs (smaller target, further form starting position) which, in turn, may require greater activation of the corticospinal pathway. Performance of simple motor skills and isometric single joint exercise activate similar motor regions within the brain, primarily M1, as well as induce homogeneous changes within the corticospinal tract (increase in excitability and decrease in inhibition) (Garry et al. 2004; Perez

et al. 2004; Toma and Nakai 2002). It is possible that initial activation of the pathway during the contraction resulted in interference and a reduction in optimal output required to perform that task when accuracy demands are high, leading to a decline in motor performance (increase in MT). Furthermore, evidence shows that isometric exercise can affect grasping stability during fine motor control by altering directional coordination of each individual digit that is involved in the movement as well as control of force direction in each digit (Hu et al. 2018). This can lead to a detriment in dexterous manipulation during performance of fine motor tasks.

4.5.3. Sustained single joint fatigue

Similar to previous work, older adults displayed a longer time to task failure compared to the young when performing an intermittent sustained submaximal isometric contraction (Bilodeau et al. 2001b; Hunter et al. 2005; Hunter et al. 2008; Yoon et al. 2013; Yoon et al. 2012) which is likely attributed to the age-related loss of type II fibres in older adults resulting in a greater proportion of fatigue resistant type I fibres (Andersen 2003; Lexell et al. 1983). However contrary to previous work (Hunter et al. 2005; Hunter et al. 2008; Yoon et al. 2008; Yoon et al. 2012), older adults produced greater force at baseline compared to the young in the current study (Table 4.1). This may once again be attributed to the homogeneous physical activity levels reported between age groups (Table 4.1 – *see chapter three*). Therefore, whilst the older adults likely had some degree of a decline in type II fibres, increased physical activity may have minimised the age-related loss of muscle mass associated with fibre death (Goodpaster et al. 2008) and maintained upper limb tension (Trappe et al. 2003) causing them to be stronger compared to the young.

MT improved post fatiguing exercise during SMT at lower ID levels indicating an improvement in speed of movement when accuracy demands are low and increased post fatiguing exercise at higher IDs when accuracy demands were high in both age groups indicating a decline in speed of movement. These results mirror those reported following

performance of a brief muscle contraction (Fig 4.5). The lack of difference in MT between control and fatiguing measures suggest that activation of the corticospinal pathway irrespective of duration or amount of fatigue can induce changes in task performance during a SMT task. Furthermore, no reported difference in coordination of simultaneously active hand muscles recruited during non-fatiguing and fatiguing contractions has been identified (Danna-Dos Santos et al. 2010). This further reduces the likelihood of observable differences in task performance between control and fatiguing contraction. Alternatively, this finding may also be attributed to the fact that the fatiguing intervention was not aimed at the primary musculature involved in performance of Fitts task (intrinsic hand muscles) but rather the elbow flexor muscles. However, studies have shown that inducing fatigue in one muscle group can lead to force decrements in a rested non-exercised group (Aboodarda et al. 2015; Halperin et al. 2014; Kennedy et al. 2013). Nevertheless, the likelihood that the intrinsic hand muscles did not experience at least some fatigue is quite low given that activation of the wrist and hand muscles was necessary to maintain elbow flexion during exercise (*see Fig 4.1*), although the force of the wrist was not measured. As such, implementation of an arm reaching task (which directly involved the fatigued muscles), like the rotary pursuit task (Ammons 1955) may provide more insight into motor performance of the fatigued muscle. Finally, the outcomes of this study may also be a direct reflection of the complexity of task administered. Lower complexity elicits the likelihood of a 'ceiling' effect thereby reducing the probability of observing a more significant detriment in performance post fatiguing exercise. Implementing more complex tasks such as the SVIPT task (Reis et al. 2008) for future studies may offer more insight into the impact of fatigue on skill performance during a speed-accuracy trade-off task.

4.5.4. Age related difference in MT

With ageing comes a detriment in movement and processing times due to changes within the CNS such as attenuated cortical (Oliviero et al. 2006) and motoneuron excitability

(Lexell 1997), reduction in size of the cortex (Raz et al. 2007) as well as motor cortical (Dickstein et al. 2007) and spinal degradation (Sale and Semmler 2005; Scaglioni et al. 2002). As such, an attenuation in motor performance is often observed. For example, studies have identified a decline in MT during Fitts type tasks in older adults compared to the young (Goggin and Meeuwsen 1992), which can been attributed to an age-related difference in strategy during movement. While younger adults place a greater emphasis on speed of movement, older adults focus on accuracy of movement at the expense of speed (Seidler-Dobrin et al. 1998; Seidler et al. 2010). This is also observed at baseline in the current study (Table 4.3) with older adults requiring more time to complete the task at each ID compared to the young. However once normalised to baseline, a similar magnitude of fatigue resulted in no age-related difference in MT, contrary to the original hypothesis. One possible explanation for this finding is the age range of older adults selected for this study (60 - 70 years). Evidence shows that older adults aged 60 - 70 yrs fatigue relatively similarly to young adults while older adults aged 70 - 80 yrs experience a greater degree of fatigue compared to the young (Sundberg et al. 2018b). Therefore, a lack of difference in magnitude of fatigue between young and older adults may result in a similar impact on motor performance (MT). Furthermore, in a study that aimed to expand on the age-related differences in psychomotor ability between young and older adults (age range: 21 – 90 years) decrements in performance such as precision control, arm-hand steadiness, manual dexterity and wrist finger speed were only apparent in the older group with a mean age of 80 years (Cheong et al. 2013). This further suggests that age-related differences in motor performance remain more prominent in an older age group (Charness and Bosman 1994; Wilkinson and Allison 1989).

4.5.5. Conclusion

The current findings suggest no age-related differences in motor performance post fatiguing exercise. However, an improvement in MT was observed following a short

contraction when accuracy demands were low indicating a beneficial effect of single-joint isometric exercise on task performance in young and older adults. This may be attributed to an exercise induced augmentation in activation of the corticospinal pathway post exercise, as well as increased activation of the visual cortex and higher brain centres. On the other hand, MT increased following a short contraction when accuracy demands were high suggesting a detrimental effect of fatigue when task performance requires greater fine motor control. Interference of the corticospinal pathway resulting in a reduction of cortical output may be the primary contributor for this finding. Interestingly, similar observations were made post fatiguing exercise suggesting that activation of the corticospinal pathway is sufficient to elicit changes in motor performance irrespective of amount and duration of fatigue. Nevertheless, additional investigation into the impact of fatigue on motor performance in older adults, particularly very old adults (>85yrs), is required to provide a more functional representation of fatigability differences between young versus older adults. Greater understanding of the impact of fatigue on performance in the ageing population allows for the development of interventions that may be used to circumvent its negative effects.

CHAPTER V

PRIMING TDCS DOES NOT FACILITATE SUBSEQUENT TDCS EFFECTS ON FATIGABILITY AND MOTOR PERFORMANCE IN YOUNG AND OLDER ADULTS.

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Overall percentage (%)	55%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	15/11/2021

Co-Author Contributions

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

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

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

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

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5. PRIMING TDCS DOES NOT FACILITATE SUBSEQUENT TDCS EFFECTS ON FATIGABILITY AND MOTOR PERFORMANCE IN YOUNG AND OLDER ADULTS.

5.1. ABSTRACT

Metaplastic induced shifts in cortical excitability via tDCS have been shown to improve learning and motor performance in young and older adults. However, this is yet to be applied in the context of fatigability. Fifteen young and older adults enrolled in three counterbalanced double blinded neuromodulation sessions; sham primed sham tDCS (stDCS – stDCS), sham primed anodal tDCS (stDCS – atDCS) and cathodal primed anodal tDCS (ctDCS – atDCS). In each session, participants received a 15 min priming treatment (sham/cathodal) followed by a 15 min active treatment (sham/anodal) during a 15% MVC of the elbow flexor muscles until task failure. MEP amplitude and LICI (ISI 100 ms) were measured in the biceps brachii before and after priming, as well as post fatiguing exercise. Movement time (MT) during a Fitts type task was also measured prior to tDCS stimulation and after exercise. Cathodal priming did not impact corticospinal excitability or LICI relative to sham priming in young and older adults. However, suppression in corticospinal excitability and greater attenuation in LICI was observed following anodal stimulation applied during exercise (demonstrated by stDCS – atDCS) relative to sham. In addition, cathodal primed anodal tDCS did not impact corticospinal excitability or LICI relative to sham primed anodal tDCS, suggesting no added benefit of cathodal priming in both age groups. No improvement in time to task failure or MT was observed either following cathodal primed or sham primed anodal application, despite the noted change in excitability, suggesting no parallel between tDCS induced changes in excitability and fatigability/task performance in both young and older adults.

Key Words: ageing, cortical excitability, fatiguing exercise, intracortical inhibition, metaplasticity, motor performance, TMS, tDCS.

5.2. INTRODUCTION

It has been well documented that neuromuscular fatigue enhances corticospinal excitability and decreases intracortical inhibition in young healthy adults as a possible compensatory mechanism to boost neural drive to the muscle and maintain force output (Benwell et al. 2007b; Benwell et al. 2006a; Hunter et al. 2016a; Otieno et al. 2019; Otieno et al. 2021; Otieno et al. 2022; Vucic et al. 2011). This thesis provides novel evidence demonstrating that older adults show a similar decline in GABA mediated inhibition following single-joint isometric exercise (*see chapter three*) suggesting no impact of ageing on GABA modulation with fatigue. While chapter two (Otieno et al. 2021) alluded to a possible age-related compensatory reduction in $GABA_B$ mediated inhibition, this might be attributed to the varying amounts of fatigue seen between young and older adults. This was later addressed in chapter three (Otieno et al. 2022) where an identical attenuation in GABA mediated inhibition was identified following a similar amount of fatigue in both age groups. This indicates a retention in the ability to modulate intracortical inhibitory processes with ageing. The effects of fatigue on excitatory and inhibitory circuits also provide an opportunity for the application of interventions such as tDCS (Nitsche and Paulus 2000) to minimise/manipulate fatigability (Abdelmoula et al. 2016; Cogiamanian et al. 2007; Oki et al. 2016; Williams et al. 2013).

TDCS is a form of non-invasive neuromodulation that uses weak direct currents (1-2 mA) delivered via electrodes placed directly on the scalp (Nitsche and Paulus 2000). Unlike TMS, tDCS does not directly stimulate corticospinal axons causing them to discharge, instead it acutely modulates resting membrane potential of the underlying tissue thereby adjusting ongoing neuronal firing activity (Liebetanz et al. 2002). Excitability of the tissue placed directly under the anode electrode occurs via subthreshold membrane depolarisation whereas inhibition of the tissue placed directly under the cathode electrode occurs via subthreshold

hyperpolarisation (Nitsche and Paulus 2000; Nitsche et al. 2007; Nitsche et al. 2003b; Priori et al. 1998; Rothwell 2010; Stagg and Nitsche 2011). Due to its ability to elicit sustained and transient changes in cortical excitability; referred to as neuroplasticity (Bindman et al. 1964; Bindman et al. 1962; Nitsche and Paulus 2001), tDCS has become a major tool used in the modulation of cortical mechanisms involved in fatigability. When applied directly over M1 in healthy young (Abdelmoula et al. 2016; Cogiamanian et al. 2007; Williams et al. 2013) and older adults (Oki et al. 2016), anodal tDCS has been shown to decrease fatigability (prolong time to task failure) in some cases, likely mediated by the modulation of fatigue-related changes in corticospinal excitability. However, no study has investigated the impact of anodal tDCS on fatigability in both age groups simultaneously. Furthermore, inconsistencies exist in the literature with some studies reporting no impact of anodal tDCS on fatigability (Kan et al. 2013; Muthalib et al. 2013; Radel et al. 2017). This has been attributed to methodological disparities such as sample size differences, timing of stimulation and single versus double blinded studies further limiting tDCS' clinical application. Beyond simple stimulation parameters, the magnitude and direction of effects evoked by tDCS also depend on the history and level of synaptic activity at the time of stimulation (Davis and Bezprozvanny 2001; Turrigiano and Nelson 2004).

The CNS endeavours to maintain stable neural function via homeostatic regulation (Davis and Bezprozvanny 2001; Turrigiano and Nelson 2004) making it possible for excitatory stimuli to decrease excitability when applied with/directly after another excitatory stimulus (Huang et al. 2008; Thirugnanasambandam et al. 2011). As such, discrepancies between studies could also be attributed to inter-individual variability driven by differing histories of synaptic activity between participants. This has been supported by studies that show ~50% of participants do not show the expected potentiation/depression of corticospinal excitability

following atDCS or ctDCS i.e., do not respond to stimulation (Puri et al. 2015; Strube et al. 2016; Wiethoff et al. 2014).

An interesting technique has now been developed to minimise inter-individual variability and facilitate behavioural outcomes by lowering neuronal activity in M1 and decreasing the threshold for induction of LTP-like processes (Christova et al. 2015; Fujiyama et al. 2017). According to the Bienenstock-Cooper-Munro theory of homeostatic metaplasticity, plasticity at a synapse is bidirectional resulting in either LTP or LTD (Bienenstock et al. 1982). If previous synaptic activity of the underlying tissue is low, then synaptic modification threshold decreases thus favouring LTP induction over LTD. On the other hand, if synaptic activity of the underlying tissue is high then synaptic modification threshold increases thus favouring LTD induction over LTP. The resulting effects showed greater improvement in skill acquisition and an increased response to anodal tDCS amongst participants when preceded by cathodal tDCS priming in both young and older adults (Christova et al. 2015; Fujiyama et al. 2017). However, this is yet to be established in the context of fatigability.

The current study aimed to investigate the impact of tDCS induced metaplasticity on corticospinal excitability, $GABA_B$ mediated inhibition and fatigability in young and older adults. The study also aimed to determine the resulting impact on performance of a SMT task post fatiguing exercise in both age groups. It was hypothesised that cathodal primed anodal tDCS would enhance corticospinal excitability, decrease $GABA_B$ mediated inhibition and improve fatigability, as well as motor task performance similarly in young and older adults compared to sham primed anodal tDCS.

5.3. METHODS

Fifteen right-handed young (25.3 ± 3.7 yrs; 8 females) and fifteen older adults (68.9 ± 5.1 yrs; 8 females) were recruited for participation in this study from the university community

and social media. Handedness was assessed using the Edinburgh Handedness inventory (Oldfield 1971). Participants were excluded based on current use of psychoactive medication (e.g., sedative, antidepressants, and antipsychotics) or history of neurological and/or psychiatric disease. Self-reported subjective physical activity (work index, sport index, leisure-time index) was assessed in all participants (Table 5.1), using an activity questionnaire (adapted from Baecke et al. 1982) where participant scores ranged from 1 (sedentary) to 5 (active) for each index. All procedures were approved by the University of Adelaide Human Research Ethics Committee and conducted in accordance with the Declaration of Helsinki. Each participant provided written, informed consent prior to participation.

Table 5.1: Participant characteristics.

	Young	Old
N	15	15
Females	8	8
Weight (kg)	65.7 ± 9.7	81.1 ± 19.5*
Height (cm)	171.1 ± 11.4	168.4 ± 7.7
Handedness	0.96 ± 0.5	0.97 ± 0.1
Work Index	2.4 ± 0.5	2.6 ± 0.5
Sport index	2.3 ± 0.7	2.4 ± 0.4
Leisure-time index	2.9 ± 0.5	2.9 ± 0.7

Values are shown as mean ± SD. *P<0.05 when compared to the young participants. Handedness ≥ 0.5 indicates right-handed individuals.

5.3.1. Experimental set-up

Elbow flexor force in newtons was evaluated using a Biodex Multi-Joint System (Biodex, Inc, Shirley, NY). Each participant sat upright with their right arm slightly abducted and their elbow resting comfortably on a padded support flexed to 90° so that the supinated

forearm was horizontal to the ground. The shoulders were restrained by two nylon straps to minimize shoulder movement. Forces detected by the biodex were recorded online using a Power 1401 A-D converter and Spike 2 software [Cambridge Electronics Design (CED), Cambridge, UK]. The force exerted in the vertical direction was displayed on a 17-in. monitor located 0.9 m in front of the participant.

Figure 5.1: Experimental set up: *Elbow flexor force was evaluated using a Biodex Multi-joint system. Participant sat upright with elbow-joint strapped to padded surface for upward elbow flexion. Screen placed directly in front of participant for force feedback. Shoulders and left knee retrained by nylon straps to minimise shoulder and leg movement. TMS was applied to the left primary motor cortex.*

5.3.2. Electrical Recordings

EMG signals were recorded using surface electrodes (Ag-AgCl, 8-mm diameter) that were placed in a monopolar configuration (over the middle of the muscle belly and the tendon) of biceps brachii and triceps brachii (Hunter et al. 2008). EMG signals were amplified (×300) and band-pass filtered (16 –1,000 Hz) [model CED 1902, Cambridge, UK]. Force (1,000 Hz) and EMG (2,000 Hz) signals were sampled with a CED 1401 computer interface and Spike2 software (CED).

5.3.3. Experimental protocol

The study was comprised of three randomised sessions (sham primed sham tDCS (stDCS – stDCS), sham primed anodal tDCS (stDCS – atDCS), cathodal primed anodal tDCS (ctDCS – atDCS)) with two age groups (young and older adults). Both participant and experimenter were blinded to session type in each instance (double blinded). Fig 5.2 illustrates the fatiguing protocol during each session. Maximum force and EMG were determined by calculating the average value of three, 3 – 5 s MVCs of the elbow flexors. Prior to the fatiguing task, participants received *a set of stimulations* which consisted of twenty single pulse TMS, twenty paired-pulse TMS and two electrical stimulations of the brachial plexus. This is due to the evidence shown where averaging less than 20 simultaneous MEP responses results in increased variability (Biabani et al. 2018). All stimulations were delivered during 5% of the maximal recorded EMG during MVC in a non-fatigued muscle (*see chapter three*). This was maintained at an absolute level (5% of maximum EMG measured at baseline) at all time points. Participants were then instructed to complete the SMT task for baseline measures. Once

completed, participants received priming tDCS treatment (stDCS/ctDCS) for 15 min at rest. A 15 min rest period was then provided following stimulation during which *a set of stimulations* was administered at 5 min post priming. In order to fatigue the muscle, participants performed a submaximal sustained contraction at 15% MVC to task failure. stDCS/atDCS was applied during the 15% contraction for a total of 15 min. If tDCS application was completed prior to task failure, contraction intensity was increased by 5% every 5 min until participant approached task failure (defined as a 5% drop in target force for >3 s in both age groups). A verbal cue provided by the experimenter indicated when participants should commence exercise or rest, and participants were asked to refrain from using other muscles. Visual feedback of force output (displayed on a computer screen) and verbal encouragement was provided throughout the protocol. Immediately post-exercise, participants performed two brief 5 s MVCs with a 10 s rest period between contractions, followed by a *set of stimulations* at 5% EMG contraction and SMT task respectively. A 10 min recovery period was then provided followed by a final *set of stimulations* during a 5% EMG contraction. To conclude the session, participants were instructed to perform two final MVCs to measure force recovery.

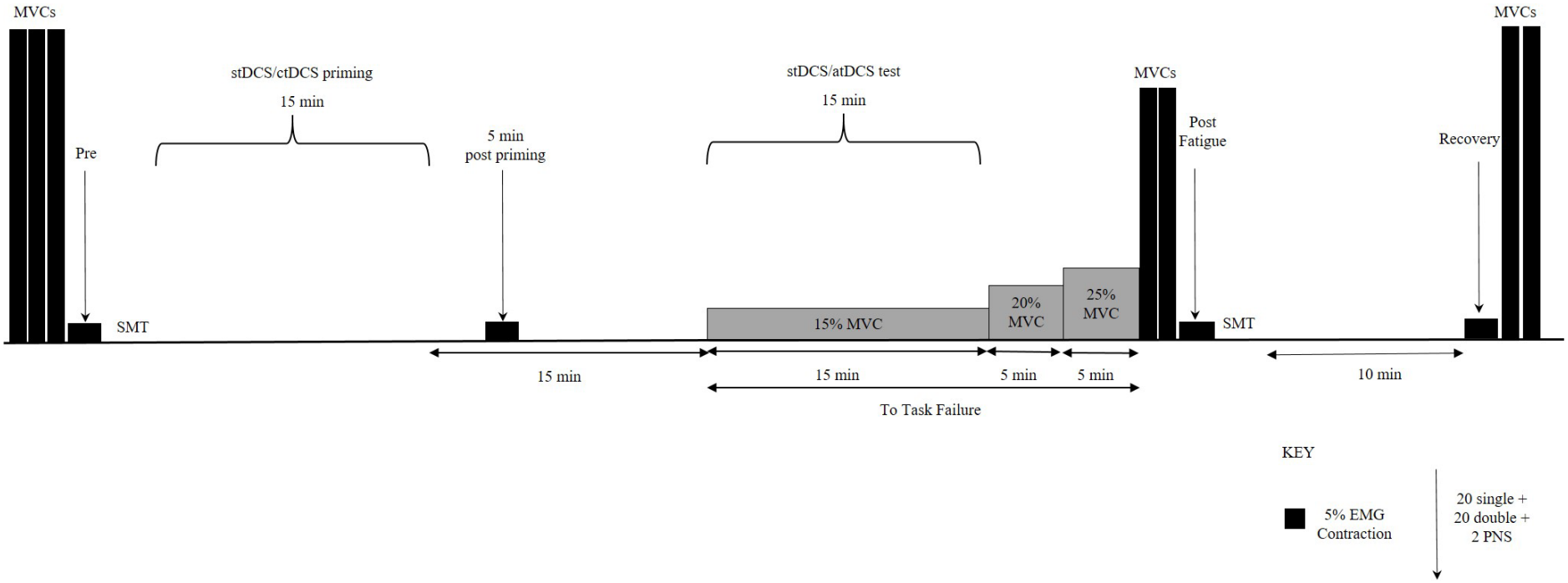

Figure 5.2: Experimental protocol schematic A) Baseline; *Participants performed three 3-5 s MVCs and received a set of stimulations (solid arrow) during 5% EMG contraction (solid black box) prior to exercise. They were then instructed to perform the SMT task followed by priming application (stDCS/ctDCS) for 15 min. A 15 min break was then provided where a set of stimulations was administered 5 min post priming. In order to fatigue the muscle, participants were required to perform a submaximal contraction at 15% MVC to task failure whilst receiving stDCS/atDCS during exercise. If tDCS application concluded prior to task failure (5% drop in force for >3 s), contraction intensity was increased by 5% every 5 min until task failure was reached. Two more 3-5 s MVCs were performed immediately post exercise followed by a set of stimulations and SMT task. A 10-minute recovery period was then provided, and participants received the final set of stimulations (solid arrow) during 5% EMG contraction and performed two final 3-5 s MVCs*

5.3.4. *Electrical stimulation of the brachial plexus*

A constant-current stimulator (model DS7AH, Digitimer, Welwyn Garden City, Hertforshire, UK) was used to deliver single stimuli (100 μs duration) to the brachial plexus to produce a maximal compound muscle action potential (maximum M-wave: M_{max}) of the biceps brachii muscle. A cathode was placed in the supraclavicular fossa and an anode on the acromion. The stimulation intensity was determined by increasing the current in increments of 10 mA until the peak-to-peak M-wave amplitude plateaued (M_{max}). To ensure supramaximal activation of the muscles, the stimulation intensity was set at 120% of the intensity that produced the largest M-wave response at rest. These measurements were performed prior to any contractions or TMS. Due to reported feelings of severe discomfort, three older adults did not receive brachial plexus stimulation. Two more older participants were excluded from analysis due to shift in electrode position during the protocol.

5.3.5. *Transcranial magnetic stimulation*

TMS was applied to the left primary motor cortex using a figure-of-eight coil (external wing diameter 9 cm) with two monophasic Magstim 200^2 magnetic stimulators connected through a Bistim (Magstim, Dyfed, UK). The coil was placed tangentially to the scalp at an angle of 45° to the sagittal plane, with the handle pointed laterally and backwards, producing an anteriorly directed current flow in the brain. The coil was placed on the scalp over the region that produced the largest response in the active biceps brachii muscle at a fixed stimulator intensity (60–65% maximum stimulator output). This location was marked on the scalp using a pen for reference, and the coil was manually placed on the optimal spot by the experimenter, with coil position continually checked throughout the experiment. TMS was delivered at a rate of 0.2 Hz with 10% variance between trials.

AMT was obtained in biceps brachii with the TMS coil placed over the optimal location. AMT was defined as the lowest stimulus intensity that produced a visible MEP response relative to background EMG in three out of five stimulations during a 5% EMG contraction. LICI was also assessed in an active muscle. Test MEP was set at an intensity that elicited an MEP closest to 50% of M_{max} (NB: on average MEP of 33% M_{max} was obtainable in most participants during the 5% EMG contraction). Suprathreshold conditioning intensity (110%, 120%, 130% and 140% AMT) that a) elicited a SP closest to 150 ms (Table 5.3) and b) evoked closest to 50% inhibition of test MEP response at baseline (*see chapters two and three*) was selected for experimentation (McNeil et al. 2011a; Otieno et al. 2021). Interstimulus interval (ISI) was set at 100 ms.

5.3.6. Transcranial direct current stimulation

Current was induced by a battery-driven constant-direct current stimulator (NeuroConn DC Stimulator Plus, Germany) and delivered through a pair of 35 cm^2 square rubber electrodes placed inside pre-saline soaked sponges. The active electrode was placed on the representation field of the right elbow flexor muscles (determined by TMS as described above) and the reference electrode placed on the contralateral supraorbital region (Nitsche and Paulus 2000). During anodal tDCS and cathodal tDCS, an initial 8 s ramp-up and ramp-down period was implemented with current delivered at 1.5 mA for 15 min. During sham tDCS, a similar 8 s ramp-up and ramp-down period was implemented with current delivered for 30 s to induce initial sensations associated with tDCS stimulation, but no corticospinal excitability changes (Oki et al. 2016). To ensure double blinding of both experimenter and participant, position of the red sponge and blue sponge on the participant's scalp remained unchanged during each session. However, polarity of the sponges was altered by a third person directly on the DC stimulator during cathodal tDCS such that the red sponge was the reference electrode and the blue sponge the active electrode. This was communicated to both the experimenter and

participant prior to commencement of the first session. Self-reported sensation measures confirmed no difference in sensation between sessions (not reported).

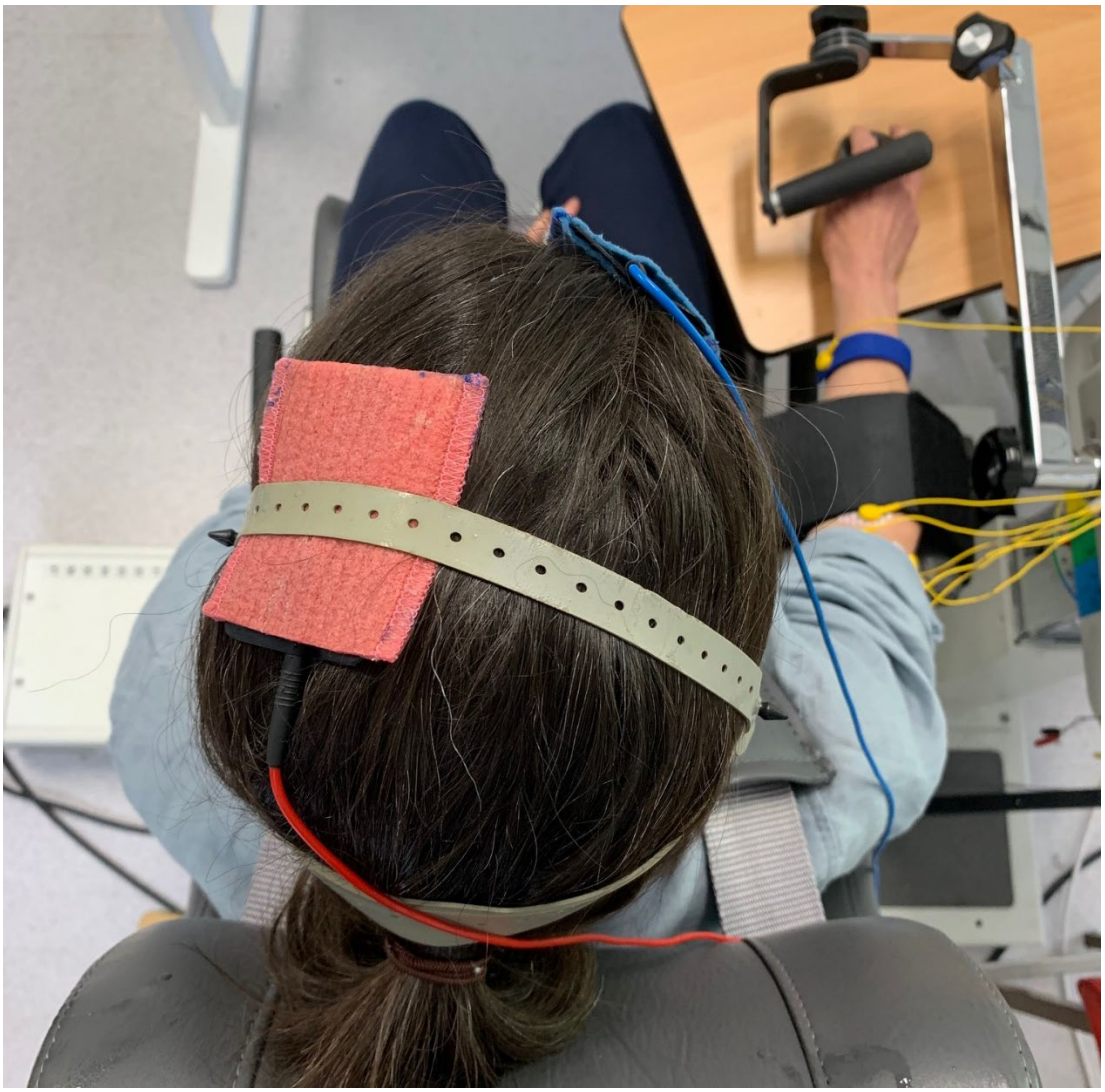

Figure 5.3: Anodal tDCS configuration: *Anode (red sponge) placed directly on M1 hotspot determined by TMS. Cathode (blue sponge) placed contralateral supraorbital region. Polarity of the sponges was changed by a third person on DC stimulator during cathodal tDCS without shifting position on the participant's scalp to ensure blinding of experimenter and participant.*

5.3.7. Simple Movement Time Task

Psychopy software (v3.0, Open Science Tools Ltd, Nottingham, UK) was used to set up and present visual stimuli on a monitor placed directly in front of participants. The stimuli included a white square (starting position) and a red square (target). Participants were informed

that the target would only appear on the bottom right quadrant of the screen (ten trials, e.g., Fig. 1B). ID was set using the Fitts law equation:

$$MT = a + b \log_2(2A/W)$$

The equation $\log_2(2A/W)$ provided an ID which was manipulated during experimentation by altering size of the red square (W) and distance from starting point (white square) to the target (A) (Fitts 1954) (*see Table 5.2*).

Table 5.2: ID in SMT

	ID	**ID_{range}**	**Distance to target (cm)**	**Target size (cm)**
SMT	2.6	ID_{1-3}	1.8	0.6
	3.3	ID_{3-4}	1.3	0.3
	3.6	ID_{3-4}	5.8	0.9
	3.9	ID_{3-4}	6.8	0.9
	4.1	ID_{4-5}	3.1	0.4
	4.3	ID_{4-5}	2.6	0.3
	4.4	ID_{4-5}	3.8	0.4
	5.4	ID_{5-6}	5.4	0.3
	5.7	ID_{5-6}	6.7	0.3

Using a mouse cursor, participants were instructed to first click on the white square (starting position) and wait for the appearance of the target (red square) – *see Fig. 4.2*. Once the target appeared participants were required to move towards it as quickly as possible and click again before returning to the starting position. Accurate click on target was confirmed once the target disappeared and starting position (white square) reappeared once more. This sequence was repeated until ten trials were completed. MT was recorded as the amount of time

in milliseconds between onset of cursor movement from starting position to the click on the target. To minimise anticipation, the target appeared with a random delay of 0.3 s – 3 s with IDs presented in a randomised fashion.

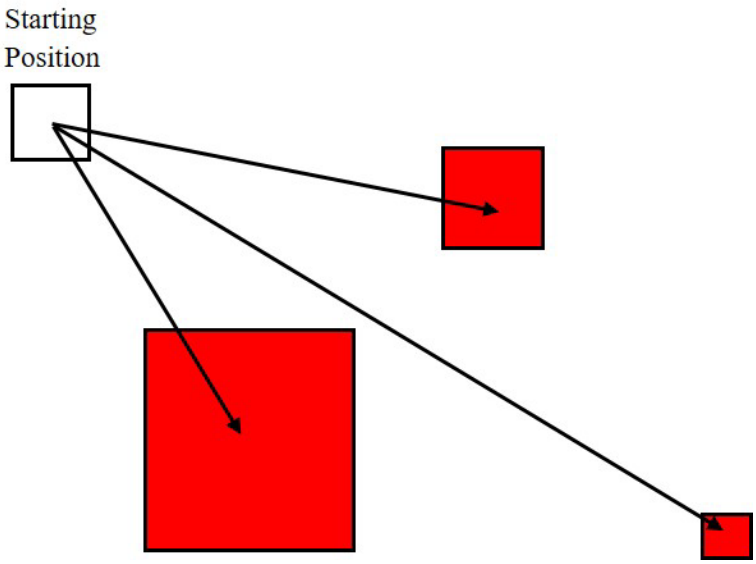

Figure 5.4: *Example of trial with arrow showing direction of movement. Target only appeared on the bottom right quadrant of the screen (three representative trials shown above however the task included ten trials in total).*

5.3.8. Data analysis

Peak force amplitude was measured during brief MVCs before and after exercise. Force during each 5% EMG contraction was measured as mean force amplitude. Voluntary EMG during each 5% EMG contraction was measured throughout the protocol as root mean squared EMG (EMG_{rms}) over a duration of 100 ms prior to stimulation. Maximum EMG (EMG_{rmsmax}) was calculated during the plateau in force of the MVCs whereby force was required to plateau for at least 2 s before termination of MVC. MEP and M_{max} amplitudes from each trial were measured as peak-to-peak in mV. Test MEPs were not expressed as a percentage of M_{max} due to the difficulty in obtaining M_{max} measurements in some of the older participants (*see section 5.3.4*). LICI was calculated as the ratio (expressed as a percentage) between the peak-to peak amplitudes of the conditioned and unconditioned MEPs (conditioned/unconditioned × 100).

Therefore, an increase in the magnitude of LICI reflects a reduction in inhibition. For SMT task, ID was subdivided into four ranges (easiest to most difficult – *see Table 5.2*) for analysis; one to three (ID_{1-3}), three to four (ID_{3-4}), four to five (ID_{4-5}) and five to six (ID_{5-6}), at each of the five time points (pre fatigue, control, fatigue, post, recovery). MT at each ID was determined using in-built scripts on the Psychopy software as the time between onset of cursor movement from starting position to click on target (Fig 5.3).

5.3.9. Statistical analysis

Linear mixed models with repeated measures (LMM_{RM}) were used to compare the effect of time (baseline versus post priming, baseline versus post fatiguing exercise and baseline versus recovery), neuromodulation (stDCS – stDCS, stDCS – atDCS, ctDCS – atDCS), and age on the magnitude of force, EMG_{rms}, LICI, MEP and M_{max} when normalised to baseline. Similarly, LMM_{RM} was used to compare the effect of age, ID, and neuromodulation on MT at baseline in young and older adults, as well as time, neuromodulation, and ID on MT in young and older adults separately. Significant interaction and main effects were investigated via Bonferroni's post hoc tests corrected for multiple comparisons. For all comparisons, normality of the data was confirmed by Shapiro-Wilk test ($P<0.05$). Two-way ANOVA was used to compare the effect of age and neuromodulation on time to task failure (TTF). Statistical significance was set at $P < 0.05$. All data in figures are presented as means and 95% confidence interval of the mean. All data in text is presented as estimated mean differences (EMD) and 95% confidence interval for the estimate, providing a non-standardised measure of effect size. All data in tables is presented as means ± SD.

5.4. RESULTS

Table 5.1 outlines participant characteristics with older and young adults showing similar physical activity scores (work, sport, and leisure-time index). Older adults were significantly heavier compared to young adults.

5.4.1. Baseline measures

Table 5.3 shows baseline corticospinal characteristics in both young and older adults. Older adults produced greater maximum force at baseline compared to the young in all three tDCS sessions. No difference between tDCS sessions was identified.

Table 5.3: Baseline corticospinal characteristics.

	Corticospinal excitability characteristics	**Young**	**Old**
stDCS –stDCS	MVC (N)	97.6 ± 95.3	111.3 ± 34.1*
	M_{max} Intensity (mA)	93.2 ± 29.9	115.4 ± 34.1*
	M_{max} (mV)	7.6 ± 2.9	8.0 ± 3.4
	CS (% MSO)	65.8 ± 9.5	67.6 ± 9.0
	TS (% MSO)	78.0 ± 7.3	76.8 ± 7.2
	AMT (% MSO)	49.9 ± 7.0	49.9 ± 7.7
	Conditioned MEP (% unconditioned MEP)	46.9 ± 32.5	48.0 ± 31.1
	Unconditioned MEP (mV)	3.0 ± 1.6	2.1 ± 2.0
	5% EMG_{rms} (mV)	0.07 ± 0.03	0.06 ± 0.03
stDCS –atDCS	MVC (N)	95.2 ± 27.5	114.6 ± 36.9*
	M_{max} Intensity (mA)	88.0 ± 23.0	117.2 ± 34.2*
	M_{max} (mV)	8.3 ± 2.4	8.0 ± 4.3
	CS (% MSO)	64.1 ± 8.8	64.7 ± 6.6
	TS (% MSO)	79.7 ± 6.7	76.6 ± 6.7
	AMT (% MSO)	49.6 ± 7.0	52.1 ± 7.7

	Conditioned MEP (% unconditioned MEP)	46.6 ± 30.4	46.7 ± 32.1
	Unconditioned MEP (mV)	2.7 ± 1.2	1.9 ± 1.5
	5% EMG_{rms} (mV)	0.07 ± 0.02	0.05 ± 0.05
ctDCS –atDCS	MVC (N)	93.1 ± 34.3	114.7 ± 38.9*
	M_{max} Intensity (mA)	95.2 ± 25.4	116.3 ± 27.5*
	M_{max} (mV)	8.8 ± 3.2	7.7 ± 4.1
	CS (% MSO)	64.7 ± 6.9	64.9 ± 5.2
	TS (% MSO)	78.2 ± 7.0	75.6 ± 7.1
	AMT (% MSO)	47.5 ± 5.5	51.1 ± 6.5
	Conditioned MEP (% unconditioned MEP)	41.1 ± 28.4	43.6 ± 31.8
	Unconditioned MEP (mV)	2.9 ± 1.7	2.2 ± 1.9
	5% EMG_{rms} (mV)	0.07 ± 0.03	0.06 ± 0.04

Values are shown as mean ± SD. *$P < 0.05$ compared to young adults. MSO, Maximum Stimulator Output; CS, Conditioning Stimulus; TS, Test Stimulus.

5.4.2. Time to Task Failure

There was no main effect of neuromodulation ($F_{2,23}$ = 0.58, *P = 0.165*), age ($F_{1,44}$ = 1.96, *P = 0.563*) or interaction between neuromodulation and age ($F_{2,4}$ = 0.16, *P = 0.851*) on TTF (Fig 5.5).

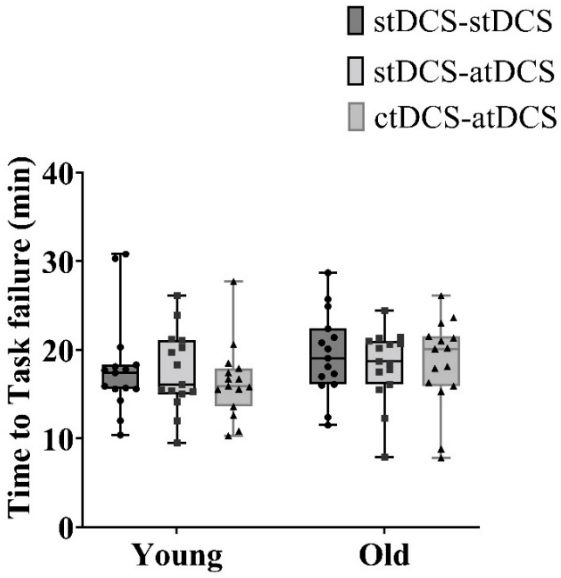

Figure 5.5: *Time to task failure (min) during stDCS – stDCS, stDCS – atDCS and ctDCS – atDCS in the elbow flexors in young and older adults. The lower and upper edges of each box show the 25th – 75th percentiles, respectively, whereas the horizontal line within the box shows the median. The whiskers span all data points (individual participant time to task failure) included within each box.*

5.4.3. MVCs

Post fatigue: MVC force decreased with time ($F_{1,20}$ = 248.51, *P<0.001*) when normalised to baseline, however no main effect of age ($F_{1,21}$ = 1.02; *P = 0.324*) or neuromodulation ($F_{2,390}$ = 0.48, *P = 0.617*) was observed. No interaction between age and neuromodulation ($F_{2,390}$ = 0.63, *P = 0.532*), neuromodulation and time ($F_{2,390}$ = 0.47, *P = 0.628*) or time, age, and neuromodulation ($F_{2,390}$ = 0.65, *P = 0.521*) was observed (Fig 5.6). However,

there was an interaction between age and time ($F_{1,390}$ = 5.46, *P = 0.020*) with a decrease in MVC force post fatiguing exercise in both young and older adults with an EMD of 29% (95% CI [26, 32], *P<0.001*) and 33% (95% CI [30, 36], *P<0.001*) respectively. Older adults also displayed a greater decline in MVC force compared to the young with an EMD of 4% (95% CI [0.5, 7], *P = 0.026*).

Recovery: A decline in MVC force was observed with time ($F_{1,22}$ = 57.83, *P<0.001*) when normalised to baseline, however no main effect of age ($F_{1,16}$ = 3.05, *P = 0.100*) or neuromodulation ($F_{2,30}$ = 2.40, *P = 0.108*) was observed. Nevertheless, an interaction between age, time, and neuromodulation ($F_{2,363}$ = 16.55, *P<0.001*) was identified. Young adults displayed a decrease in MVC force during stDCS – stDCS, stDCS – atDCS, ctDCS – atDCS with an EMD of 16% (95% CI [12, 20], *P<0.001*), 7% (95% CI [3, 11], *P = 0.001*) and 9% (95% CI [5, 13], *P<0.001*) respectively. Older adults displayed a similar decline in MVC force during all three tDCS sessions with an EMD of 12% (95% CI [8, 16], *P<0.001*), 15% (95% CI [12, 19], *P<0.001*) and 15% (95% CI [11, 19], *P<0.001*) respectively. Younger adults also showed a greater decline in maximum force compared to older adults during stDCS – stDCS with an EMD of 4% (95% CI [1, 8], *P = 0.007*). On the other hand, older adults showed a greater decline in maximum force compared to the young during ctDCS – atDCS with an EMD of 6% (95% CI [3, 9], *P<0.001*).

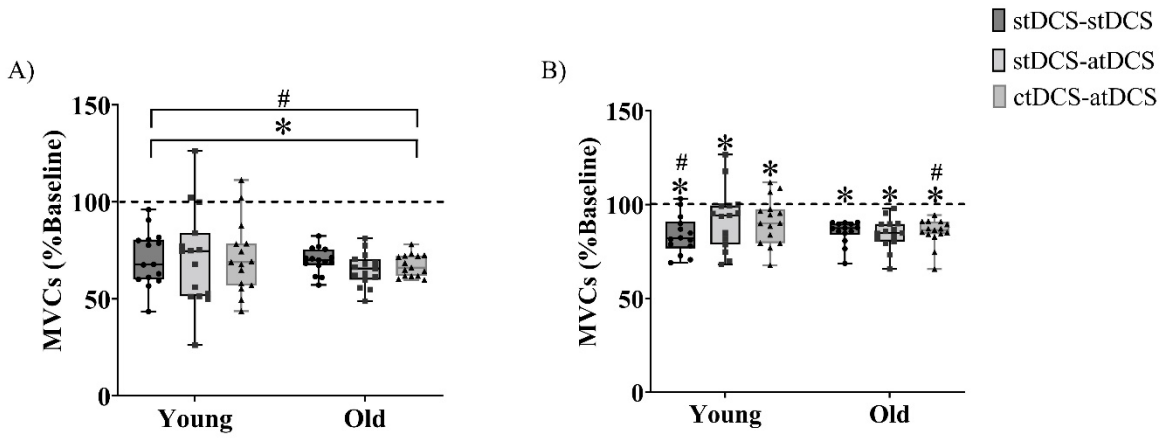

Figure 5.6: *MVC force (%Baseline) (A) Post Fatigue and (B) Recovery in the elbow flexors in young and older adults. The lower and upper edges of each box show the 25th – 75th percentiles, respectively, whereas the horizontal line within the box shows the median. The whiskers span all data points (individual participant responses) included within each box. *P < 0.05 compared to baseline; #P < 0.05 young vs. older adults.*

5.4.4. EMG$_{rms}$ during 5% EMG contraction

Post Priming: While there was a main effect of time ($F_{1,20} = 12.59$, *P = 0.002*), no main effect of age ($F_{1,21} = 0.28$, *P = 0.600*) or neuromodulation ($F_{2,34} = 0.64$, *P = 0.532*) on EMG_{rms} during 5% EMG contraction was observed when normalised to baseline. Furthermore, no interaction between age and time ($F_{1,3468} = 1.15$, *P = 0.284*), age and neuromodulation ($F_{1,3505} = 1.37$, *P = 0.254*), neuromodulation and time ($F_{2,3468} = 1.45$, *P = 0.235*) or interaction between time, age, and neuromodulation ($F_{2,3468} = 1.68$, *P = 0.187*) on EMG_{rms} during 5% EMG contraction was identified.

Post Fatigue: There was no main effect of time ($F_{1,20} = 3.25$, *P = 0.087*), age ($F_{1,22} = 0.15$, *P = 0.703*) or neuromodulation ($F_{2,35} = 1.23$, *P = 0.304*) on EMG_{rms} during the 5% EMG contraction when normalised to baseline. Furthermore, no interaction between age and time ($F_{1,3475} = 0.59$, *P = 0.444*), age and neuromodulation ($F_{1,3511} = 1.79$, *P = 0.167*), neuromodulation and time ($F_{2,3475} = 2.28$, *P = 0.102*) or interaction between time, age, and neuromodulation ($F_{2,3475} = 2.01$, *P = 0.135*) was identified.

Recovery: While there was a main effect of time ($F_{1,18}$ = 62.92, *P<0.001*), no main effect of age ($F_{1,25}$ = 1.64, *P = 0.212*) or neuromodulation ($F_{2,35}$ = 0.04, *P = 0.964*) was seen on EMG_{rms} during 5% EMG contraction when normalised to baseline. Furthermore, no interaction between age and time ($F_{1,3464}$ = 5.92, *P = 0.05*), age and neuromodulation ($F_{2,3491}$ = 0.78, *P = 0.458*) neuromodulation and time ($F_{2,3462}$ = 0.75, *P = 0.473*) or interaction between time, age, and neuromodulation ($F_{2,3462}$ = 0.93, *P = 0.394*) was identified.

5.4.5. Corticospinal excitability measures

Post priming: There was a main effect of time ($F_{1,21}$ = 11.22, *P = 0.003*), but no main effect of age ($F_{1,15}$ = 0.01, *P = 0.975*) or neuromodulation ($F_{2,30}$ = 1.49, *P = 0.242*) on LICI when normalised to baseline (Fig 5.7A). Furthermore, no interaction between age and neuromodulation ($F_{2,2762}$ = 0.09, *P = 0.913*), neuromodulation and time ($F_{2,3078}$ = 3.05, *P = 0.556*), age and time ($F_{1,2984}$ = 0.04, *P = 0.849*) or time, age, and neuromodulation ($F_{2,3078}$ = 0.43, *P = 0.650*) was identified, indicating no change in amount of inhibition post priming (Fig 5.7A). There was a main effect of time on MEP amplitude when normalised to baseline ($F_{1,19}$ = 12.81, *P = 0.002*) however no main effect of age ($F_{1,18}$ = 0.39, *P = 0.539*) or neuromodulation ($F_{2,35}$ = 0.38, *P = 0.690*) was observed (Fig 5.7D). Furthermore, no interaction between age and neuromodulation ($F_{2,3208}$ = 1.69, *P = 0.185*), neuromodulation and time ($F_{2,3175}$ = 2.08, *P = 0.125*), age and time ($F_{1,3161}$ = 2.37, *P = 0.124*) or time, age, and neuromodulation ($F_{2,3175}$ = 0.51, *P = 0.598*) was identified (Fig 5.7D).

Post Fatigue: While there was a main effect of time ($F_{1,20}$ = 49.07, *P<0.001*), no main effect of age ($F_{1,19}$ = 0.30, *P = 0.588*) or neuromodulation ($F_{2,31}$ = 3.01, *P = 0.064*) was observed on LICI when normalised to baseline. No interaction between age and time ($F_{1,2982}$ = 0.85, *P = 0.357*), age and neuromodulation ($F_{2,2900}$ = 0.89, *P = 0.413*) or time, age, and neuromodulation ($F_{2,3081}$ = 0.003, *P = 0.997*) was identified either. However, an interaction between neuromodulation and time ($F_{2,3081}$ = 12.16, *P<0.001*) was observed. LICI increased

post fatigue when normalised to baseline in both age groups during stDCS – stDCS, stDCS – atDCS and ctDCS – atDCS with an EMD of 43% (95% CI [23, 64], *P<0.001*), 79% (95% CI [59, 99], *P<0.001*) and 54% (95% CI [34, 75], *P<0.001;* Fig 5.7B) respectively, indicating a decline in inhibition post fatigue in both young and older adults. Furthermore, a greater increase in LICI was observed during stDCS – atDCS compared to stDCS – stDCS in both age groups with an EMD of 35% (95% CI [11, 60], *P<0.001;* Fig 5.7B) indicating a greater decrease in inhibition during stDCS – atDCS session.

There was no main effect of time ($F_{1,21} = 3.43$, *P = 0.078*), age ($F_{1,15} = 1.96$, *P = 0.181*) or neuromodulation ($F_{2,30} = 3.36$, *P = 0.048*) on MEP amplitude when normalised to baseline. No interaction between age and neuromodulation ($F_{2,3187} = 1.30$, *P = 0.273*), age and time ($F_{1,3215} = 20.30$, *P = 0.061*) or time, age, and neuromodulation ($F_{2,3197} = 1.27$, *P = 0.287*) was identified either. However, an interaction between neuromodulation and time ($F_{2,3197} = 21$, *P<0.001*) was observed. MEP amplitude decreased post fatigue in both age groups during stDCS – atDCS and ctDCS – atDCS with an EMD of 12% (95% CI [3, 20], *P = 0.008*) and 12% (95% CI [3, 20], *P = 0.009;* Fig 5.7E) respectively. Furthermore, a greater decrease in MEP amplitude was identified in both age groups during stDCS – atDCS and ctDCS – atDCS compared to stDCS – stDCS with an EMD of 10% (95% CI [2, 17], *P = 0.005*) and 9% (95% CI [1, 16], *P = 0.014;* Fig 5.7E) respectively.

Recovery: While there was a main effect of time ($F_{1,21} = 33.41$, *P<0.001*), no main effect of age ($F_{1,15} = 1.34$, *P = 0.266*) or neuromodulation ($F_{2,32} = 1.36$, *P = 0.271*) on LICI was observed when normalised to baseline. However, an interaction between time, age, and neuromodulation ($F_{2,3050} = 6.25$, *P = 0.002*) was identified. Younger adults displayed an increase during stDCS – stDCS, stDCS – atDCS and ctDCS – atDCS from baseline with an EMD of 46% (95% CI [23, 70], *P<0.001*), 61% (95% CI [37, 84], *P<0.001*) and 37% (95%

CI [14, 61], *P = 0.002;* Fig 5.7C) respectively indicating a continued attenuation in inhibition at recovery. Older adults displayed a similar increase compared to baseline during stDCS – stDCS, stDCS – atDCS and ctDCS – atDCS with an EMD of 39% (95% CI [14, 64], *P = 0.003*), 70% (95% CI [46, 95], *P<0.001*) and 82% (95% CI [58, 107], *P<0.001;* Fig 5.7C) respectively indicating a similar attenuation in inhibition at recovery. Furthermore, older adults showed a greater increase in LICI during stDCS – atDCS and ctDCS – atDCS compared to stDCS – stDCS with an EMD of 38% (95% CI [7, 69], *P = 0.011*) and 58% (95% CI [27, 89], *P<0.001;* Fig 5.7C) respectively. Finally older adults showed greater LICI compared to the young during ctDCS – atDCS with an EMD of 54% (95% CI [35, 73], *P<0.001;* Fig 5.7C) suggesting less inhibition in older adults compared to the young.

There was a main effect of time ($F_{1,19}$ = 9.88, *P = 0.005*), age ($F_{1,17}$ = 4.61, *P = 0.047*) and neuromodulation ($F_{2,35}$ = 35.33, *P = 0.014*) on MEP amplitude when normalised to baseline. An interaction with time, age and neuromodulation was also identified ($F_{2,3161}$ = 11.64, *P<0.001*). Young adults displayed an increase in MEP amplitude normalised to baseline during stDCS – stDCS and ctDCS – atDCS with an EMD of 15% (95% CI [9, 20], *P<0.001*) and 17% (95% CI [12, 23], *P<0.001;* Fig 5.7F) respectively. A similar increase was observed in older adults during stDCS – stDCS with an EMD of 15% (95% CI [9, 21], *P<0.001*) along with a contradictory decrease during stDCS – atDCS with an EMD of 8% (95% CI [2, 13], *P = 0.008;* Fig 5.7F). Furthermore, younger adults showed a greater decrease in unconditioned MEP amplitude during stDCS – atDCS compared to stDCS – stDCS and ctDCS – atDCS with an EMD of 12% (95% CI [3, 20], *P = 0.005*) and 14% (95% CI [6, 23], *P<0.001;* Fig 5.7F) respectively. Older adults also showed a greater decrease in MEP during stDCS – atDCS and ctDCS – atDCS compared to stDCS – stDCS with an EMD of 25% (95% CI [16, 34], *P<0.001*) and 17% (95% CI [8, 26], *P<0.001;* Fig 5.7F) respectively. Young adults also displayed greater MEP amplitude compared to the older adults during stDCS – atDCS and ctDCS – atDCS with

an EMD of 11% (95% CI [6, 17], *P<0.001*) and 18% (95% CI [12, 23], *P<0.001;* Fig 5.7F) respectively.

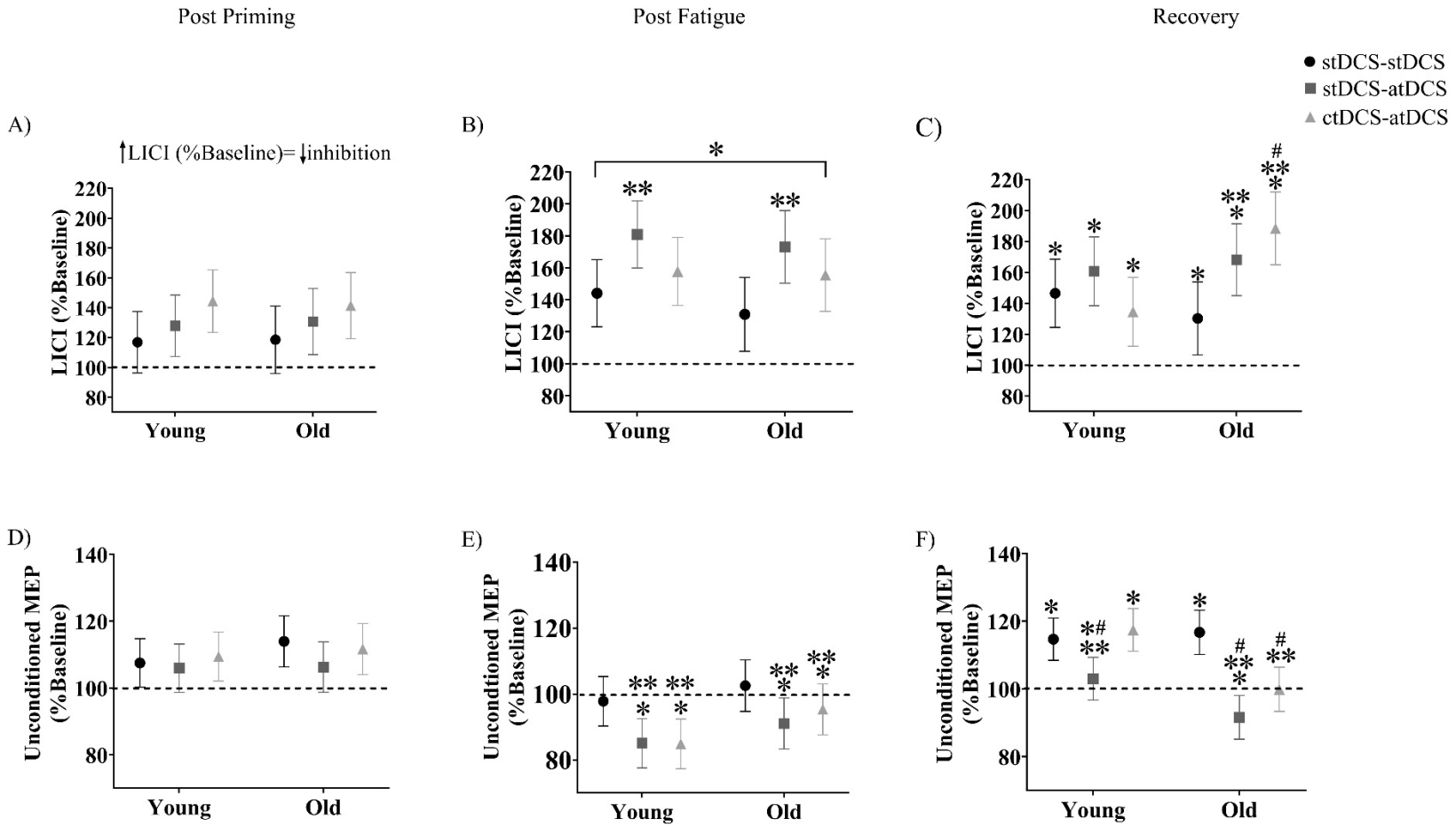

Figure 5.7: *LICI (%Baseline) and single pulse MEP amplitude (%Baseline) during stDCS – stDCS (circles), stDCS – atDCS (squares) and ctDCS – atDCS (triangles) in the elbow flexors in young and older adults. *P < 0.05 compared to baseline; #P < 0.05 young vs. older adults; **P < 0.05 compared to stDCS – stDCS; *#P < 0.05 compared to ctDCS – atDCS.*

5.4.6. M_{max}

Post Priming: There was no main effect of time ($F_{1,13} = 0.85$; $P = 0.373$), age ($F_{1,22} = 0.43$; $P = 0.517$), neuromodulation ($F_{2,27} = 0.50$; $P = 0.619$) or interaction between time, age, and neuromodulation ($F_{2,235} = 0.18$, $P = 0.834$) on M_{max}.

Post Fatigue: There was no main effect of time ($F_{1,247} = 3.63$; $P = 0.058$), age ($F_{1,22} = 0.34$; $P = 0.564$), neuromodulation ($F_{2,27} = 0.33$; $P = 0.719$) or interaction between time, age, and neuromodulation ($F_{2,247} = 0.60$, $P = 0.551$) on M_{max}.

Recovery: There was no main effect of time ($F_{1,12} = 0.000$; $P = 0.988$), age ($F_{1,22} = 0.39$; $P = 0.537$), neuromodulation ($F_{2,27} = 0.18$; $P = 0.836$) or interaction between time, age, and neuromodulation ($F_{2,234} = 0.44$, $P = 0.643$) on M_{max}.

5.4.7. Simple movement time task

5.4.7.1. Baseline measures

Similar to chapter four, older adults required more time to complete the task compared to the young at baseline (age effect: $F_{1,27} = 11.86$, $P = 0.002$) at all IDs (Table 5.4). Both age groups also required more time to complete the task at $ID_{3\text{-}4}$, $ID_{4\text{-}5}$ and $ID_{5\text{-}6}$ compared to $ID_{1\text{-}3}$ (ID effect: $F_{3,792} = 114.52$, $P<0.001;$ Table 5.4). No difference between sessions was observed (neuromodulation effect: $F_{2,38} = 0.66$, $P = 0.525$).

Table 5.4: MT at baseline in young and older adults.

	ID	Young (ms)	Old (ms)
stDCS - stDCS	ID_{1-3}	850.1 ± 175.1	956.3 ± 170.5*
	ID_{3-4}	966.9 ± 209.5$^{\#}$	1140.3 ± 181.8*$^{\#}$
	ID_{4-5}	994.8 ± 196.7$^{\#}$	1274.6 ± 230.3*$^{\#}$
	ID_{5-6}	1341.9 ± 624.8$^{\#}$	1483.1 ± 322.5*$^{\#}$
stDCS - atDCS	ID_{1-3}	751.2 ± 133.4	984.1 ± 198.1*
	ID_{3-4}	931.1 ± 194.9$^{\#}$	1176.6 ± 263.7*$^{\#}$
	ID_{4-5}	1023.0 ± 196.7$^{\#}$	1181.0 ± 221.1*$^{\#}$
	ID_{5-6}	1287.6 ± 412.2$^{\#}$	1458.6 ± 291.0*$^{\#}$
ctDCS - atDCS	ID_{1-3}	894.3 ± 249.5	985.4 ± 198.6*
	ID_{3-4}	946.5 ± 199.1$^{\#}$	1156.8 ± 220.7*$^{\#}$
	ID_{4-5}	1041.2 ± 223.1$^{\#}$	1218.5 ± 243.6*$^{\#}$
	ID_{5-6}	1236.3 ± 233.7$^{\#}$	1416.7 ± 339.6*$^{\#}$

Values are shown as mean ± SD. *$P<0.05$ compared to young; $^{\#}P < 0.05$ compared to ID_{1-3}.

5.4.7.2. Post fatigue measures

ID_{1-3}: While there was a main effect of time on MT when normalised to baseline ($F_{1,22} = 5.25$, $P = 0.032$), no main effect of age ($F_{1,113} = 0.11$, $P = 0.740$), neuromodulation ($F_{2,41} = 1.09$, $P = 0.347$) or interaction between time, age, and neuromodulation ($F_{2,108} = 1.12$, $P = 0.309$) was observed.

ID_{3-4}: There was no main effect of time ($F_{1,22} = 0.52$, $P = 0.477$), age ($F_{1,648} = 0.42$, $P = 0.518$), neuromodulation ($F_{2,35} = 0.78$, $P = 0.465$) or interaction between time, age, and neuromodulation ($F_{2,629} = 0.92$, $P = 0.400$) on MT when normalised to baseline.

ID$_{4\text{-}5}$: There was no main effect of time ($F_{1,23} = 3.47$, $P = 0.075$), age ($F_{1,16} = 0.40$, $P = 0.538$), neuromodulation ($F_{2,31} = 1.83$, $P = 0.177$) or interaction between time, age, and neuromodulation ($F_{2,442} = 0.34$, $P = 0.712$) on MT when normalised to baseline.

ID$_{5\text{-}6}$: There was no main effect of time ($F_{1,21} = 0.06$, $P = 0.138$), age ($F_{1,289} = 0.16$, $P = 0.689$), neuromodulation ($F_{2,32} = 0.27$, $P = 0.767$) or interaction between time, age, and neuromodulation ($F_{2,278} = 0.07$, $P = 0.933$) on MT when normalised to baseline.

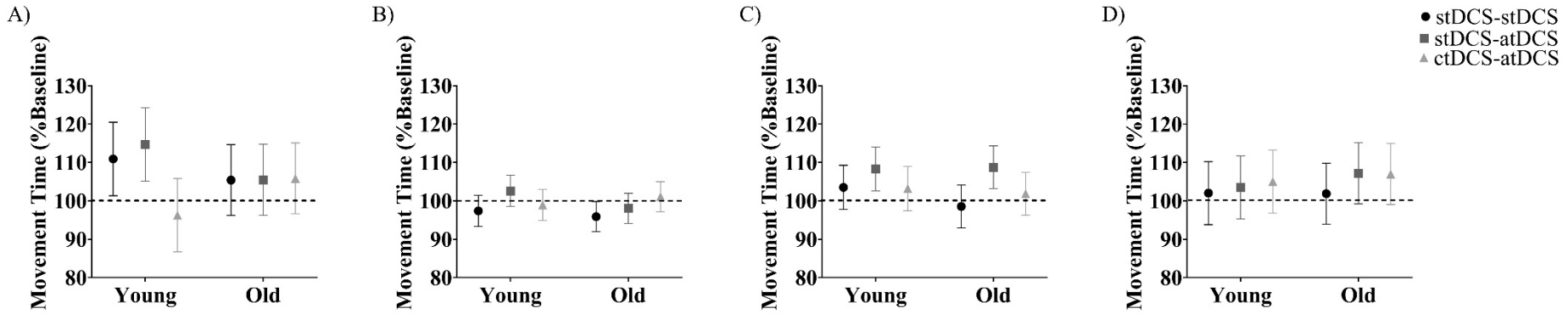

Figure 5.8: *MT (%Baseline) during stDCS – stDCS (circles), stDCS – atDCS (squares) and ctDCS – atDCS (triangles) at A) ID_{1-3}, B) ID_{3-4}, C) ID_{4-5} and D) ID_{5-6} in the elbow flexors in young and older adults.*

5.5. DISCUSSION

This study aimed to investigate how tDCS induced metaplastic changes interact with corticospinal excitability, $GABA_B$ mediated inhibition, fatigability, and motor skill performance in young and older adults. Cathodal priming had no effect on corticospinal excitability or LICI in both age groups. Furthermore, while anodal tDCS (stDCS – atDCS) elicited a similar suppression in corticospinal excitability and greater decline in $GABA_B$ mediated inhibition in both age groups relative to sham stimulation (stDCS – stDCS) immediately post exercise, cathodal primed anodal tDCS (ctDCS – atDCS) did not alter corticospinal excitability or $GABA_B$ mediated inhibition relative to anodal tDCS (stDCS – atDCS). These findings suggest no added benefit of priming with cathodal tDCS on corticospinal excitability during fatiguing exercise. No improvement in time to task failure or motor skill performance was identified in all three tDCS sessions in both age groups

5.5.1. Effects of primed anodal tDCS on corticospinal excitability post fatiguing exercise

As hypothesised (Hunter et al. 2016a; Otieno et al. 2021), corticospinal excitability did not change post fatigue following sham tDCS (demonstrated by stDCS – stDCS) in both young and older adults when measured during a 5% EMG contraction. However contrary to previous work (Oki et al. 2016; Williams et al. 2013), anodal tDCS application (demonstrated by stDCS – atDCS) unexpectedly decreased unconditioned MEP amplitude in both young and old post fatiguing exercise relative to sham suggesting a decrease in corticospinal excitability in both age groups. While previous studies have reported an increase in MEP amplitude following anodal tDCS application, it is important to note that single pulse MEP measures were collected during the fatiguing contraction (Oki et al. 2016; Williams et al. 2013), not immediately post exercise. Given the enhanced excitation observed during simultaneous application of anodal tDCS and single-joint isometric exercise, one possible mechanism underlying the unexpected

suppression in excitability in the current study could be neuronal counter-regulation (homeostatic regulation) driven by activation of hyperpolarising potassium channels to prevent over excitation. For example, anodal tDCS driven post-synaptic membrane depolarisation can mediate an NMDA receptor-mediated augmentation of synaptic strength presumably via an increase in intracellular Ca^{2+} levels (Liebetanz et al. 2002). This influx of Ca^{2+} ions is sufficient to activate the hyperpolarising potassium channels (Misonou et al. 2004) thereby leading to a decrease in excitability.

Interestingly, no age-related difference in excitability modulation was observed following anodal tDCS. Previous work shows a delayed response to anodal tDCS (15 – 30 minutes) in older adults relative to the young (increase observed immediately after tDCS application in the young) (Fujiyama et al. 2014; Ghasemian-Shirvan et al. 2020). This has been attributed to an age-related decline in the ability to induce LTP-like mechanisms in older adults compared to the young following anodal tDCS. However, this discrepancy between studies may be associated with the commonly reported high inter-individual variability response to tDCS, particularly in the ageing population (Li et al. 2015). Healthy ageing is associated with natural processes such as brain atrophy which can lead to differences in synaptic connectivity, myelination, and neurotransmission (Anderson and Rutledge 1996; Zimerman and Hummel 2010). Furthermore, visible changes in structure mediated by brain atrophy such as increased distance between the skull and brain and increased cerebrospinal fluid (CSF) can cause variation in response to tDCS amongst older participants due to shunting of current as a result of CSF's high conductivity relative to brain tissue (Li et al. 2015). It is therefore plausible that age-related differences in anatomy amongst individuals could contribute to the null findings observed in this study.

In both age groups, unconditioned MEP amplitude remained unchanged following cathodal priming relative to sham suggesting no impact of priming on corticospinal excitability.

One reason for the lack of change observed may be the timing of TMS stimulation. Given that changes in MEP vary overtime (Wischnewski and Schutter 2015), it remains possible that stimulation at a single time point (5 min post ctDCS) was not sufficient to detect changes in corticospinal excitability. However, this is not the first study to report confounding results regarding cathodal stimulation. While some studies have identified a decline in corticospinal excitability following ctDCS in young and old (Ghasemian-Shirvan et al. 2020; Sidhu 2021), other studies report no alteration in excitability in both age groups (Strube et al. 2016; Wiethoff et al. 2014). Fujiyama and colleagues report a similar lack of impact of ctDCS priming at 1.5 mA for 10 min (Fujiyama et al. 2017) prior to anodal tDCS application. It was suggested that this relatively short application of ctDCS (priming) was required to amplify the subsequent effect of atDCS without overtly increasing corticospinal excitability (Siebner et al. 2004; Ziemann and Siebner 2008). However, unlike Fujiyama and colleagues, cathodal primed anodal stimulation did not elicit greater changes in corticospinal excitability post fatiguing exercise relative to sham primed anodal tDCS. This brings to question whether induction of metaplasticity did in fact occur given the notable lack of change in unconditioned MEP observed post priming. One reason for this discrepancy may be different ISIs (rest periods) between priming and test protocols. Although the current study offered a 15-minute delay following cathodal stimulation, Fujiyama and colleagues applied anodal tDCS immediately after TMS measures were collected (ISI = 0). It is possible that metaplastic changes were indeed induced in the current study however the prolonged break might have dissipated any neuronal alterations prompted by cathodal priming leading to no amplification of anodal tDCS effects. This has also been shown in a study where application of subsequent tDCS treatment with no break prolonged the aftereffects of tDCS from 60 to 90 minutes (Monte-Silva et al. 2010). On the other hand, tDCS effects were attenuated or abolished with increasing delays between the two doses (Monte-Silva et al. 2010). Not only does this further highlight the impact

of inter-individual variability between studies but also highlights the difference in effectiveness between different non-invasive brain stimulation techniques.

Greater MEP amplitude size is recorded at recovery in both age groups following sham tDCS (stDCS – stDCS), indicating an increase in excitability. The mechanism underlying this is unclear given that previous work shows no change in unconditioned MEP amplitude at recovery when measured during an EMG contraction (see chapter two and Brownstein et al. 2020; Hunter et al. 2016a). However, this may suggest possible post-contraction facilitation (similar to that observed in older adults at recovery in chapter two). In addition, older adults show continued attenuation in MEP amplitude at recovery compared to the young following both sham and cathodal primed anodal tDCS, suggesting an age-related decline in the ability of anodal tDCS to modulate excitability during exercise.

5.5.2. Effects of primed anodal tDCS on $GABA_B$ mediated inhibition post fatiguing exercise

Consistent with our findings in chapter three, LICI declined similarly in both age groups post fatiguing exercise following sham stimulation (demonstrated by stDCS – stDCS) suggesting a decline in $GABA_B$ mediated inhibition regardless of age. Furthermore, both age groups displayed a greater decline in $GABA_B$ mediated inhibition post fatigue following anodal stimulation (demonstrated by stDCS – atDCS) relative to sham. This finding could be attributed to the attenuation in GABA concentration mediated by anodal tDCS application which may exacerbate the decline in LICI observed (Stagg and Nitsche 2011).

On the other hand, cathodal priming tDCS did not alter LICI in both young and older adults, as demonstrated by previous work. When applied over M1 at 1.5 mA for 20 min in ten young healthy adults, Tremblay and colleagues also showed that cathodal stimulation does not affect LICI (Tremblay et al. 2013a). Stimulation at a single time point (5 min post cathodal tDCS) may not have been sufficient to detect changes in corticospinal excitability since MEP changes do vary over time with non-invasive brain stimulation (Wischnewski and Schutter

2015). However, cathodal tDCS has been shown to directly affect glutamate not GABA levels (Stagg and Nitsche 2011), which could also explain the lack of change observed, since LICI is known to reflect $GABA_B$ mediated mechanisms. Indeed, cathodal primed anodal stimulation also showed no impact on LICI post fatigue relative to sham primed anodal stimulation, suggesting that there is no added benefit of cathodal priming prior to anodal stimulation on LICI measurements.

Both young and older adults continue to show attenuated LICI at recovery in all three tDCS conditions. However, unlike young adults, older adults also exhibit recovery in the amount of LICI following anodal stimulation relative to sham, indicating an age-related decline in the ability to modulate $GABA_B$ inhibitory activity for at least ten minutes after exercise *(see chapter two*). Interestingly, older adults also show greater attenuation in LICI following cathodal primed anodal stimulation application relative to sham; contrary to the young. Given that this was not observed immediately post exercise, a delayed response to cathodal primed anodal stimulation in older adults could explain this outcome. Older adults tend to have a 15 – 30 min delayed response to anodal stimulation (Fujiyama et al. 2014; Ghasemian-Shirvan et al. 2020), following cathodal priming, which has been attributed to the age-related decline in ability to induce LTP-like plasticity (Clayton et al. 2002; Foster and Kumar 2002; Sailasuta et al. 2008).

5.5.3. Fatigability and motor skill performance

Contrary to previous work (Oki et al. 2016; Williams et al. 2013), anodal tDCS application (demonstrated by stDCS – atDCS session) did not increase in TTF in young and older adults despite the notable change in corticospinal excitability measures and LICI post fatiguing exercise. The increase in TTF observed in young and older adults (Oki et al. 2016; Williams et al. 2013) was previously attributed to an increase in supraspinal drive mediated by the prolonged facilitation of corticospinal excitability by anodal tDCS application

(Cogiamanian et al. 2007). However, further study showed no direct parallel between changes in corticospinal excitability and an increase in TTF (Abdelmoula et al. 2016); a finding that is echoed in the current study. Nevertheless, the lack of change in TTF, despite noted changes in corticospinal excitability above, highlights the complex cortical network involved in fatigue development during exercise outside the traditional excitation/inhibition pathways. In any case, the discrepancies between studies may be attributed to a myriad of methodological factors. For instance, although tDCS was applied during contraction, previous work delivered anodal tDCS for a total of 20 minutes at 1.5 mA while the current study administered tDCS for a duration of 15 minutes. It is possible that 15 minutes was not sufficient to elicit neuromuscular changes that could improve TTF. One other point of consideration is the exercise model implemented. While previous work implemented a stronger contraction to task failure (20% MVC for both young and old), the current study utilised a 15% MVC for 15 minutes (to match duration of anodal tDCS) with increasing contraction strength at the end of stimulation (5% increase every 5 min). On average, young adults took ~25 min to approach task failure at 20% MVC (Williams et al. 2013) while older adults took ~15 – 20 min to approach task failure at the same contraction strength (Oki et al. 2016). Therefore, it is possible that a greater contraction strength coupled with longer anodal tDCS duration was required to accurately assess time to task failure and identify changes following anodal tDCS. Nevertheless, one other study identified a lack of change in TTF when anodal tDCS was applied during contraction (Radel et al. 2017) further highlighting the inter-individual differences that affect behavioural outcomes amongst studies. Cathodal primed anodal stimulation also elicited no change in TTF suggesting no direct benefit of cathodal priming prior to atDCS on fatigability in young and older adults.

As hypothesised, older adults required more time to complete the SMT task at baseline (see chapter four, Goggin and Meeuwsen 1992). This can be attributed to the age-related slowing in MT mediated by the greater emphasis on accuracy rather than speed in older adults

(Goggin and Meeuwsen 1992). However, unlike chapter four, single joint fatiguing exercise (indicated by stDCS – stDCS session) did not affect MT. Though unclear, the likely candidate for this discrepancy could be a potential learning effect. Contrary to chapter four, participants in the current study were required to perform the SMT task three separate times over a span of three weeks. The brief amount of time provided between sessions (at least one week) likely promoted learning amongst participants thereby causing a ceiling effect given that data shows that learning a fine motor task can be retained over several weeks (up to 29 days after initial session) (Reis et al. 2009). This in turn makes it difficult to identify whether anodal tDCS or cathodal primed anodal tDCS affected task performance in young and older adults. Therefore, increased time between sessions may be recommended for future assessment.

5.5.4. Conclusion

This study provides novel insight into the impact of cathodal primed anodal tDCS on fatigue mediated corticospinal excitability changes in young and older adults. A similar suppression in corticospinal excitability and attenuation in $GABA_B$ mediated activity following anodal tDCS was identified in both age groups, indicating an age-related retention in ability to modulate corticospinal excitability measures with tDCS application during fatigue. Furthermore, cathodal tDCS priming elicited no change in corticospinal excitability in both young and older adults, with no reported difference in corticospinal excitability modulation following cathodal primed anodal tDCS. This suggests no beneficial effect of priming with cathodal tDCS prior to anodal stimulation during fatigue in both age groups. Despite the noticeable effect of anodal tDCS on excitability and inhibition, anodal tDCS also had no impact on fatigability or motor task performance. Furthermore, priming with cathodal tDCS revealed no added benefit on fatigability or motor task performance in both age groups. These findings point to a more complex cortical network involved in the development of fatigue and task performance outside of the well-known excitatory/inhibitory pathways within M1. A deeper

understanding of these networks is required to will provide an opportunity for the optimisation of tDCS as a potential clinical tool used to minimise fatigability and its subsequent effect on motor task performance.

6. GENERAL DISCUSSION

The effect of fatigue on the excitation/inhibition balance has been widely studied in young adults, with evidence pointing to an increase in corticospinal excitability and decrease in intracortical inhibition to sustain force output as task difficulty increases (Benwell et al. 2007b; Benwell et al. 2006a; Hunter et al. 2016a; Maruyama et al. 2006; Opie et al. 2020; Otieno et al. 2019; Otieno et al. 2021; Vucic et al. 2011). Despite the abundance of literature on the age-related differences in resting intracortical inhibitory measures (SICI and LICI) (Bhandari et al. 2016; Cirillo et al. 2010; Cirillo et al. 2011; McGinley et al. 2010; Oliviero et al. 2006; Opie and Semmler 2014a; Peinemann et al. 2001; Rogasch et al. 2009; Sale et al. 2016; Smith et al. 2009), very little has been done to identify the fatigue related changes in corticospinal excitability and inhibition with ageing. Furthermore, despite similarities in excitability/inhibitory modulation in M1 following exercise induced fatigue and motor skill performance, the impact of fatigue on motor skill performance remains largely understudied, particularly within the older age group. This is of great significance given that increased fatigability is a major contributor to the reduced ability to perform everyday tasks and, as such, lead an independent life in older adults. Within this thesis, I have attempted to identify any age-related differences in modulation of excitatory/inhibitory processes following single joint isometric exercise, and further investigated fatigue's impact on motor skill performance in young and older adults. I also investigated the possible clinical application of tDCS as an intervention to minimise fatigability and its subsequent effect on motor skill performance through the induction of homeostatic metaplasticity.

6.1. Age related difference in corticospinal excitability measures and motor skill performance with fatigue

The lack of age - related differences in corticospinal excitability, GABA mediated inhibition and motor performance with fatigue remains a common theme within the thesis, particularly when a similar amount of fatigue is induced in young and older adults (*see chapter*

three, four and five). However, this might be attributed to the age-range selected for the older cohort (60 – 70 years old). Data shows that skeletal muscle atrophy and neuromuscular degeneration increases with advanced age (>70 years) (Hunter et al. 2016b; Purves-Smith et al. 2014; Spendiff et al. 2016). It is this progressive decline in muscle function that exacerbates an increase in fatigability in both older males and females. Nevertheless, studies show that older adults aged 60 – 70 years old experience a lesser magnitude of fatigue compared to older adults aged 70 and above (Sundberg et al. 2018b). This might explain the lack of difference in corticospinal excitability and motor performance measures seen in the current thesis. Furthermore, the older cohort that opted to participate in these studies were not characterised by the decreased physical activity levels and sedentary behaviour commonly observed with ageing (Martin et al. 2014). Most older adults actually reported homogeneous physical activity levels to the young and produced greater force during maximum contractions. Therefore, whilst the older adults likely had some degree of a decline in type II fibres, increased physical activity might have minimised the age-related loss of muscle mass associated with fibre death (Goodpaster et al. 2008) and maintained upper limb tension (Trappe et al. 2003), contributing to the similarity in corticospinal excitability measures and motor performance between young and older adults (*see chapters three and four*). Research also shows that detriments in precision control, arm-hand steadiness, manual dexterity and wrist finger speed (Cheong et al. 2013) are more apparent in very old adults (80 years and above) thereby affecting performance in fine motor tasks. Recruitment of much older adults is therefore recommended for future work to accurately determine the impact of age-related neuromuscular degeneration on corticospinal measures and task performance with fatigue.

One other important consideration is the type of exercise performed. While an isometric single joint exercise was implemented in each chapter, older adults in fact experience less fatigue compared to the young (Yoon et al. 2013; Yoon et al. 2012). However, this is not

observed during dynamic exercise. Due to the age-related loss in type II muscle fibres, older adults experience increased fatigability when performing dynamic/whole body exercise compared to the young (Sundberg et al. 2018b; Yoon et al. 2013; Yoon et al. 2012). It is this increase in fatigability that greatly impacts their ability to perform everyday tasks given that most work requires dynamic activity. Therefore, as a next step, it would be beneficial to assess the age-related differences in corticospinal excitability and intracortical inhibition (SICI and LICI) during/after dynamic or whole-body exercise in order to identify any variation in neural mechanisms that may govern the observed difference in fatigability. Although some studies show a decline in paired-pulse TMS measures during whole body exercise in healthy young adults (Sidhu et al. 2013b), it is yet to be established in the older population. Nevertheless, this thesis provides novel evidence of similar modulatory processes between young and older adults when measuring corticospinal excitability during and after fatigue.

6.2. Impact of muscle and exercise type on age related changes in corticospinal excitability with fatigue

In chapters two and three, I investigated the impact of single joint fatiguing exercise on corticospinal excitability measures (SICI, LICI, SP and MEP) with divergent findings between studies (Otieno et al. 2021). While chapter two reported an age-related compensatory decline in LICI (Otieno et al. 2021), chapter three identified a similar decline in LICI post fatiguing exercise in both age groups (Otieno et al. 2022). However, younger adults experienced greater fatigue in chapter two while both age groups experienced a similar amount of fatigue in chapter three. One major contributor for this finding may be differences in the exercise model implemented. It is well known that older adults are characterised by decreased fatigability (mediated by an increase in proportion of type I fibres) (Andersen 2003; Lexell et al. 1983) during isometric single-joint exercise. Older adults require more time on average to approach a congruent amount of fatigue to their younger counterparts when performing an isometric single-joint exercise (Hunter et al. 2005; Yoon et al. 2013; Yoon et al. 2012). This is further

echoed in chapters two and three where a prolonged timed contraction (15 min) was not sufficient to elicit a similar amount of fatigue in both young and old. The implementation of a time to task failure exercise is necessary to obtain an equivalent magnitude of fatigue. Furthermore, chapters two and three also highlight the preference of a force contraction over an EMG contraction when designing suitable exercise models for age-related comparisons. These observations emphasise the importance of exercise model selection when investigating the impact of neuromuscular fatigue on corticospinal excitability measures between age groups.

One other important consideration for the discrepancy between studies is the muscle of interest i.e., FDI versus elbow flexor muscles. It is well established that differing muscle groups exhibit alternate neuromuscular properties. For example, hand muscles are characterised by a larger cortical representation within M1 relative to the neighbouring elbow flexor muscles. Furthermore, intrinsic hand muscles are known to receive greater input from monosynaptic corticospinal projections (de Noordhout et al. 1999) while upper limb muscles are postulated to receive greater input from small diameter corticospinal fibres and indirect inputs mediated by spinal interneurons (Colebatch et al. 1990). We see this reflected in LICI measures where studies show varied outcomes in different muscle groups. Opie and colleagues identified an age-related decline in LICI measured from the FDI muscle at baseline (Opie and Semmler 2014b) while McGinley and colleagues showed a contradictory increase in baseline LICI measured in the forearm muscles (McGinley et al. 2010). A similar age-related increase in baseline LICI was observed in older adults when measured in the biceps brachii (Otieno et al. 2022), contrary to the lack of difference seen at baseline in chapter two (FDI muscle) (Otieno et al. 2021). Fatiguing studies also show disparities between different muscle groups. For example, Benwell and colleagues reported a decline in LICI post fatiguing exercise (Benwell et al. 2007b) following single joint isometric exercise of the FDI muscle while McNeil and

colleagues reported an increase in LICI when measured in the elbow flexor muscles (McNeil et al. 2011b; McNeil et al. 2009). Taken together, these findings highlight the influence of the varied muscle/muscle groups studied on corticospinal excitability measures with fatigue. Investigation of different muscle groups is necessary for future work when attempting to identify a holistic impact of fatigue on corticospinal excitability in older adults.

6.3. SP versus LICI

In order to obtain a holistic view of the impact of fatigue on $GABA_B$ mediated activity, chapter three assessed the impact of fatigue on SP and LICI modulation following single-joint fatiguing exercise in young and older adults (Otieno et al. 2022). This is because previous pharmacological evidence shows that both SP and LICI reflect $GABA_B$ mediated pathways (Chen et al. 1999; McDonnell et al. 2006) . However, differing outcomes between the two measures brings to question whether SP and LICI truly reflect similar cortical mechanisms. While previous studies report an increase in SP duration (Benwell et al. 2007b; Brownstein et al. 2020; McKay et al. 1996; Mileva et al. 2012; Taylor et al. 2000; Yoon et al. 2013; Yoon et al. 2012), no change was seen during or post fatiguing exercise in both age groups in chapter three, despite similarities in the exercise model selected (single joint submaximal isometric contraction held to task failure) and muscle group (elbow flexors). This contradicts the decline in LICI observed post fatiguing exercise (*see chapters one and two*) demonstrating the divergent pathways involved in both measures. It was previously argued that the spinal influence on the earlier part of SP (Inghilleri et al. 1993) was the major contributor for the discrepancy between SP and LICI, since LICI was thought to be largely mediated by cortical mechanisms (Werhahn et al. 1999)). However, evidence now shows that both LICI (measured at ISI = 100 ms) and SP are influenced by changes in spinal excitability (McNeil et al. 2009). This brings to question once again if SP and LICI reflect similar cortical inhibitory mechanisms. An attempt has been made to address this by investigating the relationship

between GABA concentration and SP/LICI measures through the use of magnetic resonance spectroscopy (MRS) (Tremblay et al. 2013b). Interestingly, no direct relationship between GABA concentration levels and LICI/SP was evident. In contrast, SP had a direct correlation to glutamate levels but not GABA. Although a close relationship exists between glutamate and GABAergic activity within M1 (Tremblay et al. 2013b), these findings highlight the need for caution when linking changes in SP and LICI to $GABA_B$ mediated activity. Nonetheless, it should also be noted that TMS protocols reflect GABA receptor activity while MRS mostly reflects extracellular and intracellular GABA concentrations (Maddock and Buonocore 2012). Therefore, further assessment of the neural underpinnings of SP and LICI is required to obtain an accurate evaluation of the impact of fatigue on $GABA_B$ mediated mechanisms.

6.4. *TDCS as a potential clinical intervention for reduced fatigability*

A current concern exists within the literature regarding the clinical application of tDCS, given the well-known variability (within-subjects and between studies) in physiological and behavioural outcomes. We see this echoed in chapter five where no beneficial effects of tDCS or cathodal primed tDCS application were observed in young and older adults during fatigability and task performance measurements, despite previous work alluding to an improvement in both behavioural measures (Abdelmoula et al. 2016; Cogiamanian et al. 2007; Okano et al. 2015; Oki et al. 2016; Williams et al. 2013). Therefore, optimisation of tDCS as a potential clinical tool remains a primary concern within the field. Chapter five shows that beyond simple stimulation parameters and history of synaptic activation, other factors must be taken into consideration when addressing the variability of tDCS among studies, such as inter-individual variations in brain anatomy (Datta et al. 2010; Li et al. 2015). Anatomical differences, like skull and cerebrospinal thickness, sulcal depth and cortical folding can influence the spread and intensity of the current reaching the brain following direct stimulation (Datta et al. 2009; Laakso et al. 2015; Rush and Driscoll 1968; Seibt et al. 2015). For example,

skull conductivity strongly affects the amount and direction of current that penetrates into the brain (Bestmann and Ward 2017; Datta et al. 2010; Opitz et al. 2015). This variation in electric field (E-field) intensity at a target region has been estimated to be around 100% amongst individuals (Laakso et al. 2015). Seeing as the E-field intensity directly affects the physiological and behavioural effects of stimulation (Indahlastari et al. 2021; Kim et al. 2014), this substantial variation alone could explain the discrepancy in outcomes across individuals and studies. Therefore, the conventional application of a fixed intensity of tDCS stimulation (1 – 2 mA) ignores how much current reaches the brain in each individual leading to differences in E-fields within the cortex (Bestmann and Ward 2017; Feng et al. 2018). One study shows that application of tDCS with fixed dose (1 & 2 mA) increases variability of E-field intensity by more than 100% across individuals whereas individualised dose-control application ensured the same E-field intensity was delivered to M1 in all individuals (Evans et al. 2020). Consequently, future work could implement the use of individualised dose-control application to minimise variable outcomes between studies (Indahlastari et al. 2021; Kim et al. 2014). Age-related atrophy of the brain is another important consideration when assessing age-related effects of tDCS. Loss of brain tissue can lead to increased distance between the skull and brain causing an increase in the proportion of CSF (Lockhart and DeCarli 2014). Given the high conductivity of CSF, this can be problematic because it may cause shunting of current and add to the ever-increasing variability of response, particularly amongst older individuals.

One other important consideration is sample size. Evans and colleagues showed that variability in E-field intensity increases with small sample size numbers (N = 15 – 30) following a fixed 1 mA dose and decreases significantly with larger sample sizes (N = 50) (Evans et al. 2020), thereby highlighting the need for larger sample size in future work. Furthermore, variations in parameters such as duration of stimulation and amount of time between stimulation doses also remains a major concern. Chapter five highlights this

discrepancy as studies that applied a longer duration of tDCS (~20 min) observed a significant increase in TTF (Oki et al. 2016; Williams et al. 2013) while no improvement was identified when a briefer duration of tDCS was implemented (~15 min) in both age groups. Differing outcomes are also observed when comparing anodal tDCS effects at different ISIs between priming and active stimulation. While Fujiyama saw an improvement in skill acquisition in young and older adults with no delay between priming and active tDCS (Fujiyama et al. 2017), chapter five found no effect when an ISI of 15 min was applied in both age groups. Monte-Silva and colleagues also observed similar contradictions at different ISIs between stimulations (Monte-Silva et al. 2010). Application of two identical periods of cathodal stimulation (9 min application of cathodal tDCS at 1 mA) with no delay between tDCS application (ISI = 0 min) prolonged the aftereffects from 60 to 90 min after tDCS. However, a prolonged delay of 20 min between cathodal tDCS treatments prolonged tDCS-induced after-effects for ≤120 min (Monte-Silva et al. 2010) contrary to the lack of effect seen in chapter five following an ISI of 15 min. Nevertheless, it should be noted that Monte-Silva applied tDCS at rest not during contraction. This further highlights the need for consensus regarding the appropriate amount of delay between priming and active dosages, particularly during contraction, to elicit improvements in behavioural outcomes.

Finally, the impact of sex differences on excitability measures during fatigue remains an important consideration with neuromodulation. Ansdell and colleagues show changeable SICI measures across a eumenorrheic menstrual cycle due to fluctuating hormones (Ansdell et al. 2019b) given that oestrogen is known to have excitatory effects while progesterone has an inhibitory influence (Smith et al. 1989; Smith and Woolley 2004). Therefore, caution is needed when utilising neuromodulatory techniques in females since the capacity for neuromodulation may be increased in young eumenorrheic females at certain times of the menstrual cycle (Ansdell et al. 2019b). In line with this, there also needs to be consideration for post-

menopausal older females who are/are not using hormone replacement therapy. In any case menstrual cycle in young female participants should be controlled for in future studies to obtain a clearer image of the impact of neuromodulation on fatigue.

As it stands, tDCS is considered a potential neurostimulation tool particularly in the clinical setting due to several factors. It is relatively inexpensive, safe (no seizures reported so far) and somewhat easy to use, making self-administration possible. However, interindividual variability across studies remains relatively high with ~50% of participants not responding to tDCS (Puri et al. 2015). Furthermore, there is currently no widely used method of individualised dosing available. If tDCS is to remain a viable method of treatment, particularly in fatigue prevention, development of an individualised model of dosing and consensus regarding tDCS parameters like duration of stimulation and delay is required.

7. CONCLUDING REMARKS

This thesis provides novel evidence regarding similar modulation of corticospinal excitability and intracortical inhibitory processes with fatigue in young and older adults. Studies also showed a similar change in task performance following single joint fatiguing exercise in both age groups, which suggests a retention in the ability to maintain motor skill performance with ageing. Interestingly, anodal tDCS application induced a similar suppression in corticospinal excitability and greater decline in $GABA_B$ mediated inhibition post fatiguing exercise in young and older adults. However, cathodal primed anodal tDCS elicited no change in excitability or inhibitory measures. Furthermore, tDCS showed no beneficial effects on fatigability or task performance in both age groups. This suggests no added benefit of cathodal priming on corticospinal excitability, fatigability, or motor performance. This also highlights the complexity of the networks and pathways involved in fatigue development and motor performance outside of the conventional excitation/inhibition balance in M1. Greater understanding of these networks will promote further development of interventions that can be

used to minimise fatigability and its subsequent negative effect on everyday living, particularly in older adults, in order to improve quality of life and encourage independent living.

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