INTERVENTIONS TO MANIPULATE NEUROPLASTICITY IN AGEING



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Thesis abstract

Ageing is associated with a decline in brain and skeletal muscle function, which leads to loss of independence and a reduction in general health status. Neuroplasticity refers to the capacity of the nervous system to change in response to pathological (injury or disease) or physiological events (learning from experience). Age-related decline in neuroplasticity limits motor function improvements and learning, which has an impact on functional recovery following brain injury in older adults. Evidence suggests that exercise and motor training can enhance brain function and minimise age-associated decline in the neuromusculoskeletal system. Therefore, the experimental studies included in this thesis aimed to evaluate neuroplasticity, including alterations in the central nervous system and neuromuscular system in healthy older adults through various interventions.

Chapter 2 (conducted at the University of Nottingham) examined neuromuscular function and the cross-limb transfer effect in older adults, where these changes were assessed following an acute unilateral resistance exercise. This study provides insights into functional properties (force production) and motor unit characteristics using high-density surface electromyography (HDsEMG) and intramuscular electromyography (iEMG). This study demonstrated that acute unilateral resistance exercise leads to a bilateral reduction in force production in healthy older adults, indicating a cross-limb transfer effect. The motor unit level adaptations were evident only in the exercised limb, and the mechanisms underlying the functional changes in the non-exercised limb remain uncertain.

Chapter 3 (conducted at the University of Adelaide) examined 'age-related changes in brain and motor function', during which the impact of a novel non-invasive brain

stimulation technique (i.e., theta-gamma transcranial alternating current stimulation; theta-gamma tACS) on motor performance was evaluated and compared between young and older adults. The study utilised a unilateral ballistic thumb task performed concurrently with stimulation applied to the contralateral primary motor cortex. Both young and older adults showed improvement in motor performance with theta-gamma tACS, with effects lasting up to 60 minutes. Furthermore, corticospinal excitability (assessed using transcranial magnetic stimulation; TMS) was greater following theta-gamma tACS compared to sham stimulation. These findings suggest that theta-gamma tACS may offer a promising approach to counteract some aspects of motor deficits associated with ageing.

The final experimental chapter (Chapter 4, conducted at the University of Adelaide) evaluated 'ageing, neuromuscular function and cross-limb transfer effect', integrating these approaches by assessing the potential of tACS to influence ballistic motor performance and cross-limb transfer in healthy older adults. Three experimental groups received stimulation to primary motor cortex (theta-gamma tACS, theta tACS, or sham tACS) during performance of a unilateral ballistic thumb task. The study results indicated that ballistic motor performance improved in all tACS conditions, with the greatest effect following theta tACS for both hands. Training-induced corticospinal excitability was increased for theta tACS only, while there was a correlation between the trained and untrained gains in motor performance that was significant for theta-gamma tACS only. These findings suggest tACS has the potential to facilitate ballistic motor performance and promote cross-limb transfer effect in older adults.

Overall, this thesis provides novel insights into techniques that could be used to mitigate some aspects of age-related neuromuscular deficits and further supports the understanding of neurophysiological modifications associated with ageing and motor performance following different interventions. These findings have potential implications for rehabilitation following neurological disorders (e.g., stroke) and orthopaedic conditions (e.g., single-limb fractures), where non-invasive brain stimulation or training of the unaffected limb can be used to facilitate motor performance gains of the affected limb. This thesis also emphasises the necessity for deeper insights into the neural mechanisms underlying cross-limb transfer of resistance exercise and ballistic motor performance, as well as the requirement to develop more effective non-invasive brain stimulation interventions to improve motor performance in older adults.

Thesis Supervisory Panel: A/Prof John Semmler, A/Prof Mathew Piasecki, Dr George Opie, and Professor Philip Atherton

Thesis Examination Panel: A/Prof Martin Sale, Dr Justin Andrushko, and Dr Katie Robinson

Thesis submission date: 25th September 2025

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List of publications

This thesis contains a combination of published peer-reviewed journal articles and text written in manuscript form, under the Academic Program Rules of the University of Adelaide, Australia, and the University of Nottingham, United Kingdom.

Peer-reviewed publications arising from this thesis

- **Gamage, N.N.,** Altheyab, A., Guo, Y., Phillips, B.E., Opie, G.M., Semmler, J.G., Atherton, P. and Piasecki, M., 2025. Bilateral neuromuscular adaptation to acute unilateral resistance exercise in healthy older adults. *GeroScience*, pp.1-17. https://doi.org/10.1007/s11357-025-01693-8
- **Gamage, N.N.,** Liao, W.Y., Hand, B.J., Atherton, P.J., Piasecki, M., Opie, G.M. and Semmler, J.G., 2025. Theta-gamma transcranial alternating current stimulation enhances ballistic motor performance in healthy young and older adults. *Neurobiology of Aging*. 152. pp.1-12. https://doi.org/10.1016/j.neurobiolaging.2025.04.010
- **Gamage, N.N**., Liao, W.Y., Atherton, P.J., Piasecki, M., Opie, G.M. and Semmler, J.G., 2025. Transcranial alternating current stimulation improves ballistic motor performance in trained and untrained limbs of healthy older adults. *GeroScience*, pp.1-20. https://doi.org/10.1007/s11357-025-02064-z

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- Guo, Y., Jones, E.J., Smart, T.F., Altheyab, A., **Gamage, N.**, Stashuk, D.W., Piasecki, J., Phillips, B.E., Atherton, P.J. and Piasecki, M., 2023. Sex disparities in age-related neuromuscular decline: unveiling female susceptibility from early to late elderly. *bioRxiv*, pp.2023-06. https://doi.org/10.1101/2023.06.13.544761
- Guo, Y., Jones, E.J., Altheyab, A., **Gamage, N.N.**, Phillips, B.E., Atherton, P.J. and Piasecki, M., 2025. Task Complexity and Limb Dominance Modulate the Effects of Ageing on Neuromuscular Function. bioRxiv, pp.2025-09. https://doi.org/10.1101/2025.09.01.673460

For abstracts and presentations arising from this thesis, refer to the appendix.

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- **1st Prize- Academic Excellence in Postgraduate Research Award 2025** International Student Awards 2025, organised by the 'StudyAdelaide' ($ 500)
  Links: Featured in the University of Adelaide- News, International Student Award winners 2025- StudyAdelaide Website

2024

- **1st Prize- Academic Excellence in Postgraduate Research Award 2024** International Student Awards 2024, organised by the 'StudyAdelaide' ($ 500)
  Links: Featured in the University of Adelaide- News, International Student Award winners 2024- StudyAdelaide Website

- **1st Prize- Three Minute Thesis (3 MT) Competition (Joint PhD)** Adelaide-Nottingham Alliance ($ 200)
  Link: Featured in the University of Nottingham- Twitter

2023

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Dedication

Firstly, to my beloved father,

Rear Admiral (Rtd) Muditha M. H. Gamage (Late)

Losing you unexpectedly in the 2nd year of my PhD was the hardest moment of my life. I was so close to giving up, but your last words to me,

'Finish your PhD with flying colours', became my guiding light.

You were and are my greatest strength, my biggest supporter, and the quiet voice behind all my achievements.

I will continue to carry your pride, discipline, and kindness with me always.

I know you were always proud of me,

But you would be the proudest today if you were here.

This thesis is for you, my loving Thaththa!

&

Secondly, to my first child,

Who is growing quietly inside me

Thank you for making the final months of my PhD extra memorable!

Declaration

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

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Signed: Nishadi Gamage

Date: 25/09/2025

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List of Abbreviations

1 RM	one repetition maximum
ACh	Acetylcholine
AMT	active motor threshold
BMI	body mass index
CE	cross-education
CI	confidence interval
CoV	coefficient of variation
CSA	cross-sectional area
DQEMG	decomposition-based quantitative electromyography
EHI	Edinburgh handedness inventory
EMG	electromyography
FR	firing rate
FS	force steadiness
GLMM	generalised linear mixed models
HDsEMG	high-density surface electromyography
HD tACS	high-definition transcranial alternating current stimulation
iEMG	intramuscular electromyography
IPAQ	International physical activity questionnaire
KE	knee extensor
M1	primary motor cortex
MEP	motor-evoked potential
MEQr	morningness-eveningness questionnaire
MoCA	Montreal cognitive assessment

MRI	magnetic resonance imaging
MU	motor unit
MUFR	motor unit firing rate
MUP	motor unit potential
MVC	maximal voluntary contraction
NF	near fibre
NIBS	non-invasive brain stimulation
NMJ	neuromuscular junction
PAC	phase-amplitude coupling
PTP	post-training performance
RE	resistance exercises
RMT	resting motor threshold
RPE	rate of perceived exertion
SD	standard deviation
SICI	short-interval intracortical inhibition
tACS	transcranial alternating current stimulation
TG	theta-gamma
TMS	transcranial magnetic stimulation
VAS	visual analogue scale
VL	vastus lateralis

CHAPTER 1: BACKGROUND AND LITERATURE REVIEW

1.1.General introduction and thesis overview

Advances in medical care and lifestyle interventions have led to an escalation in the percentage of older people (Rechel et al., 2013), with life expectancy estimated to increase by 6.9 years for women (to 89.0 years) and 8.5 years for men (84.5 years) by the year 2060 (Francis et al., 2017). Many factors, including reduced mortality rates, increased longevity, and decreased fertility or childbearing, account for the growing ageing population (see Harper, 2014 for review). This global ageing trend has implications for societies, economies, and has a greater strain on healthcare systems (Phillips, 2007). Furthermore, this also raises concerns for chronic disease management, healthcare service delivery, and lifestyle modifications that demand innovative intervention strategies.

Ageing is accompanied by numerous structural, functional, and biochemical transformations that occur in the neuromuscular system, resulting in a decline in cognitive, motor performance, and other brain functions (see Seidler et al., 2010 for review). These alterations can negatively impact the functional independence and overall quality of life in older individuals. The capacity of the brain to change (i.e., plasticity) also declines with advancing age (Semmler et al., 2021), which affects motor function in older adults (Ward, 2006, Zapparoli et al., 2022), along with a diminished ability to relearn motor skills following neurological injury (Laaksonen and Ward, 2022). Furthermore, age-related reduction in skeletal muscle function is demonstrated to result in detrimental outcomes such as mobility disorders (McPhee et al., 2013), frailty (McPhee et al., 2016), increased fall rates (Gale et al., 2016) and an overall decline in general health status (see Wilkinson et al., 2018 for review). Importantly, evidence suggests that the structure and function of the

neuromusculoskeletal system can be improved through various interventions targeted to manipulate brain and neuromuscular function, with such changes occurring through mechanisms related to nervous system plasticity.

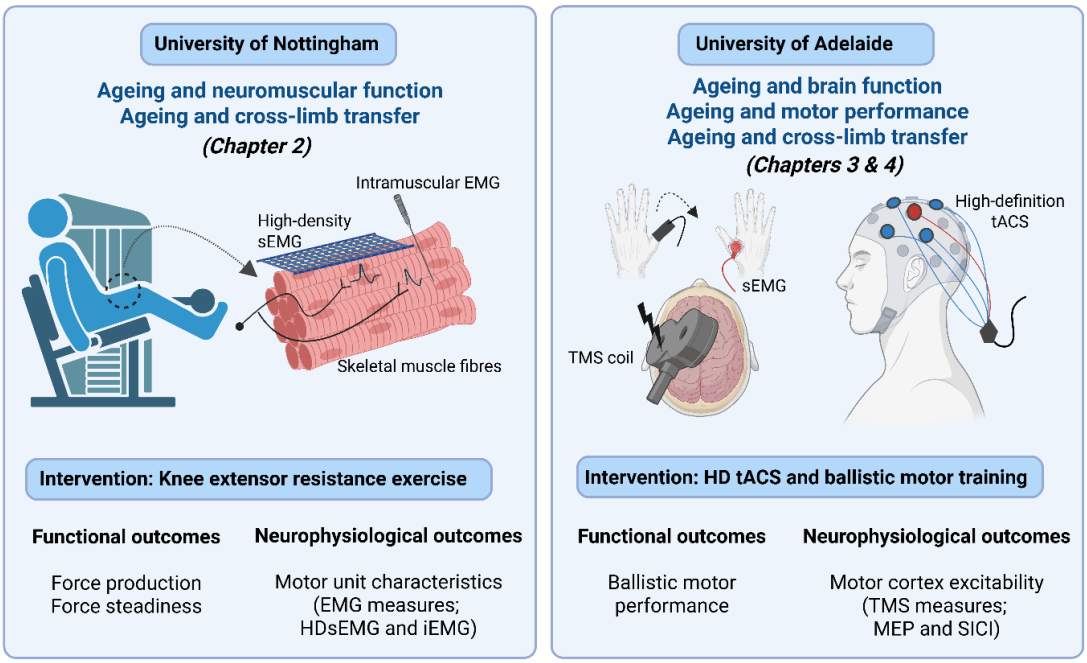


Figure 1.1. Thesis overview and outline of human experimental studies. *Abbreviations: EMG, electromyography; HDsEMG, High-density surface electromyography; iEMG, intramuscular electromyography; sEMG, surface electromyography; TMS, transcranial magnetic stimulation; HD tACS, high-definition transcranial alternating current stimulation; MEP, motor-evoked potential; SICI, short-interval intracortical inhibition. Figure created using BioRender.com.*

The three human experimental studies in this thesis aim to advance the understanding of the cortical and neuromuscular mechanisms underlying various interventions that enhance neuroplasticity in healthy older adults (Figure 1.1). The effects of ageing on cross-limb transfer, neuromuscular performance, and brain function will be explored using various techniques. One experimental study (Chapter 2) utilised electromyography (EMG) techniques to assess the electrical activity of muscles. A combination of minimally invasive intramuscular EMG and high-density surface

EMG was used to comprehensively assess motor units at different depths to assess motor unit adaptation following an acute resistance exercise intervention. The other two experimental studies (Chapters 3 and 4) used non-invasive brain stimulation (NIBS) to assess and modify human brain function. A combination of NIBS techniques, including TMS and transcranial alternating current stimulation (tACS), was employed to assess and induce changes in neuroplasticity in older adults. As part of the Adelaide-Nottingham Alliance, the experiment in Chapter 2 was conducted at the University of Nottingham, United Kingdom, and the experiments in Chapters 3 and 4 were conducted at the University of Adelaide, Australia.

1.2.Neuromuscular physiology and electromyography (EMG)

1.2.1.The neural basis of human movement control

Multiple regions of the nervous system are associated with movement control. The human motor cortex is located at the posterior of the frontal lobe, and it comprises a distinct number of cortical regions, including primary motor cortex (M1; Brodmann's area 4), supplementary motor area (SMA), and premotor area (PMA). M1 has the potential for structural and functional reorganisation, which is one of the focuses of this thesis. However, the adult brain's capacity for cortical reorganisation remains debated (Makin and Krakauer, 2023, Schone et al., 2025). While several brain areas are involved in muscular function, the M1 is widely recognised due to its predominant role in the execution of movements, and is considered a main target for NIBS and rehabilitative interventions (Sanes and Donoghue, 2000). The PMA and M1 areas are densely populated with excitatory and inhibitory neurons, and these neurons not only connect to other cortical regions but also form a long axonal connection with the lower motor neurons in the ventral horn of the spinal cord (Kandel et al., 2000). The SMA and PMA are primarily involved in planning and organising movements, and these areas send signals to M1 for motor execution, with M1 function being assessed with TMS in Chapters 3 and 4.

Motor neurons transmit signals from the central nervous system to muscles (Figure 1.2.A). The cell body of a motor neuron is located in the motor cortex, brainstem, or spinal cord, with its axon projecting onto muscles. These motor neurons are generally classified as upper and lower motor neurons. The upper motor neurons integrate excitatory and inhibitory signals from the cortex through descending pathways and synapse on interneurons and lower motor neurons located in the ventral horn of the

spinal cord. The activation/discharge of a motor neuron generates an action potential (Figure 1.2.B) that propagates along the axon towards distal axonal branches and neuromuscular junctions (NMJ) (Figure 1.2.C). When an action potential reaches the NMJ, it evokes the release of the neurotransmitter acetylcholine, which depolarises the muscle fibre membrane and generates a muscle fibre action potential (MFAP), of which multiple sum to generate a MU potential (MUP), and a coordinated muscle contraction (Heckman and Enoka, 2004).

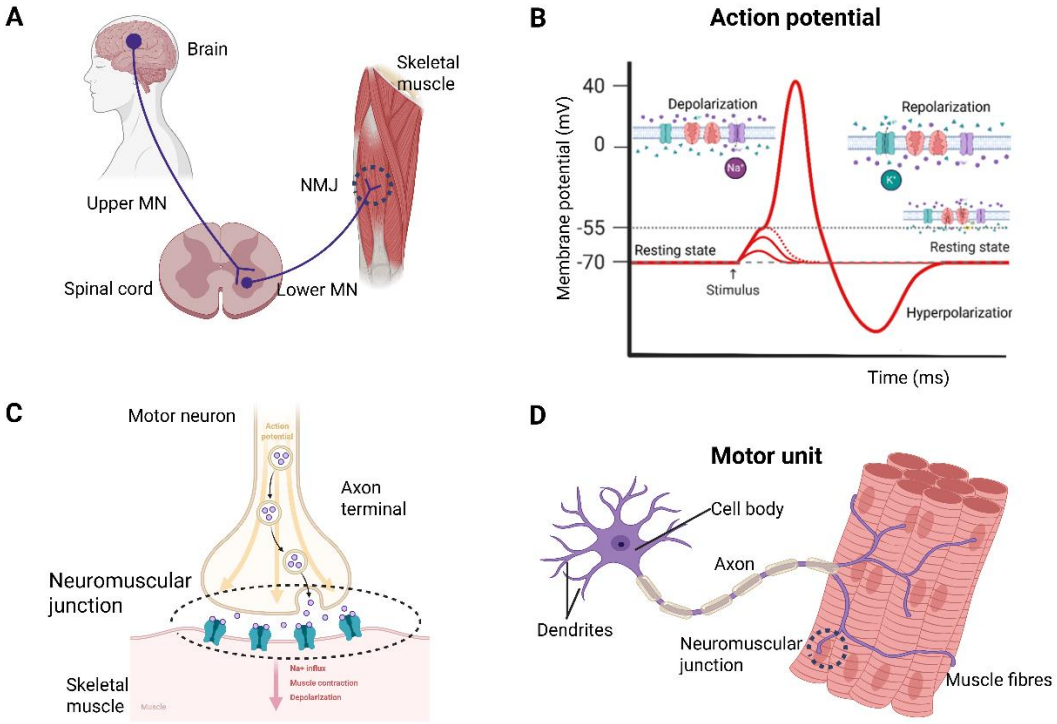


Figure 1.2. Basics of neuromuscular physiology. Neural input to the skeletal muscles (A); mechanism of an action potential (B); neuromuscular junction (NMJ) (C); and components of a motor unit (MU) (D). *Abbreviations: MN, motor neuron; NMJ, neuromuscular junction. Figure created using BioRender.com.*

The main functions of the human motor system include the generation of force and power, movement production, and posture control, all of which contribute to functional independence. The components of the muscular system (muscle units) and

the nervous system (motor neurons) are engaged in the control of movement, and both share a basic functional unit termed the motor unit (MU) (Heckman and Enoka, 2004). The human MU is considered the final component of the neuromuscular system, which consists of a motor neuron in the ventral horn of the spinal cord, its axon, and skeletal muscle fibres that the axon innervates (Sherrington, 1925) (Figure 1.2.D). In a MU, the number of muscle fibres can range from as few as ten to several thousand, depending on the size of the muscle (Heckman and Enoka, 2004). More details about MUs will be described in the following sections.

1.2.2.Rate coding and motor unit recruitment

The force exerted by a muscle during a voluntary contraction is governed by two processes: (i) the rate at which each motor neuron discharges action potentials (rate coding or firing rate), and (ii) the number of motor units activated (MU recruitment) (Enoka and Duchateau, 2017). The MUs are recruited from small to large, using an orderly method termed *Henneman's size principle* (Henneman, 1957). This feature has a functionally important role during muscle contractions, which allows graded application of force, facilitating both fine motor manipulation and high force application. This is also important for efficient energy use. The smaller, slower, more fatigue-resistant MUs are recruited during low-intensity contractions. During higher contraction intensities, in addition to smaller MUs, larger, faster, fatigable MUs are also recruited. Numerous physiological and environmental factors, including age, muscle fatigue, contraction intensity, injury status, exercise training and temperature, could affect MU behaviour (Ota et al., 2025).

1.2.3.Motor unit activity recording using electromyography

Recording the electrical activity of muscles provides information on how the nervous system generates and controls movement, and how this process may change in certain circumstances, such as ageing. This can be achieved using electromyography (EMG; Figure 1.3), which uses electrodes to detect MU potentials (MUPs). The MUPs are the summation of muscle fibre potentials (MFPs) generated by propagating muscle fibre action potentials (Piasecki et al., 2021b). The EMG electrodes can be positioned on the skin to record electrical activity from a larger portion of muscle (surface or sEMG) or can be inserted into the muscle to record action potentials within a relatively smaller volume (intramuscular or iEMG). A combination of both iEMG and sEMG techniques allows the assessment of MUs at different depths, providing invaluable information on MU adaptations following exercise interventions. These methods will be described in the following sections.

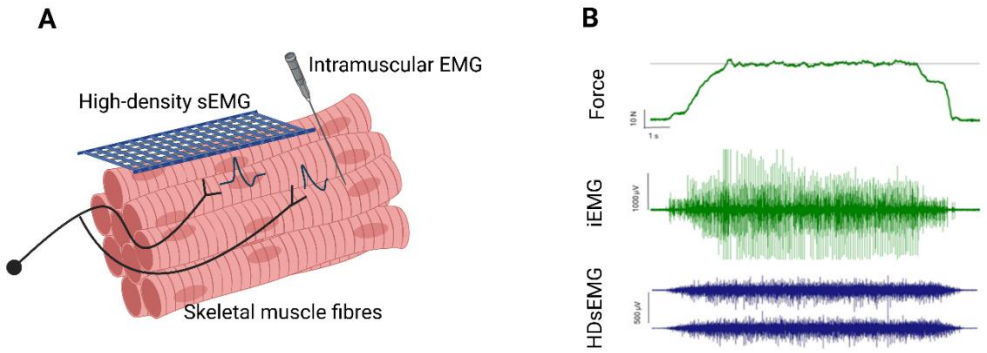


Figure 1.3. Intramuscular and high-density surface electromyography. Schematic of electrode positioning over a motor point identified using low-intensity electrical stimulation of the muscle of interest (e.g., Vastus lateralis for Chapter 2) (A); Raw data recordings showing examples of a force trace (upper) with corresponding iEMG trace (middle) and a subset of 2 HDsEMG channels (lower) obtained during a sustained isometric knee extension at 25% of MVC, which were undertaken in Chapter

2 (B). *Abbreviations: iEMG, intramuscular electromyography; HDsEMG, high-density surface electromyography*. *Figure created using BioRender.com*.

1.2.3.1.Intramuscular electromyography (iEMG)

The iEMG technique is used to record individual MUPs and is generally undertaken using concentric needle electrodes (Figure 1.4.A/B) or fine wire electrodes (Stashuk, 1999a). Compared to concentric needle electrodes, fine wire electrodes may minimise participant discomfort, but they cannot be accurately repositioned between the contractions. In contrast, a concentric needle, being a firm structure, can be adjusted between and during contractions at varying muscle depths. The iEMG techniques allow for accurate sampling of MUs from the deeper regions of the muscles or deeper muscles, specifically for individuals with greater subcutaneous tissue (e.g., thigh muscles of obese or elderly) (Piasecki and Stashuk, 2023). Sampling MUs at different depths of a muscle can be achieved through needle repositioning (Figure 1.4.C) by rotating the bevel 180° and slightly retracting (~5 mm) the needle (Jones et al., 2021), which was conducted during experiments in Chapter 2.

The iEMG procedures also enable near-fibre (NF) recordings representing the contributions of the fibres closest to the recording electrode (Piasecki et al., 2021b). A near-fibre MUP (NFM) is the acceleration of a MUP and is calculated by high-pass filtering a MUP template waveform (Piasecki et al., 2021a). Fibres that are close to the needle detection surface mainly contribute to NFM (Piasecki et al., 2021a, Stashuk, 1999b). Although NFM parameters can provide similar details as conventional MUP analysis, NF parameters have the potential to provide more detailed information related to NMJ transmission variability, as they are dependent on the activity of MU fibres closer to the needle detection surface (Allen et al., 2015).

However, there are several disadvantages of iEMG. First, due to possible participant discomfort, only low to moderate forces can be assessed. Second, iEMG electrodes cannot be repositioned at the exact location across experimental sessions. Third, only a small number of MUs can be reliably identified during voluntary contractions (Martinez-Valdes and Negro, 2023). Additionally, iEMG signal decomposition and analyses could be time-consuming (see chapter 2 for details of iEMG acquisition and analysis).

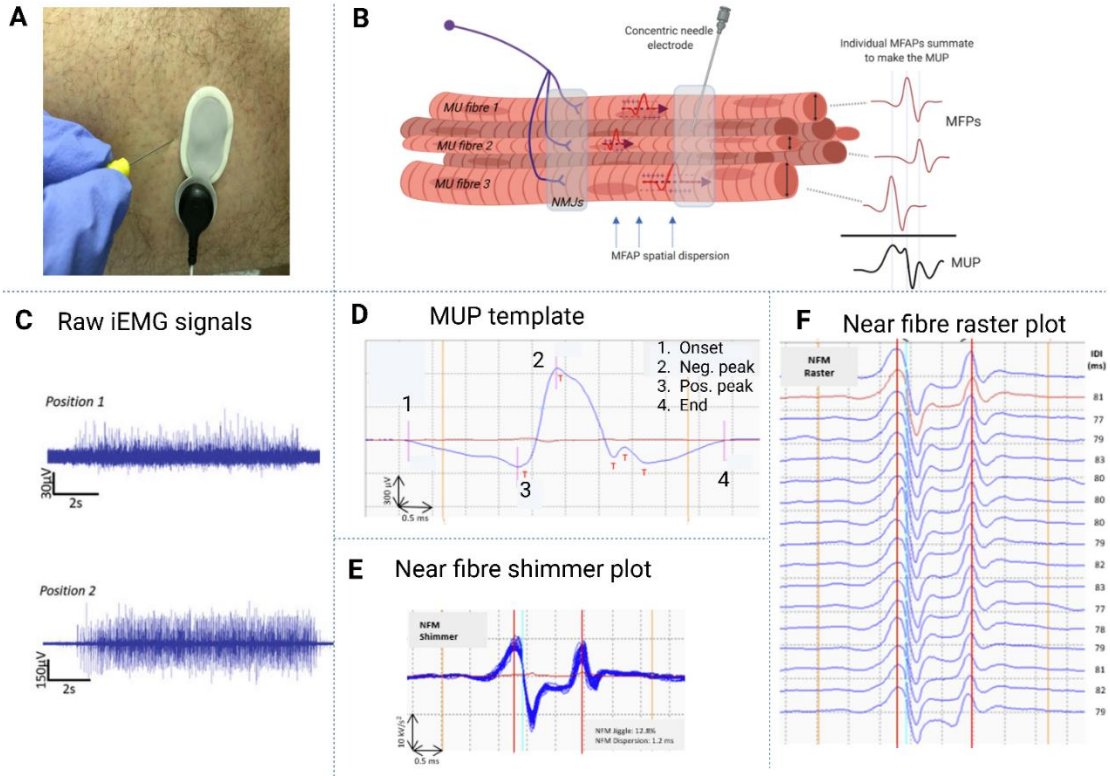


Figure 1.4. Intramuscular electromyography (iEMG). Schematic of intramuscular electrode (concentric needle) insertion into a motor point of vastus lateralis muscle (A) and schematic representation of a motor unit potential (MUP) with contributions from three MU fibres (B). Muscle fibre action potentials (MFAP) commence at the neuromuscular junction and propagate along the muscle fibres. Corresponding muscle fibre potentials (MFPs) may be distinctly detected by the concentric needle electrode due to the spatial dispersion and variability in detection time of MFPs from MU fibres 1, 2, and 3. This variability can occur due to variable NMJ locations, transmission times, and MFAP conduction times, influencing the final MUP shape. Raw iEMG

recordings (C) obtained during two knee extensor voluntary contractions at 10% of maximal voluntary contraction. Needle position 1 shows visible spikes with a poor signal-to-noise ratio, while needle position 2 (obtained with a different depth and direction that happen to be closer to the signal origin) shows improved signal-to-noise ratio resulting in more visible spikes. An individual motor unit potential (MUP) template (D) and corresponding near fibre MUP (NFM) shimmer (E) and raster (F) plots. The MUP template shows the onset (1), negative peak (2), positive peak (3), and end (4) of the MUP. The corresponding NFM has two distinct peaks, shown as a raster plot of consecutive observations and overlaid as a shimmer plot. *Abbreviations: MUP, motor unit potential; MU, motor unit; MFAP, muscle fibre action potential; NMJ, neuromuscular junction; MFP, muscle fibre potential; iEMG, intramuscular electromyography. Figure panels B and C adapted from Piasecki and Stashuk (2023) and recreated using BioRender.com.*

1.2.3.2.Surface electromyography (sEMG)

In contrast to iEMG, sEMG is a noninvasive technique that uses electrodes attached to the skin to record electrical activity associated with neuromuscular activation. This technique can be further divided into conventional and high-density (HDsEMG) variants. Conventional sEMG includes monopolar sEMG (i.e., single recording electrode and a reference electrode) and bipolar sEMG (i.e., two recording electrodes and a reference electrode). Monopolar recording preserves all information from EMG signal, but is very sensitive to electromagnetic noise due to the lack of common mode rejection, whereas bipolar electrode arrangements have the potential to eliminate electromagnetic noise that interferes with the EMG signal (Beck et al., 2007, Mohr et al., 2018).

In contrast, HDsEMG involves tens or hundreds of electrodes applied in an array over a large section of muscle, or across multiple muscles concurrently (Martinez-Valdes et al., 2016). There are different sizes and arrangements of HDsEMG electrodes, and

the type of HDsEMG electrode used depends on the muscle of interest. For example, smaller grids are used for hand muscles, while larger grids are commonly used for lower limb muscles. A 64-channel, 5 × 13 grid (Figure 1.5.A) was used to assess lower limb muscles in Chapter 2 (see Chapter 2 for details of HDsEMG methods).

Multi-electrode HDsEMG offers a wide detection area and accounts for the spatial and temporal characteristics of the signal, enabling comprehensive assessment compared to conventional sEMG (Holobar et al., 2009, Martinez-Valdes and Negro, 2023). Additionally, HDsEMG enables the tracking of the same populations of MUs following an intervention, which can be used to evaluate changes in MU plasticity and MU firing characteristics (Figure 1.5.E), such as MU recruitment/de-recruitment thresholds, mean firing rate, firing rate variability, and firing rate at recruitment/de-recruitment (Martinez-Valdes and Negro, 2023). However, sampling deeper MUs with HDsEMG is unfeasible due to signal attenuation caused by the interference from subcutaneous tissue. Therefore, I have used both iEMG and HDsEMG in Chapter 2 to assess MUs of the vastus lateralis muscle before and after an exercise intervention, assessing MUs at different depths (using iEMG) and tracking MUs from pre- to post-interventions (using HDsEMG).

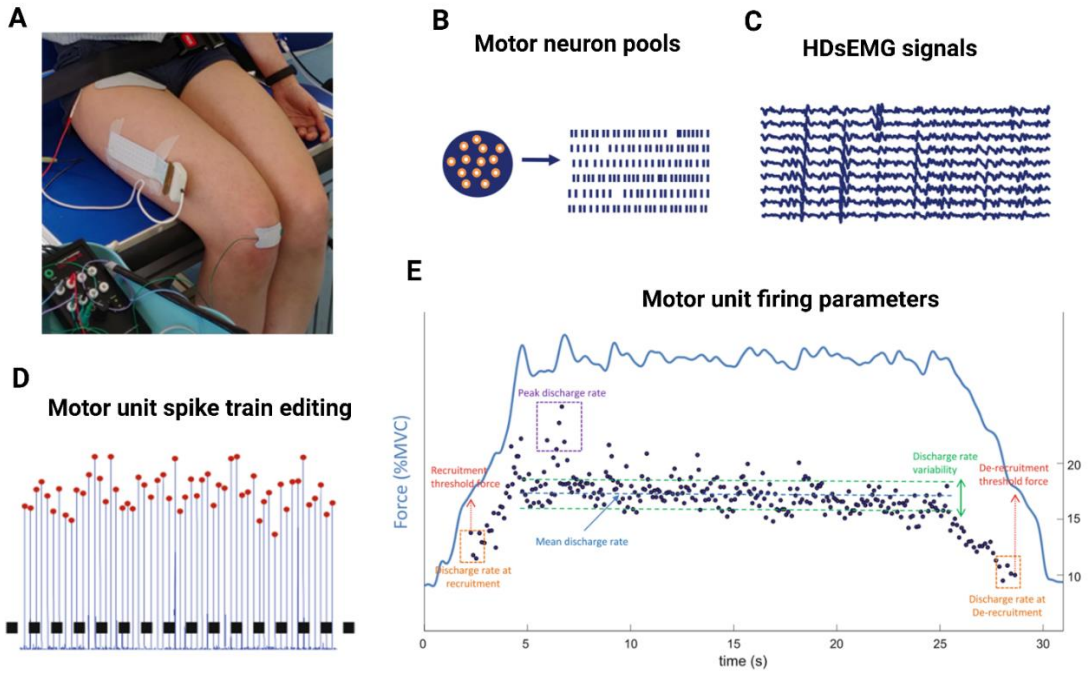


Figure 1.5. High-density surface electromyography (HDsEMG). Schematic indicating 64 channel HDsEMG electrode positioning over vastus lateralis muscle (A), Motor neuron pools generate multiple action potentials (B), raw HDsEMG recording (C), HDsEMG clustering methods and accuracy methods used to identify the firing rates of the decomposed motor unit (D), The most common MU firing measures (E) indicating recruitment and de-recruitment threshold forces (red) representing the force level at which the MU begins and ends firing action potentials. Firing rate at recruitment and de-recruitment (orange), indicating the average firing rate calculated from the first and last five firings. Mean firing rate (blue dashed line) showing the average firing rate on the sustained section of a particular muscle contraction. Firing rate variability (green dashed line) shows the fluctuations in firing frequency that can be calculated as the standard deviation or coefficient of variation in instantaneous firing rate. *Abbreviations: HDsEMG, High-density surface electromyography. Figure panels B-E adapted from Martinez-Valdes and Negro (2023) and recreated using BioRender.com.*

1.3.Cortical excitability and non-invasive brain stimulation

1.3.1.Development of non-invasive brain stimulation

Initial investigations on brain stimulation primarily used electrical stimulation applied to the exposed cortex of epilepsy patients undergoing surgical intervention (Penfield and Boldrey, 1937, Penfield and Rasmussen, 1950). Although the practicality of these invasive techniques was limited, they provided valuable information for understanding the organisation of the human brain and how it contributes to motor control. Later, Merton and Morton showed that the human motor cortex could be non-invasively stimulated using transcranial electrical stimulation (tES) by applying very brief, high-voltage electrical currents through electrodes attached to the scalp covering the motor cortex (Merton and Morton, 1980). They showed that stimulation of the arm and leg areas of the motor cortex generated twitch-like movements of the contralateral hand and foot muscles, respectively (Merton and Morton, 1980). To counteract the high resistance of the human scalp and skull, tES required a large current, which led to participant discomfort and pain. Although tES was the first successful technique to stimulate the human motor cortex non-invasively, the high level of discomfort arising from this method restricted its potential application. As a transformative substitute, Barker et al. (1985) established transcranial magnetic stimulation to address the drawbacks associated with tES (Barker et al., 1985).

1.3.2.Transcranial magnetic stimulation (TMS)

TMS discharges a brief electrical current from high-capacitance devices into a copper coil (Figure 1.6), resulting in an electromagnetic pulse that penetrates the scalp and skull onto the cortex (Barker et al., 1985). When TMS is applied over M1 as a single pulse, it activates cortical interneurons that synapse onto corticospinal neurons, which

activate spinal motor neurons through the corticospinal pathway (Barker and Shields, 2017). Subsequently, this generates a muscle twitch in the targeted peripheral muscles on the contralateral side that can be measured as a motor-evoked potential (MEP) (Figure 1.6). Standard surface EMG can be used to record the MEP response, the amplitude of which reflects the overall excitability of the corticospinal pathway (Rossini et al., 2015).

The introduction of TMS addressed the drawbacks associated with tES, as the magnetic fields in TMS can bypass the resistance of the scalp and skull (Barker et al., 1985). TMS is a non-invasive, painless, tolerable, and safe technique that was initially used in research settings to explore the function of the cerebral cortex. Currently, in addition to its research usage under standard guidelines (Rossini et al., 2015), different forms of TMS are also applied in various clinical settings, such as stroke rehabilitation (Dionisio et al., 2018), Alzheimer's disease (Sabbagh et al., 2019), depression (Sonmez et al., 2019) and migraine (Barker and Shields, 2017).

TMS is commonly applied as (i) single-pulse TMS, (ii) paired-pulse TMS, or (iii) repetitive TMS (rTMS; trains of repetitive TMS pulses). rTMS is used to non-invasively induce neuroplasticity in M1 (Goldsworthy et al., 2021), and has been discussed in recent reviews (see Klomjai et al., 2015 for review). I have used single-pulse and paired-pulse TMS to assess cortical excitability and inhibition in healthy adult M1 (Chapters 3 and 4), which will be discussed in the following sections.

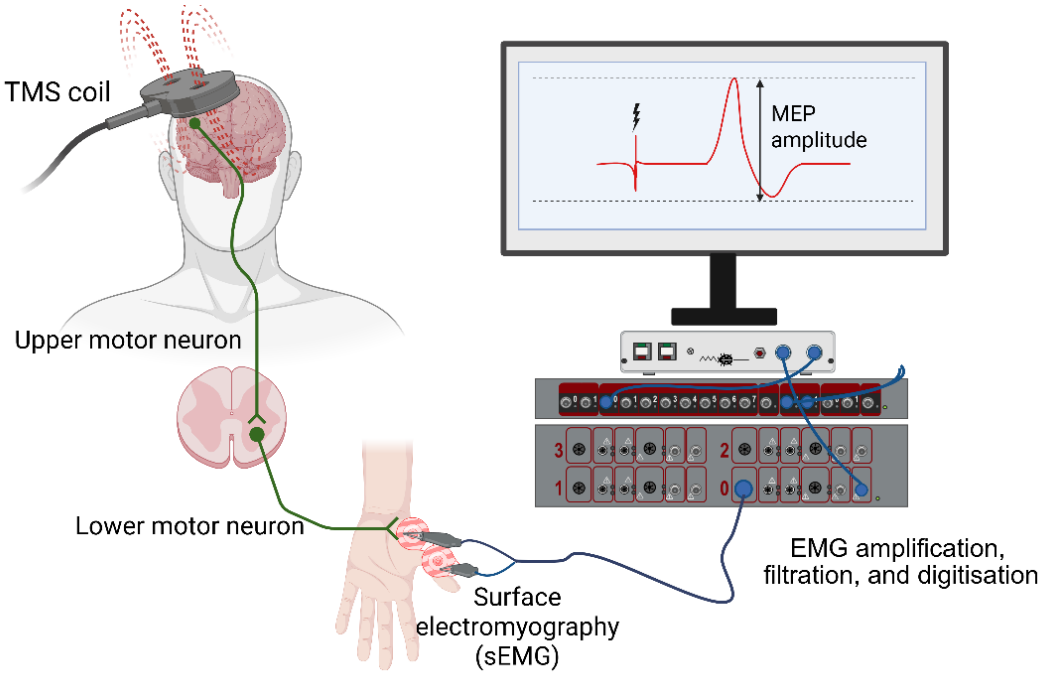


Figure 1.6. Transcranial magnetic stimulation (TMS). TMS produces action potentials in the underlying cortical neurons, which activate spinal motor neurons through the corticospinal pathway, resulting in a muscle twitch in the muscle of interest (e.g., abductor pollicis brevis), which is recorded using surface electromyography electrodes. *Abbreviations: TMS, transcranial magnetic stimulation; EMG, electromyography; MEP, motor-evoked potential. Figure created using BioRender.com.*

1.3.3.Single-pulse TMS

Single-pulse TMS is used to assess cortical excitability within M1. When a single TMS pulse is applied to M1, TMS-generated peak-to-peak MEP amplitude (Figure 1.7.A) is commonly expressed relative to stimulus intensity (percentage of maximum stimulation output, % MSO) and is commonly assessed in TMS studies before and after an intervention. The MEP amplitude provides an indirect measure of the excitability of the neural components activated by TMS, including intracortical circuits, corticospinal neurons, and spinal motor neurons (Rothwell, 1997). There is high trial-to-trial variability of MEP amplitudes, possibly due to ongoing fluctuations

in neurophysiological processes such as oscillatory brain rhythms (Sauseng et al., 2009), baseline muscle activation (Darling et al., 2006), and attentional focus and arousal levels (Mars et al., 2007). Due to this variability in MEP amplitude responses, it is necessary to acquire multiple MEP measures to better capture cortical excitability (Spampinato et al., 2023, Vallence et al., 2023).

There are numerous single-pulse measures, including cortical motor threshold, input/output curve, and silent period that quantify different features of cortical excitability. The input/output curve (stimulus-response curve) is the sigmoidal curve demonstrating the relationship between the MEP amplitude and stimulus intensity (Möller et al., 2009, Pitcher et al., 2003). The silent period is the period of EMG silence that appears following MEP produced by suprathreshold single-pulse TMS during tonic contraction of the target muscle (Fuhr et al., 1991) and has several cortical and spinal inhibitory contributions (Inghilleri et al., 1993). The input/output curve and silent period will not be discussed further (see Rossini et al., 2015 for review), but motor thresholds, which are used in Chapters 3 and 4, will be discussed in detail below.

1.3.3.1.Cortical Motor thresholds (MT)

The cortical motor threshold (MT) is the lowest intensity of motor cortex stimulation needed to elicit a motor response in the target muscle (Rossini et al., 2015). The MT reflects corticospinal and spinal motor neuronal excitability and allows the TMS intensity to be standardised for each individual (Shimazu et al., 2004). This MT can be recorded while the target muscle is in a resting (resting MT) or active (active MT) state. Resting motor threshold (RMT) is the smallest stimulus intensity (% MSO) needed to generate a peak-to-peak MEP amplitude of ≥ 50 μV in at least 5 out of 10 consecutive trials in the relaxed target muscle (Rossini et al., 2015). Active motor

threshold (AMT) is the lowest stimulus intensity required to produce a peak-to-peak MEP amplitude of ≥ 200 μV in at least 5 out of 10 consecutive trials during a low-intensity (~10% of maximum) contraction of the target muscle (Rossini et al., 2015). The stimulation intensity needed for AMT is lower than that for RMT (by ~ 20%) due to differences in cortical and spinal excitability during muscle activation (Garry and Thomson, 2009).

1.3.4.Paired-pulse TMS

Paired-pulse protocols consist of two TMS stimuli delivered at specific interstimulus intervals (ISI) to measure intracortical excitability. The first pulse, the *conditioning stimulus (CS),* is considered to activate the intracortical circuit being assessed. The second pulse, the *test stimulus (TS),* produces a MEP response (termed the 'test MEP') in the target muscle. When the CS is delivered before the TS, the effects of the two stimuli summate at the corticospinal neuron, modifying the test MEP response, resulting in a decrease (inhibition) or increase (facilitation) in MEP response, relative to single-pulse TMS given alone (Rossini et al., 2015).

The specific circuit that is activated (excitatory or inhibitory) is contingent upon the intensities of CS, TS, and ISI. For example, short-interval intracortical inhibition (SICI) can be assessed with an ISI of 1-6 ms (Kujirai et al., 1993), and intracortical facilitation, which is considered to be mediated by glutamatergic interneurons (Liepert et al., 1997), can be assessed with an ISI of 10-15 ms between CS and TS. I will discuss the paired-pulse paradigm of SICI in detail, as I have used SICI in two experimental studies (Chapters 3 and 4).

First described by Kujirai and colleagues, SICI (Figure 1.7) includes the suppression of a suprathreshold TS applied at short intervals of 1-6 ms following a subthreshold

CS (Kujirai et al., 1993). SICI is a common measure of intracortical inhibition and is thought to reflect activity of gamma-aminobutyric acid type A ($GABA_A$) receptors (Di Lazzaro et al., 2000). Numerous intrinsic factors (e.g., age, pathology) and extrinsic interventions (e.g., exercise, motor skill learning, and pharmacological interventions) can modify SICI. For example, pharmacological studies on $GABA_A$ receptor agonists have been shown to modulate SICI (Di Lazzaro et al., 2007, Ziemann, 2008). Previous studies have shown a reduction in SICI in the context of skill acquisition (Coxon et al., 2014, Liepert et al., 1998) and this change is considered important for modulating cortical plasticity associated with training (Cirillo et al., 2011). Despite some inconsistent findings in SICI among young and older adults, alteration in GABAergic inhibition is still considered a key marker of M1 plasticity (Ziemann et al., 2001). In Chapters 3 and 4, I measured SICI before and after tACS to evaluate the changes in GABAergic inhibition and M1 plasticity.

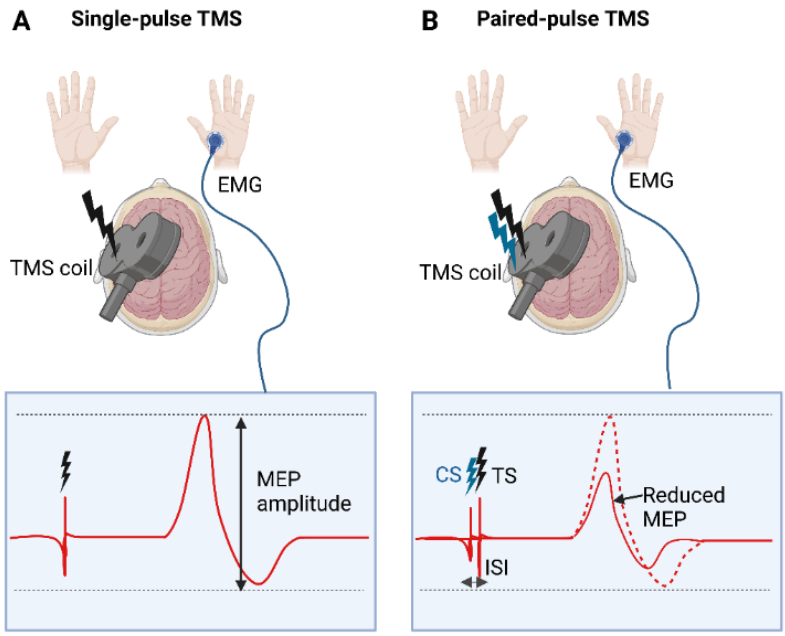


Figure 1.7. Schematic showing TMS paradigms. Single-pulse TMS (A) produces a test motor-evoked potential (MEP) for corticospinal excitability assessment; An example of a paired-pulse TMS paradigm (B) used to assess short-interval intracortical

inhibition (SICI). Horizontal dashed line indicates the amplitude of the test MEP (unconditioned). *Abbreviations: TMS, transcranial magnetic stimulation; EMG, electromyography; MEP, motor-evoked potential; CS, conditioned stimulus; TS, test stimulus; ISI, inter-stimulus interval. Figure created using BioRender.com.*

1.4.Neuroplasticity

Neuroplasticity broadly refers to the process that involves structural and functional changes in the nervous system in response to pathological (injury or disease) or physiological (learning from experience) events (Mateos-Aparicio and Rodríguez-Moreno, 2019, Semmler et al., 2021). Neuroplasticity is a universal process that underpins the capacity of the nervous system to adapt, acquire skills, retain information, and facilitate functional recovery following brain injuries (e.g., stroke and traumatic brain injuries). The mechanisms of cortical plasticity are typically divided into rapid short-term adaptations and long-term structural reorganisation (see section 1.4.1 on *mechanisms of cortical plasticity*). These structural and functional modifications in neuronal cells and synapses can occur over a wide range of time frames, ranging from minutes to months (Semmler et al., 2021). Although neuroplasticity can occur throughout the lifespan, the capacity for adaptation is considered to decrease with advancing age (Semmler et al., 2021). Plasticity is underpinned by complex neurobiological processes, and interventions that influence these (e.g., exercise, brain stimulation) have the potential to influence plasticity induction. Therefore, numerous interventions, such as non-invasive brain stimulation, deep brain stimulation, exercise, and training, have been commonly used to modulate neuroplasticity (see Cramer et al., 2011 for review). The experiments conducted in this thesis aimed to manipulate neuroplasticity in healthy older adults.

1.4.1.Mechanisms of cortical plasticity

In humans, plasticity may manifest through the rapid modification of synaptic strength and efficacy (Bachtiar and Stagg, 2014), typically referred to as synaptic plasticity, and occurs through various activity-dependent mechanisms (Mateos-Aparicio and Rodríguez-Moreno, 2019). Additionally, plasticity may also arise from long-term structural modifications in neural networks, such as neurogenesis and synaptogenesis (Gould et al., 1999, Kleim et al., 2004). These mechanisms will be briefly outlined below.

1.4.1.1.Short-term cortical plasticity

Cortical plasticity in the short term can be rapidly induced from the unmasking of pre-existing connections and activation of silent synapses (Jacobs and Donoghue, 1991), excitability changes of post-synaptic neurons (Woody et al., 1991), or modulating synaptic strength in an activity-dependent manner (Donoghue and Hess, 1996). These mechanisms are outlined briefly in the section below.

Silent synapses (i.e., functional synapses, but currently not active) are described as connections between neurons with no alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) responses (Liao et al., 1995), and the unmasking of these silent synapses within M1 can lead to rapid plastic modifications in response to motor inputs (Jacobs and Donoghue, 1991). Silent synapses can be rapidly activated through the insertion of postsynaptic AMPA receptors (Liao et al., 1999), enhanced excitatory neurotransmitter release, or a reduction in tonic inhibition (Chen et al., 2002).

Tonic inhibition is mediated by GABA, which is the brain's primary inhibitory neurotransmitter. There are two main GABA receptors: ionotropic $GABA_A$ and metabotropic $GABA_B$. $GABA_A$ receptors, located on the postsynaptic cells, act on chloride membrane channels. When GABA molecules bind to $GABA_A$ receptors, an influx of chloride ions occurs, resulting in membrane hyperpolarisation, leading to an inhibitory postsynaptic potential (IPSP). The IPSP decreases the ability of the membrane potential to reach the threshold required for action potential generation (Krnjević and Schwartz, 1967). In contrast, $GABA_B$ receptors are located on both pre- and postsynaptic membranes and are coupled to potassium and calcium channels through G-proteins. When $GABA_B$ receptors are activated, they generate IPSP through increasing potassium conductance or decreasing calcium conductance, producing slow synaptic inhibition (Kerr and Ong, 1996). Previous studies have examined the role of GABA-mediated inhibition on cortical plasticity, with changes in GABAergic inhibition considered a key indicator of M1 plasticity (Kullmann et al., 2012, Ziemann et al., 2001) (see section 1.4.3 on *motor learning and use-dependent plasticity*).

Another mechanism underlying short-term cortical plasticity occurs through the modulation of synaptic strength in an activity-dependent manner (Donoghue and Hess, 1996). The ability to alter synaptic efficacy was first shown in the rabbit hippocampus (Bliss and Lømo, 1973), with high-frequency repetitive stimulation on the hippocampus generating increased excitatory postsynaptic potentials (EPSPs) lasting up to several hours after stimulation (Bliss and Lømo, 1973). This enhanced synaptic efficacy resulting from repeated high-frequency stimulation was described as long-term potentiation (LTP) (Bliss and Lømo, 1973). Conversely, repetitive stimulation at lower frequencies reduces synaptic efficacy and is referred to as long-term depression (LTD) (Dudek and Bear, 1992). Previous research has demonstrated LTP and LTD-

like processes within several brain areas, including the motor cortex (Hess and Donoghue, 1996, Hess and Donoghue, 1994). These LTP and LTD processes are not limited to glutamatergic synapses, but are also observed at GABAergic synapses (Kano et al., 1992, Morishita and Sastry, 1996).

Under normal conditions, synaptic transmission at excitatory glutamatergic synapses involves the release of the neurotransmitter glutamate, which binds to AMPA receptors located on the postsynaptic membrane. This will allow sodium influx, leading to depolarisation at the dendritic synapse, generating an EPSP, which, if sufficiently large, will lead the cell to fire (Caporale and Dan, 2008). While various cellular processes account for LTP and LTD, the most extensively studied are those related to N-methyl-D-aspartate (NMDA) receptors (Malenka and Bear, 2004).

The induction of LTP requires the activation of NMDA receptors in addition to AMPA receptors. At the resting state, the NMDA receptor has a magnesium ion block within the NMDA pore that interrupts the current through the NMDA receptor. This NMDA receptor channel activation requires a concurrent release of glutamate by the presynaptic terminal and sufficiently large AMPA-receptor-mediated depolarisation of the postsynaptic membrane. Subsequently, the magnesium block in the NMDA receptor will get displaced, allowing rapid calcium influx through the receptor. The subsequent increase in intracellular calcium is considered to be a sufficient trigger for the strengthening of synaptic transmission through LTP (Bear and Malenka, 1994). In contrast, the low postsynaptic calcium influx is thought to activate a protein phosphatase cascade leading to LTD, whereby the active synapses are decreased in effectiveness (Bear and Malenka, 1994). During LTD, the intracellular cascade

decreases the AMPA receptor opening potential (Isaac, 2001) and is also involved in the elimination of these receptors from the membrane (Malinow and Malenka, 2002).

Generally, the induction of LTP requires various properties, including associativity, cooperativity, input specificity, and involvement of NMDA and GABA receptors (Bi and Poo, 2001, Bliss and Collingridge, 1993, Paulsen and Sejnowski, 2000). According to the principle of associativity, LTP can be induced when a weak synaptic input is coactivated with a strong synaptic input (Bi and Poo, 2001, Bliss and Collingridge, 1993), or through the synchronous activation of another input to the same post-synaptic cell (Buonomano and Merzenich, 1998). This phenomenon is commonly referred to as Hebbian Plasticity, in line with Hebb's postulate, which suggests that synaptic connections between two cells are increased when two cells fire together repeatedly (Hebb, 1949). Cooperativity requires several axons to be activated simultaneously and is considered an intensity threshold for inducing LTP (Bliss and Lømo, 1973). Therefore, on the post-synaptic membrane, NMDA receptor activation occurs when magnesium channel block is reduced, allowing calcium influx to reach the threshold for activation (Bliss and Collingridge, 1993). The input specificity of LTP indicates that only the inputs that are active along the pathway are potentiated (Andersen et al., 1977). The involvement of NMDA receptors is essential for cortical plasticity, and it also frequently requires a reduction in GABA-mediated inhibition (Bliss and Collingridge, 1993).

1.4.1.2.Long-term cortical plasticity

Neurogenesis and synaptogenesis are some of the key processes involved in facilitating long-term neuroplastic changes. Neurogenesis refers to the generation of

new neurons, and synaptogenesis is the generation of new synapses. These processes were initially assumed to be limited to the early development in the mammalian brain, but research indicates they also occur in adulthood. For example, adult rodents have shown neurogenesis in cognitive brain areas following an associative learning task (Gould et al., 1999), whereas synaptogenesis within the motor cortex and cerebellum has been demonstrated in response to motor skill learning tasks (Black et al., 1990, Kleim et al., 1996).

In the adult animal motor cortex, extensive motor training induces reorganisation of movement representation and synaptogenesis and is considered to occur during late, but not early phases of motor skill learning (Kleim et al., 2004). Plasticity within M1 during early phases may not require synaptogenesis, but instead reflect modifications in neuronal excitability (Aou et al., 1992) and enhanced efficacy of existing synapses (Rioult-Pedotti et al., 1998). Previous studies have shown that more than a week (~10 days post-training) is required to demonstrate motor map reorganisation and synaptogenesis in response to continual motor training over the 10 days in adult animal models (Kleim et al., 2004). Similarly, in professional musicians, repetitive and prolonged motor practice results in greater synaptic plasticity, likely through long-term mechanisms, such as synaptogenesis (Rosenkranz et al., 2007a).

1.4.2.Measuring neuroplasticity with TMS

Neuroplasticity may manifest as changes in cortical excitability as measured using TMS. For example, single-pulse TMS measures (see section 1.3.3 on *single-pulse TMS*) applied to the human motor cortex indicate corticospinal excitability at the time of stimulation (Chen et al., 2008, Rothwell, 1997). Subsequently, an alteration in MEP amplitude (commonly assessed before and after an intervention), is used as a non-

invasive method to indirectly assess corticospinal plasticity (Pascual-Leone et al., 1998), and this change in MEP reflects LTP-like plasticity (Sanes and Donoghue, 2000). Additionally, paired pulse TMS measures (see section 1.3.4 on *paired-pulse TMS*) are used to assess the plasticity of intracortical inhibitory (e.g., short-interval intracortical inhibition; SICI) and facilitatory (e.g., short-interval intracortical facilitation; SICF) circuits (Kujirai et al., 1993).

Despite the common use of TMS in probing excitability within the corticospinal and intracortical systems in young and older adults (Hinder et al., 2013, Rogasch et al., 2009), its role in accurately capturing cortical plasticity remains limited due to some limitations with the technique. First, the neural populations stimulated by TMS are thought to capture only a small portion of the networks involved in motor activity (Bestmann and Krakauer, 2015). Second, since MEP size indicates the excitability of the neural pathways controlling the target muscle (Rothwell, 1997), spinal level adaptations are also thought to contribute to MEP changes (Berghuis et al., 2017). Nevertheless, despite these limitations, TMS continues to serve as a prominent method for the assessment of neuroplasticity in the motor cortex.

1.4.3.Motor learning and use-dependent plasticity

1.4.3.1.Introduction to motor learning and use-dependent plasticity

Motor learning, also known as motor skill acquisition, is a key part of our lives, and it can be described as any experience-dependent improvement in motor performance. Motor learning can range from the acquisition of novel, unknown skills to the relearning and improvement of motor skills learned in the past (Voelcker-Rehage, 2008). Initial phases of motor skill learning are thought to engage mechanisms such

as enhanced efficacy of existing synapses (Rioult-Pedotti et al., 1998) and a reduction in GABAergic intracortical inhibition (Jacobs and Donoghue, 1991). In contrast, later phases of motor training are thought to be driven by long-term modifications such as synaptogenesis (Kleim et al., 2004).

Use-dependent plasticity (also known as practice-dependent plasticity) refers to the strengthening of existing neural connections and the development of new connections in the motor cortex in response to repetitive practice of a motor skill. This phenomenon is vital for motor function recovery following brain injuries, such as stroke (Nudo et al., 2001). In humans, prolonged motor practice, exemplified by professional musicians, leads to greater synaptic plasticity, and this process is thought to result from an increase in synapses through synaptogenesis (Rosenkranz et al., 2007a). Similarly, long-term bimanual practice in humans (e.g., pianists) has shown more symmetrical motor cortex organisation, in terms of motor cortical representation, which typically exhibits a bias towards the dominant hemisphere in naïve individuals (Chieffo et al., 2016).

Alterations in motor cortex organisation are observed not only with prolonged motor practice but also following short-term (minutes) training. For example, previous studies have shown motor cortex reorganisation after short-duration practice (less than 60 minutes) of simple, repetitive ballistic thumb movement tasks (Classen et al., 1998). In human experimental studies, use-dependent plasticity can be measured using TMS, and an increase in MEP amplitude (lasting up to 60 minutes) that is generally observed following motor training is considered indicative of use-dependent plasticity (Muellbacher et al., 2001, Muellbacher et al., 2002), with mechanisms attributed to LTP-like processes (Bütefisch et al., 2000). Additionally, a reduction in GABAergic

inhibition also plays an important role in training-dependent plasticity and motor skill acquisition (Ziemann et al., 2001), which can be measured with paired-pulse TMS to assess SICI (Ziemann et al., 1996). In Chapters 3 and 4, I assessed use-dependent M1 plasticity in healthy young and older adults following a simple ballistic motor task.

1.4.3.2.Factors affecting use-dependent plasticity

Use-dependent plasticity is dependent on various factors, including the motor task performed, the duration of motor training, and participant characteristics. For example, a previous meta-analysis revealed that practicing a visuomotor but not a ballistic motor task resulted in an increase in corticospinal excitability in young and older adults (see Berghuis et al., 2017 for review). This review also identified an age-related difference in certain measures of use-dependent plasticity, including intracortical inhibitory measures such as SICI. They found a reduction in SICI with visuomotor training in older but not young adults (Berghuis et al., 2017). Additionally, the duration of the motor task could also affect use-dependent plasticity. For example, an increase in corticospinal excitability was reported following 90 seconds of pegboard training (Garry et al., 2004), while another study found a reduction in corticospinal excitability with ~15 minutes of a similar pegboard task (McDonnell and Ridding, 2006). Furthermore, the structure of training (i.e., number of trials, blocks, rests between blocks and type of contraction) could also influence use-dependent plasticity and functional measures (Lage et al., 2015). Therefore, it is important to account for these factors when interpreting use-dependent plasticity and functional outcomes following motor practice.

1.5.Brain oscillations and transcranial alternating current stimulation (tACS)

1.5.1.Brain oscillations

Brain oscillations, also known as brain waves, reflect synchronised neuronal firing, and this coordinated oscillatory activity among neuronal populations can be measured within the brain using invasive methods (e.g., stereo-electroencephalography [sEEG], electrocorticography [ECoG]), or non-invasively on the scalp with EEG and magneto-encephalography (MEG) (see Canolty and Knight, 2010 for review). Conventionally, brain oscillations have been divided into frequency bands of delta (1-4 Hz), theta (4-8 Hz), alpha (8-13 Hz), beta (13-30 Hz), and gamma (> 30 Hz) waves (Figure 1.8.A), with each associated with different functions. For example: delta waves are commonly observed in an unconscious state and are associated with deep sleep; theta waves are associated with deep meditation, light sleep, daydreaming, learning and memory; alpha waves are associated with a conscious, relaxed, light meditation state; beta waves are associated with the normal waking state, during focused concentration, and are also important for motor function (Koudelková and Strmiska, 2018). Finally, gamma waves are commonly related to motor function, learning, problem-solving, and information processing (Abhang et al., 2016, Koudelková and Strmiska, 2018). More details of brain oscillations in movement and motor performance will be discussed below (see section 1.5.5 on *effects of tACS on motor performance*).

1.5.2.Cross-frequency coupling (CFC)

In addition to their role in isolation, extensive literature has demonstrated that oscillations from different frequency bands interact with each other. Referred to as cross-frequency coupling (CFC), these interactions are thought to have an important

functional role in neuronal computation, communication, and learning (Canolty et al., 2006). In particular, the amount of CFC varies across brain regions in a task-specific manner and is linked to performance outcomes in learning tasks (Dürschmid et al., 2014). While CFC can occur between specific features of oscillations in different bands (i.e., phase, amplitude, and frequency; Canolty and Knight, 2010), one of the best studied examples is between the amplitude of a high-frequency rhythm (e.g., gamma) and the phase (i.e., a measure of the position within a full cycle of an oscillatory waveform) of a low-frequency rhythm (e.g., theta), which is known as phase-amplitude coupling (PAC; Figure 1.8.B) (Canolty and Knight, 2010, Hyafil et al., 2015). While PAC has been observed between several high-low frequency couplings, I will focus on theta-gamma PAC, which is focused on Chapters 3 and 4 due to its role in motor performance.

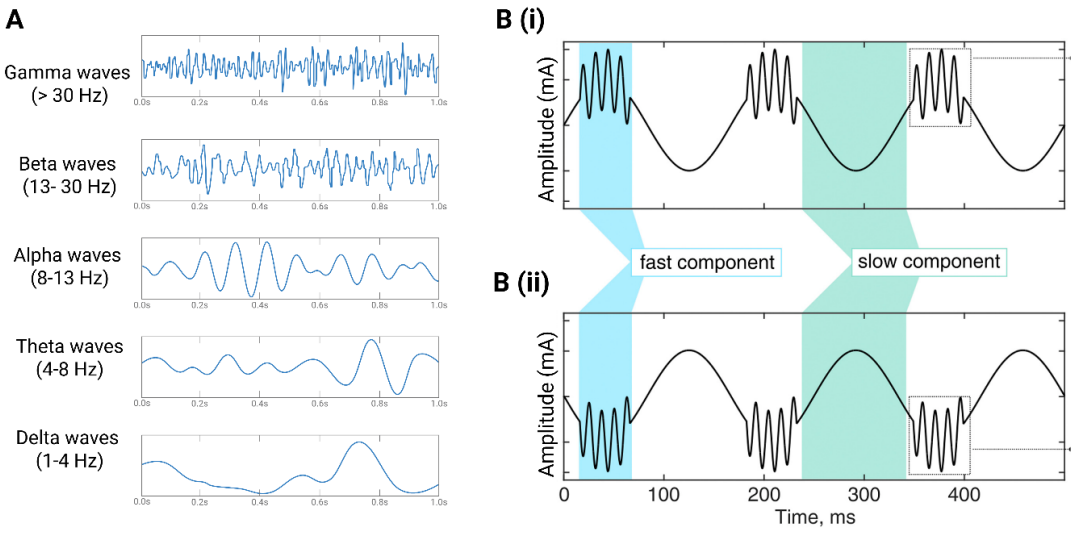


Figure 1.8. Brain oscillations and cross-frequency coupling. Brain wave frequencies (A) include delta, theta, alpha, beta, and gamma. Cross-frequency coupling transcranial alternating current stimulation (tACS) protocol examples (B). Repetitive bursts of fast alternating current (e.g., gamma) superimposed on the peak (B i) or troughs (B ii) of the continuous, slow alternating current (e.g., theta). *Figure panel A adapted from Abhang et al., 2016, and panel B adapted from Alekseichuk et al., 2016, and recreated using BioRender.com*

1.5.3.Theta-gamma phase-amplitude coupling (Theta-gamma PAC)

Emerging evidence has shown that high-frequency brain oscillations in the gamma band are frequently synchronised to low-frequency theta waves during learning (i.e., theta-gamma PAC Canolty et al., 2006, Johnson et al., 2017). This PAC between theta and gamma oscillations is frequently observed during wakefulness in the hippocampus (Tort et al., 2009), where it is known to be an important mechanism for memory processing (Colgin, 2015). Additionally, theta-gamma PAC is observed in the neocortex during information encoding and retrieval (Fell and Axmacher, 2011), and is demonstrated to be associated with working memory in humans (Alekseichuk et al., 2016, Daume et al., 2024). Importantly, these observations of theta-gamma PAC extend to the motor system (Canolty et al., 2006), where the amount of theta-gamma PAC over premotor and motor cortices has been positively related to motor learning success (Dürschmid et al., 2014), and in post-stroke motor function recovery (Rustamov et al., 2022). To mimic physiologically occurring PAC, cross-frequency tACS can be used. Given that theta-gamma tACS was applied in Chapters 3 and 4, its role in modulating motor performance will be explored further in the following sections.

1.5.4.Basics of tACS

Transcranial alternating current stimulation (tACS) is an emerging neuromodulation technique that applies low-intensity, alternating current (~0.5 - 2.0 mA peak-to-peak) at specific frequencies, via electrodes that are attached to the scalp. tACS induces a subthreshold polarisation of underlying excitable tissue that does not trigger action potentials, but modulates local brain membrane potentials, altering the likelihood of action potential generation (Vogeti et al., 2022, Vöröslakos et al., 2018) (see section

1.5.4 on the *mechanism of action of tACS*). Previous studies have used tACS to influence motor performance (see section 1.5.5 on *effects of tACS on motor performance*), memory, and cognition (McNally et al., 2024, Tavakoli and Yun, 2017). Additionally, tACS has the potential to be used as a therapeutic tool (Grover et al., 2023). For example, the application of tACS as a neuromodulatory intervention in clinical settings, such as in depression, schizophrenia, attention-deficit hyperactive disorder, and obsessive-compulsive disorder, has been considered to be beneficial, with no serious side effects or exacerbation of clinical symptoms (see Elyamany et al., 2021 for review).

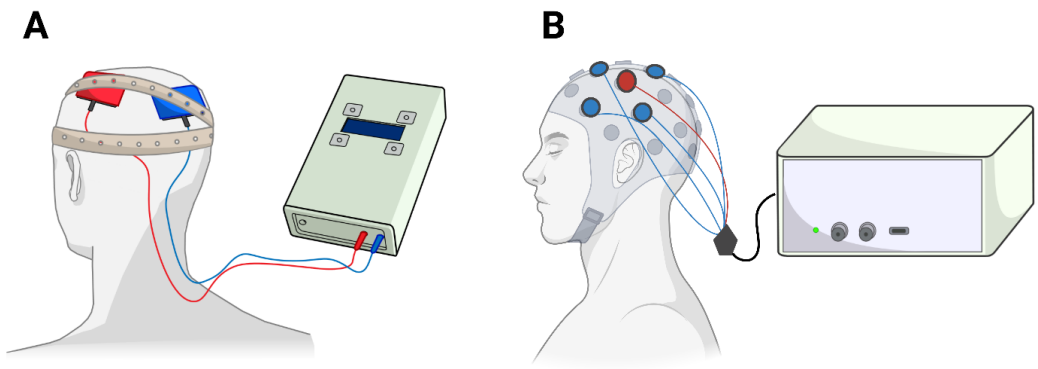


Figure 1.9. Transcranial alternating current stimulation (tACS) montages. During tACS, an alternating current flows between two or more electrodes of opposing phases (shown in red and blue). Schematic of standard bipolar tACS montage (A); Schematic example of high-density tACS (HD tACS) 4 × 1 montage (B). *Figure created using BioRender.com*.

A tACS protocol is specified using parameters such as electrode montage, stimulation intensity, duration, and frequency. For example, tACS may be applied in a wide frequency range (i.e., delta, theta, alpha, beta, or gamma; see section 1.5.1 on *brain oscillations*) and can be delivered using tACS electrodes with a variety of shapes and sizes. Conventionally, tACS is delivered using a pair of large conductive rubber

electrodes (Figure 1.9.A), with one over the targeted brain area (target electrode) and the other over a functionally unrelated area (reference electrode), with the current passing between the electrodes. During alternating current application in tACS, electrode polarity switches every half cycle (Carvalho et al., 2018). For example, in one-half of an alternating current (tACS) oscillation, one electrode acts as the anode, whereas the other acts as the cathode, with this pattern reversing in the other half cycle, i.e., the former anode now functions as the cathode and vice versa (Carvalho et al., 2018).

A limitation of the conventional electrode montage is that it activates large areas of brain, potentially resulting in confounding off-target effects. To improve focality and focus more specifically on the target area, more recent studies have used high-definition tACS (HD tACS; Figure 1.9.B). This involves a central active electrode surrounded at close proximity by several return electrodes (Wu et al., 2021). The HD tACS montage produces a much more focal electrical field, reducing off-target brain activation (Sawai et al., 2023), and is associated with reduced somatosensory side effects, such as tingling, which is advantageous in participant blinding (Heise et al., 2016). In Chapters 3 and 4, I used HD tACS as a more focal approach to modulate M1 function.

1.5.5.Mechanism of action of tACS

The precise mechanism of action of tACS remains unclear; however, increasing evidence indicates several possible explanations. There are two main categories of tACS effects: *online* (effects occurring during stimulation) and *offline* (effects that outlast the stimulation period), and these are predominantly associated with entrainment and neuroplasticity, respectively (see Elyamany et al., 2021 for review).

However, some evidence also indicates a relationship between the intensity of online effects and the magnitude of aftereffects, suggesting that the online and offline tACS effects are not completely independent (Helfrich et al., 2014, Randolph et al., 2014).

Entrainment is the temporal locking of one signal or oscillation to another (Wischnewski et al., 2023). When tACS is applied, some of the alternating current reaches the brain and modulates the membrane potential of cortical neurons in an oscillatory manner (Vöröslakos et al., 2018). This alternating membrane potential shift is strong enough to alter the probability of action potential generation (Antal and Herrmann, 2016), but this stimulation is not powerful enough to alter the firing rates of single neurons (Krause et al., 2019). Therefore, tACS is considered a sub-threshold stimulation that does not directly fire action potentials (Vosskuhl et al., 2018).

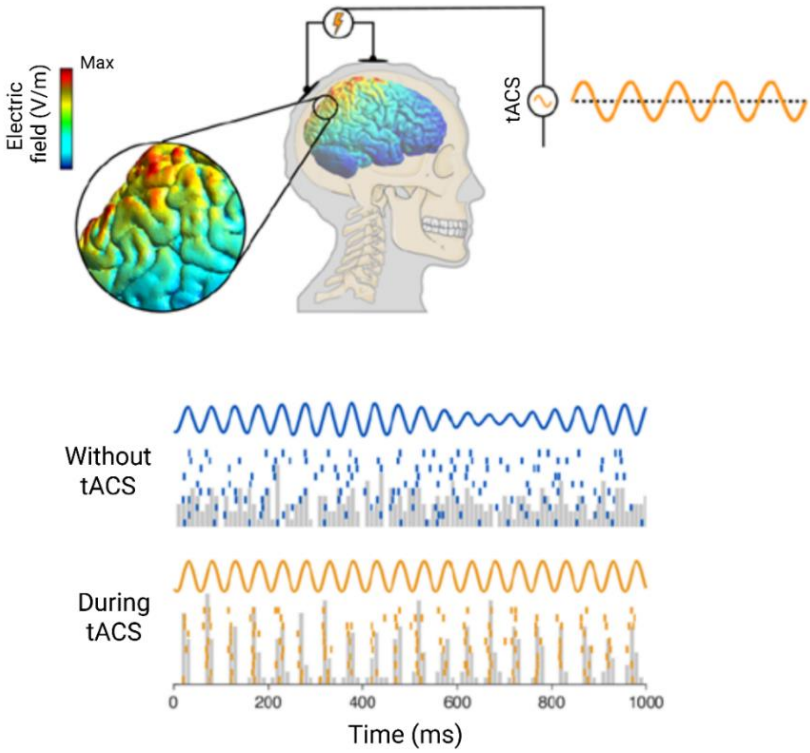


Figure 1.10. Mechanism of action of transcranial alternating current stimulation (tACS). A weak electrical current induces an oscillating electric field in the cortex. Each tick denotes the time of an action potential recorded from an awake monkey.

Without stimulation (blue trace), the activity of the neuron is arrhythmic and disorganised, whereas during tACS (orange trace), the neuron becomes entrained to the external stimulation. *Abbreviations: tACS, transcranial alternating current stimulation. Figure adapted from Krause et al., 2023, and recreated using BioRender.com*.

As a neuromodulatory intervention, tACS enables targeted modulation of endogenous brain oscillations at specific frequencies (see section 1.5.1 on *brain oscillations*) with lasting effects, which have been reported previously in motor (see McNally et al., 2024 for review) and cognitive (see Grover et al., 2023 for review) domains. Previous studies have shown that the aftereffects of tACS when concurrently performed with a behavioural task can persist up to 70 minutes following a 20-minute single tACS session (Kasten et al., 2016). Additionally, previous studies have applied tACS concurrently with different motor tasks to manipulate M1 excitability, and these will be described in the following sections.

1.5.6.Effects of tACS on motor performance

While modulation of oscillatory activity within alpha, beta, and gamma bands is often associated with motor behaviour (Davis et al., 2012, Muthukumaraswamy, 2010, Pfurtscheller et al., 2006, Pollok et al., 2014), our understanding of the specific functional relevance of these changes remains limited. However, a growing literature has attempted to address this limitation by using tACS applied with different frequencies to modulate human motor function (see Rostami et al., 2023 for review). Results of this literature, which appear to demonstrate divergent outcomes that are frequency-dependent, are briefly detailed below.

1.5.6.1.Theta tACS

Theta tACS (4-8 Hz) has been commonly used in assessing cerebellar and memory function. For example, the application of theta tACS at 4 Hz and 6 Hz supports working memory function in healthy young adults (Alekseichuk et al., 2016, Wolinski et al., 2018), but a shift to 7 Hz tACS appears to impair it (Wolinski et al., 2018). Additionally, theta tACS has been shown to influence cerebellar-related motor function, with stimulation resulting in decreased movement regularity during a rhythmic tapping task and increased movement duration of a pointing task (Guerra et al., 2024). Nonetheless, given the role of theta-oscillations as a carrier signal, which strengthens connectivity and facilitates communication between sensory and motor systems (see Bland and Oddie, 2001 for review), theta tACS may be a promising method to modulate motor behaviour. In Chapter 4, I applied theta tACS over M1 in healthy older adults to assess the role of theta tACS on ballistic motor performance.

1.5.6.2.Alpha tACS

Alpha tACS (10 Hz) over M1 has been shown to facilitate motor skill acquisition of a serial reaction time task (SRTT) during stimulation (Antal et al., 2008, Pollok et al., 2015), while some studies demonstrated impaired consolidation 6 hours after tACS (Rumpf et al., 2019) or reported no effect post-SRTT training (Krause et al., 2016, Sugata et al., 2018). Additionally, when compared to sham, alpha tACS (10 Hz) was shown to facilitate visuomotor adaptation during the early but not late stages of training (Harada et al., 2020). Overall, alpha tACS has a significant positive effect on motor function, but showed a high level of heterogeneity between the studies (Rostami et al., 2023). Interestingly, alpha tACS of an intensity of 1 mA or lower indicated

better effects on motor function compared to intensities above 1 mA (Rostami et al., 2023).

1.5.6.3.Beta tACS

Beta-band activity within the M1 has been associated with motor imagery, learning, and execution (Davis et al., 2012, Pollok et al., 2014). Considering the performance of SRTT, beta tACS (20 Hz) over M1 has been shown to improve task performance both during (Pollok et al., 2015) and after (Krause et al., 2016) stimulation, while some studies show no effect post-SRTT (Rumpf et al., 2019, Sugata et al., 2018). However, applying beta tACS (20 Hz) over M1 resulted in detrimental effects on a ballistic finger abduction task during stimulation and showed no effect on motor retention (Bologna et al., 2019). Overall, beta tACS over M1 is able to modify motor function, with an intensity of 1 mA or lower showing better effects compared to using an intensity of 2 mA (Rostami et al., 2023).

1.5.6.4.Gamma tACS

Gamma-band activity in M1 is commonly known to increase during motor preparation and execution (Gaetz et al., 2011, Muthukumaraswamy, 2010). High gamma (70 Hz) tACS over M1 has been shown to improve capacity for motor learning associated with SRTT in the second session of motor training, compared to sham (Sugata et al., 2018). Additionally, high gamma tACS (70 Hz) enhanced ballistic motor skill acquisition during stimulation, but showed detrimental effects on motor retention (Bologna et al., 2019). Nevertheless, gamma tACS over M1 has been shown to improve the velocity and acceleration of movement initiation compared with beta tACS and sham stimulation (Moisa et al., 2016). Another study reported that force generation

improved with gamma tACS, while it was impaired with beta tACS (Joundi et al., 2012). Overall, gamma tACS has been shown to improve motor function, with studies applying 2 mA gamma tACS leading to significant enhancement in motor function, while 1 mA gamma tACS showed no effect (Rostami et al., 2023). High gamma (i.e., 70-75 Hz) seems to be more effective compared to slow gamma (i.e., 40 Hz) when delivering stimulation concurrent with task performance (McNally et al., 2024).

1.5.7.Theta-gamma tACS

As described in the cross-frequency coupling section (see section 1.5.2 on *cross-frequency coupling; CFC*), theta-gamma PAC is observed in the motor system (Canolty et al., 2006), and the amount of theta-gamma PAC over motor brain regions is positively associated with motor function (Dürschmid et al., 2014, Rustamov et al., 2022). The application of theta-gamma cross-frequency tACS (theta-gamma tACS) over the prefrontal cortex has been demonstrated to boost visuomotor learning in older adults (Diedrich et al., 2024), and cognitive function in both young (Alekseichuk et al., 2016) and older adults (Reinhart and Nguyen, 2019). Importantly, theta-gamma tACS has a greater effect on working memory performance compared with theta tACS alone (Alekseichuk et al., 2016).

Considering the application of theta-gamma tACS on the motor domain, Akkad et al. (2021) showed for the first time that theta-gamma tACS (6 Hz theta and 75 Hz gamma) has the potential to modulate ballistic thumb movement in healthy young adults when applied over M1 with effects lasting for 75 minutes (Akkad et al., 2021). Importantly, theta-gamma tACS produced a greater improvement in ballistic motor performance when the gamma oscillation was nested at the peak of the theta wave (theta-gamma Peak; Figure 1.10.B.i), whereas there was no difference in performance when the

gamma oscillation was nested at the trough of the theta wave (theta-gamma Trough; Figure 1.10.B.ii) compared with sham tACS (Akkad et al., 2021).

Taken together, tACS within alpha, beta, and gamma frequencies has been shown to modify motor function, irrespective of being administered before or concurrently with motor training (Rostami et al., 2023). Theta tACS, although not explicitly used in motor areas, may also have the potential to modulate motor performance (McNally et al., 2024). Moreover, theta-gamma tACS has been demonstrated to enhance ballistic motor performance with lasting effects (Akkad et al., 2021). I have used theta tACS in Chapter 4 and theta-gamma tACS in Chapters 3 and 4 to explore their role in facilitating ballistic motor performance in healthy older adults.

1.6.Age-related alterations in the central and peripheral nervous system

1.6.1.Supraspinal-level adaptations

Healthy ageing is accompanied by structural and functional alterations to the central nervous system (see Seidler et al., 2010 for review). One of the most apparent changes associated with ageing is cortical atrophy, with total volume of the cortical area shown to be decreased (between 4-16%) with advancing age (Bartzokis et al., 2001, McGinnis et al., 2011, Raz et al., 2004). Although it was initially attributed to a reduction in the number of M1 neurons, more recent studies suggest that M1 cortex decline is associated with a volumetric decrease in premotor and primary motor cortex neuron cell body size and synaptic density (McGinnis et al., 2011, Morrison and Hof, 1997, Salat et al., 2004). Compared to young individuals, the cadaveric dissections of older adults showed a volumetric reduction of 43% in the motor cortex cell body size (Haug and Eggers, 1991), and studies suggests that this cortical decline affects motor

performance. For example, cortical atrophy has been shown to be associated with fine movements (Kennedy and Raz, 2005), while grey matter atrophy is linked to a decline in gait performance (Rosano et al., 2008).

In addition to the above changes, cortical white matter, which is primarily made of glial cells and myelinated axons and is associated with corticocortical and corticospinal connectivity, also exhibits a significant decrease in older adults (Borzuola et al., 2020). Studies have shown that a reduction in white matter integrity disrupts motor performance in several tasks, including alternating finger tapping (Sullivan et al., 2001) and fine finger movement tasks (Sullivan et al., 2010).

Ageing also affects neurotransmission, which is a vital process for transmitting chemical signals between neurons. Age-related alterations in neurotransmitters and their receptors are associated with motor and cognitive decline, with abnormalities reported in neurotransmitter regulatory systems involving GABA and glutamate (Borzuola et al., 2020). For instance, a reduction in glutamate with advancing age, which is the major excitatory neurotransmitter in the CNS, has been shown in animal models (Segovia et al., 2001) and human M1 (Kaiser et al., 2005). Previous studies have reported many discrepancies in age-associated changes in GABA, the primary inhibitory neurotransmitter in the brain (see Bhandari et al., 2016 for review). However, some studies in older adults have shown a correlation between reduced ability to modulate cortical inhibition and poorer motor function (Fujiyama et al., 2012, Levin et al., 2014).

1.6.2.Spinal-level adaptations

Among the morphological changes in the spinal region, the most critical one is spinal atrophy, largely related to the loss of spinal motor neurons (Borzuola et al., 2020). As shown in previous human studies in older adults, this decline is accompanied by a decrease in the density and diameter of myelinated and unmyelinated axons in the ventral horn of the spinal cord (Buchman et al., 2019, Terao et al., 1994, Tomlinson and Irving, 1977). Alongside these structural alterations, ageing leads to reductions in conduction velocity and responsiveness to peripheral neurons, ultimately resulting in diminished spinal motor neuron excitability (Borzuola et al., 2020, Palve and Palve, 2018).

1.6.3.Motor unit-level adaptations

1.6.3.1.Motor unit number and motor unit remodelling

When a muscle fibre gets denervated, either due to peripheral nerve injury or through the motor neuron loss, this can lead to two possible outcomes. First, the denervated muscle fibre may undergo atrophy due to lack of contractile activity and subsequently disappear due to apoptosis. Secondly, there is the collateral reinnervation or sprouting, during which the denervated fibre could be reinnervated by a nearby functioning axon. As a result of the remodelling process, older adults tend to have reduced MU number and increased MU size (Piasecki et al., 2018). If the process of reinnervation is insufficient to counter ongoing denervation, this will result in overall muscle mass loss, which is commonly observed with advancing age.

Early evidence of a reduction in MU numbers was from direct counts of neuron cell bodies in the anterior horn of the spinal cord in autopsy samples (Tomlinson and Irving,

1977). While the motor neuron numbers remained fairly steady until the age of 60, they drastically declined around the age of 75, with almost 30% fewer motor neurons innervating the lower limbs (Kawamura et al., 1977, Tomlinson and Irving, 1977). Later, indirect MU number estimates obtained using EMG methods also indicated a progressive loss of MU numbers associated with ageing (Brown et al., 1988, Piasecki et al., 2016b, Piasecki et al., 2018). Additionally, older adults also showed larger sized MUs compared to young adults (Piasecki et al., 2016a, Piasecki et al., 2016c). It is thought that the MU size increases as a result of the branching of neighboring motor neurons to reinnervate muscle fibres that were recently denervated (i.e., MU remodelling) (Hepple and Rice, 2016, Luff, 1998).

1.6.3.2.Neuromuscular junction-related adaptations

With advancing age, the neuromuscular junction (NMJ) undergoes morphological remodelling and functional impairments. Structural alterations of NMJ occur in both the presynaptic (motor nerve terminal) and postsynaptic region (muscle fibre surface and membrane), leading to a reduction in conduction velocity and reduced magnitude of muscle action potentials (Kurokawa et al., 1999, Punga and Ruegg, 2012). Furthermore, research suggests that aged mitochondria within the NMJ demonstrate changes in ATP production and calcium buffering, which may adversely affect neurotransmission (Deschenes, 2011). Additionally, there is a reduction in the number of synaptic vesicles (Wang et al., 2011) and a reduced amount of neurotransmitter release observed with ageing (Deschenes, 2011).

In humans, age-related NMJ decline is mostly reported via EMG assessments of single MU action potentials (Bromberg and Scott, 1994). Evidence suggests that advancing age results in alterations in the EMG recordings of single MU, including abnormally

increased intervals between action potentials of two fibres of the same MU (i.e., jitter) and greater variability in the overall shape of MUPs detected across consecutive firings of a single MU (i.e., jiggle) (Hepple and Rice, 2016, Hourigan et al., 2015).

In Chapter 2, I used iEMG to assess NF jiggle, which is an indicator of NMJ transmission instability, indicating the variability of temporal dispersion across MU action potentials (Piasecki et al., 2021b). This NF measure is mainly related to the activity of muscle fibres close to the detection surface of the concentric needle electrode (within ~350 μm) (Stashuk, 1999b). Hence, the contributions of distant fibres to NF MUPs are minimal, which reduces the contamination from other MUs.

1.6.3.4.Muscle fibre-level adaptations

Ageing also leads to muscle atrophy, with older adults demonstrating smaller muscle fibres in the MUs compared to young adults (Lexell and Taylor, 1991, Macaluso and De Vito, 2004, Venturelli et al., 2015). In addition, the contractile properties of muscle fibres decline with ageing, including impairments in cross-bridge kinetics (Miller et al., 2013), resulting in reduced contractile speed and ultimately lower force production (Bellumori et al., 2013) compared to young individuals.

1.7.Exercise and cross-limb transfer phenomena

1.7.1.Benefits of exercise

Numerous studies have reported the widespread benefits of exercise in humans. Engaging in regular exercise is associated with reduced cardiovascular mortality and reduced risk of cardiovascular diseases (Nystoriak and Bhatnagar, 2018), musculoskeletal diseases, metabolic diseases, and psychological disorders such as depression (Maestroni et al., 2020). Cardiovascular fitness aids in increased brain

function, specifically during cognitively challenging tasks (Colcombe et al., 2004). Additionally, improvements in the plasticity of the ageing brain are also evident with cardiovascular fitness, which is demonstrated to reduce cognitive and biological senescence among humans (Colcombe et al., 2004). Moreover, exercise is reported to slow the progressive decline in grey matter and cognitive function, which is associated with ageing (Baker et al., 2010). A recent review highlighted the advantages of performing regular strength training for the musculoskeletal system in terms of muscle, bone, tendon, cartilage, and overall health (see Maestroni et al., 2020 for review).

1.7.2.Cross-limb transfer phenomena

The transfer phenomenon (which is also termed cross-education, cross-transfer, interlimb transfer, or contralateral effect) refers to any change in motor output (such as skills and force generation) of the untrained limb following unilateral exercise training (Manca et al., 2021b). Previous studies have reported positive effects of the transfer phenomenon in various conditions affecting the upper limb, such as arm fracture, in which the active range of motion and strength have been reported to improve following training of the unaffected arm (Magnus et al., 2013). Additionally, the transfer phenomenon is effective in maintaining muscle size and strength following arm immobilisation (Farthing et al., 2009, Magnus et al., 2010). Moreover, numerous studies have demonstrated that the transfer phenomenon can be leveraged to improve the recovery of motor function among chronic stroke survivors (Dehno et al., 2021, Dragert and Zehr, 2013b). For example, unilateral resistance exercise in poststroke hemiparesis has been shown to enhance muscle strength, functionality (Morris et al., 2004, Patten et al., 2004), and movement relearning (Ellis et al., 2005).

1.7.3.Mechanisms of cross-limb transfer

The exact mechanisms of cross-limb transfer remain unclear, but existing evidence suggests that it is likely to be driven by prominent cortical mechanisms (Ruddy and Carson, 2013). Some evidence also suggests that potential contributions from other locations, such as the spinal cord, may be associated with some types of unilateral training (Dragert and Zehr, 2011).

There are two broad categories of mechanisms that have been hypothesised in the literature: (i) '*bilateral access*' and (ii) '*cross-activation*'. However, it is important to acknowledge that, although these two hypotheses may differ in theoretical explanations, they are not mutually exclusive and may operate in parallel (Carroll et al., 2006, Lee and Carroll, 2007, Lee et al., 2010, Parlow and Kinsbourne, 1989).

The first hypothesis, '*bilateral-access',* suggests that the practice-induced motor plan underlying enhanced performance with the trained limb must reside at a site within the CNS that is also accessible for control of the opposite, untrained limb (Anguera et al., 2007, Imamizu and Shimojo, 1995). One example of this proposed mechanism is the 'callosal access' hypothesis (Taylor and Heilman, 1980), which states that the motor plan created in the trained hemisphere can be accessed by the opposite hemisphere via the corpus callosum, facilitating performance gains in the untrained hand (Lee et al., 2010).

In contrast, the '*cross-activation'* hypothesis by Parlow and Kinsbourne (1989) suggests that some types of cross-limb transfer arise because unilateral motor practice produces bilateral neural changes (Parlow and Kinsbourne, 1989). According to this hypothesis, a motor task results in a 'spillover' of neural activation into the circuits that control the opposite untrained (passive/control) limb, which ultimately leads to

bilateral neural reorganisation (i.e., spillover hypothesis) (Carroll et al., 2008). Supporting this hypothesis, previous imaging studies have shown that motor areas in both hemispheres are active during unilateral motor tasks (Cramer et al., 1999, Dettmers et al., 1995, Kawashima et al., 1993, Kim et al., 1993). Additionally, previous TMS research has indicated acute alterations in the excitability of ipsilateral corticospinal pathways during unilateral muscle contraction (Hess et al., 1986, Hortobágyi et al., 2003, Stedman et al., 1998). However, cross-limb transfer is highly specific to the training contraction type and intensity, with previous studies reporting a correlation between the amount of potentiation or 'cross-facilitation' and the amount of force generated by the contractions of the opposite limb (Perez and Cohen, 2008). For example, high force contractions have been shown to facilitate ipsilateral motor cortex excitability (Hess et al., 1986, Hortobágyi et al., 2003, Stedman et al., 1998), while weak unilateral contraction leads to a suppression of ipsilateral motor cortex excitability (Leocani et al., 2000, Liepert et al., 2001, Sohn et al., 2003).

1.7.4.Importance of cross-limb transfer in ageing

Advancing age leads to skeletal fragility and decreased bone density, which results in an increased risk of falls and fractures among older adults (Ensrud, 2013, McClung, 2005), with the majority presenting with single-limb fractures (Court-Brown et al., 2012). Additionally, age-related reduction in neuroplasticity limits neuromotor functional improvements (Ward, 2006, Zapparoli et al., 2022), impairing functional recovery following strokes in older adults (Laaksonen and Ward, 2022). The loss of functional capacity during the period of injury can be attenuated by training the unaffected opposite limb (see Haggert et al., 2020 for review). Therefore, the cross-limb transfer phenomenon is particularly important in older adults and poses implications for single-limb injury rehabilitation and neurological rehabilitation

(Farthing and Zehr, 2014). I have assessed cross-limb transfer effects of healthy older adults following acute unilateral resistance exercises (Chapter 2) and unilateral ballistic motor training concurrently with tACS (Chapter 4).

1.8.Summary and aims

There is extensive literature demonstrating that healthy ageing is associated with changes in the nervous system and a decline in brain and neuromuscular function. Although the age-associated loss of motor neurons and muscle fibres is irreplaceable, the structure and function of the neuromusculoskeletal system can be improved through various interventions, including exercise and motor training. Additionally, enhancing motor function and manipulating short-term neuroplasticity has involved using diverse non-invasive brain stimulation techniques. However, most of these interventions have produced inconsistent results in older adults. For example, application of stimulation before a motor performance (i.e., priming) has been shown to enhance visuomotor skills in older adults with repetitive paired-pulse TMS (Hand et al., 2023) and transcranial direct current stimulation (tDCS) (Fujiyama et al., 2017), but not with paired associative stimulation (Opie and Semmler, 2019). Whereas, when anodal tDCS is applied to M1 during motor tasks (i.e., gating), it has been shown to improve motor performance for motor adaptation tasks (Panouillères et al., 2015), speed accuracy tasks (Goodwill et al., 2013), but not for visuomotor tasks (Mooney et al., 2019). These inconsistent results highlight the need to develop targeted interventions to generate more robust effects on motor performance and neuroplasticity in older adults.

1.8.1.Thesis aims and objectives

The overall aim of this PhD was to investigate the effects of interventions, such as resistance exercise and transcranial alternating current stimulation concurrently with ballistic motor training, to promote neuroplasticity and improve motor function in healthy older adults.

Despite the presence of inconsistent definitions and age ranges for older adults in the literature, adults aged 60 years or over were considered 'older adults' and included in the experimental studies in this thesis (Phraknoi et al., 2023).

This thesis includes several specific aims:

1. To use HDsEMG and iEMG to characterise age-related changes in the cross-limb transfer effect induced by a single bout of unilateral resistance exercise, focusing on changes in force production, force steadiness, and motor unit characteristics (Chapter 2- Published).
2. To explore the effect of HD theta-gamma tACS on ballistic motor performance in healthy young and older adults, focusing on immediate motor performance, post-training performance (~60 minutes post-training), and corticomotor plasticity explored using TMS (Chapter 3- Published).
3. To evaluate the effects of HD tACS (theta tACS and theta-gamma tACS) on motor performance improvement and cross-limb transfer effects induced by unilateral ballistic motor training in healthy older adults, assessing its potential to enhance bilateral motor performance and neuroplasticity (Chapter 4).

CHAPTER 2 (Published)

BILATERAL NEUROMUSCULAR ADAPTATION TO ACUTE UNILATERAL RESISTANCE EXERCISE IN HEALTHY OLDER ADULTS

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ORIGINAL ARTICLE

Bilateral neuromuscular adaptation to acute unilateral resistance exercise in healthy older adults

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Abstract Resistance exercise (RE) enhances functionality in older adults and has proven effective as a means of cross-education in scenarios of unilateral disuse. However, the extent to which older adults demonstrate cross-limb transfer at the motor unit (MU) level following a single bout of unilateral RE is unclear. Thirteen healthy older adults (74.9 ± 4.8 years; 5 females) underwent bilateral neuromuscular assessments pre- and post- a single bout of unilateral RE consisting of sets of 12 repetitions of leg extension of the dominant (exercise) leg, at 75% of 1 repetition maximum, performed to failure. Maximum voluntary contraction (MVC) and force steadiness (FS) were measured. Central and peripheral features of individual MU were recorded using high-density surface electromyography and intramuscular electromyography (HDs/iEMG), during contractions normalised to 25% MVC. Following unilateral RE, MVC reduced in exercise (-14.8%, $p<0.001$) and control (-6.9%, $p=0.003$) legs, with reduced FS performance in the exercise leg compared to the control ($p=0.002$). MU firing rate increased during contractions normalised to 25% baseline MVC in the exercised leg ($p<0.05$), with no adaptation in the control leg ($p>0.05$). All iEMG recorded measures of MU potentials remained unchanged in both legs (all $p>0.05$). Acute unilateral RE leads to bilateral MVC reduction in older males and females, demonstrating the cross-limb transfer effect. However, adaptation of MU features was only apparent in the exercised limb, and mechanisms underlying the force decline in the non-exercised limb remain uncertain.

Keywords Cross-education · Motor units · Neuromuscular · Electromyography · Ageing · Resistance exercise

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Introduction

Ageing is associated with a substantial decline in muscle mass, strength, and function leading to detrimental

2.1. Statement of authorship

Title of paper	Bilateral neuromuscular adaptation to acute unilateral resistance exercise in healthy older adults.
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Principal Author

Name of principal author (candidate)	Nishadi N H Gamage		
Contribution to the paper	Conceptualisation; data curation; formal analysis; investigation; methodology; project administration; validation; visualisation; writing—original draft.		
Overall percentage (%)	70%		
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	07/07/2025

Co-author contributions

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

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

Name of co-author	Abdulmajeed Altheyab		
Contribution to the paper	Conceptualisation; Methodology; writing—review and editing.		
Signature		Date	07/07/2025

Name of co-author	Yuxiao Guo		
Contribution to the paper	Methodology; writing—review and editing.		
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Contribution to the paper	Conceptualisation; methodology; resources; validation; writing—review and editing.		
Signature		Date	07/07/2025

Name of co-author	George M. Opie		
Contribution to the paper	Supervision; validation; writing—review and editing.		
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Contribution to the paper	Supervision; validation; writing—review and editing.		
Signature		Date	07/07/2025

Name of co-author	Philip Atherton		
Contribution to the paper	Conceptualisation; funding acquisition; methodology; project administration; resources; supervision; validation; writing—review and editing.		
Signature		Date	07/07/2025

Name of co-author	Mathew Piasecki		
Contribution to the paper	Conceptualisation; data curation; funding acquisition; methodology; project administration; resources; supervision (primary); validation; visualisation; writing—review and editing.		
Signature		Date	07/07/2025

2.2. Abstract

Resistance exercise (RE) enhances functionality in older adults and has proven effective as a means of cross-education in scenarios of unilateral disuse. However, the extent to which older adults demonstrate cross-limb transfer at the motor unit (MU) level following a single bout of unilateral RE is unclear. Thirteen healthy older adults (74.9 ± 4.8 years; 5 females) underwent bilateral neuromuscular assessments pre- and post- a single bout of unilateral RE consisting of sets of 12 repetitions of leg extension of the dominant (exercise) leg, at 75% of 1 repetition maximum, performed to failure. Maximum voluntary contraction (MVC) and force steadiness (FS) were measured. Central and peripheral features of individual MU were recorded using high-density surface electromyography and intramuscular electromyography (HDs/iEMG), during contractions normalised to 25% MVC. Following unilateral RE, MVC reduced in exercise (-14.8%, $P < 0.001$) and control (-6.9%, $P = 0.003$) legs, with reduced FS performance in the exercise leg compared to the control ($P = 0.002$). MU firing rate increased during contractions, normalised to 25% baseline MVC in the exercised leg ($P < 0.05$), with no adaptation in the control leg ($P > 0.05$). All iEMG recorded measures of MU potentials remained unchanged in both legs (all $P > 0.05$). Acute unilateral RE leads to bilateral MVC reduction in older males and females, demonstrating the cross-limb transfer effect. However, adaptation of MU features was only apparent in the exercised limb, and the mechanisms underlying the force decline in the non-exercised limb remain uncertain.

Keywords: Cross-education, motor units, neuromuscular, electromyography, ageing, resistance exercise

2.3. Introduction

Ageing is associated with a substantial decline in muscle mass, strength, and function, leading to detrimental outcomes such as mobility disorders, frailty, increased fall rates, and reduction in general health status (Wilkinson et al., 2018). The age-related decline in neuromuscular function is partly explained by motor unit (MU) remodelling, in which MUs generally become fewer in number and larger in size (Jones et al., 2022), and this may have implications for neuromuscular control (Pethick and Piasecki, 2022). Although the age-related loss of motor neurons and muscle fibres is irreplaceable, the structure and function of the musculoskeletal system can be improved through exercise training (McPhee et al., 2016). Therefore, performing exercises, particularly resistance training, is known to be effective for older adults and elicits significant enhancements in neuromuscular function (Peterson et al., 2010).

Cross-education (CE) describes the widely reported effect of unilateral training conferring benefit to the non-exercised contralateral limb (Altheyab et al., 2024). Although the exact mechanism of cross-limb transfer remains uncertain, it likely has cortical origins (Calvert and Carson, 2022, Ruddy and Carson, 2013). This phenomenon is effective in both the upper and lower limbs (Gabriel et al., 2006), following stroke (Dehno et al., 2021, Dragert and Zehr, 2013a) and in orthopaedic conditions (Manca et al., 2021a). Given the age-related decline in neuromotor function (Ward, 2006, Zapparoli et al., 2022) impairing functional recovery following stroke (Laaksonen and Ward, 2022), and increased risk of falls and fractures among older individuals (Ensrud, 2013, McClung, 2005), with the majority presenting with single-limb fractures (Court-Brown et al., 2012), cross-limb transfer phenomenon is particularly important for older adults (Farthing and Zehr, 2014).

Exercise-induced decline in force and/or power generation due to impaired muscle activation and/or contractile function is termed performance fatigue and originates from central and peripheral sources (Enoka and Duchateau, 2016). Similar to CE, cross-limb transfer of muscle fatigue is defined as a temporary deficit in performance of the non-exercised contralateral limb following a unilateral fatiguing protocol (Doix et al., 2013, Martin and Rattey, 2007). In young adults, higher-intensity fatiguing contractions are reported to generate greater non-local muscle fatigue effects compared to lower-intensity contractions, particularly in lower limbs (Kawamoto et al., 2014).

Ageing is not a limiting factor in some aspects of cross-limb transfer (Barss et al., 2016). However, there are several knowledge gaps regarding cross-limb transfer, including a lack of data characterising individual MU adaptations in the knee extensors (KE). Additionally, research on this phenomenon in older populations—where cross-limb transfer could be particularly valuable as an intervention—is limited, and to our knowledge, there is no available data following a single bout of unilateral KE fatiguing exercise in older individuals. Therefore, the current study aimed to evaluate the cross-limb transfer effects of acute resistance exercise (RE) on neuromuscular function, and central and peripheral features of MU adaptation in older adults. We hypothesised that both the exercised and control limbs would display reduced function, with MU firing properties altered in both, and peripheral MU features altered in the exercised limb only.

2.4. Methods

2.4.1. Ethical approval

This study was approved by the University of Nottingham Faculty of Medicine and Health Sciences Research Ethics Committee (*FHMS - 390-1121*) and was conducted in accordance with the *Declaration of Helsinki*, except for registration in a database. All participants provided written informed consent.

Seventeen healthy older adults (9 females) with a mean age (± SD) of 73.8 (± 4.9) years were enrolled in this study. Participants were excluded if they had a BMI < 18 or > 35 kg/m^2, any diagnosed neurological, musculoskeletal, cardiovascular (such as uncontrolled hypertension, recent cardiac event), metabolic disorders (such as diabetes), chronic kidney disease, malignancy (in previous 6 months), recent steroid treatment (within 6 months), respiratory diseases or if they engaged in any structured exercise training within the past 6 months. Participants were recreationally active but not involved in standard exercise programs. All participants were advised to refrain from strenuous exercises at least 48 hours before the laboratory visits. Of the 17 participants who completed the study, 13 participants (5 females, 74.9 ± 4.8 years) had sufficient data quality for high-density surface electromyography (HDsEMG) analyses, therefore data are presented for these only. Of these 13, 11 participants (4 females) were included in intramuscular electromyography (iEMG) analyses due to the low yield of MUs at one or more time points.

2.4.2. Experimental procedures

The experimental protocol and timeline are shown in Figure 2.1.A. All participants attended two laboratory visits (screening visit and study day) separated by 7-10 days.

Screening visit

During the screening visit, participants completed a comprehensive medical screening that included fasting blood tests, an electrocardiogram, blood pressure assessment, and a general health questionnaire, which allowed the exclusion of participants against pre-determined criteria as listed above. Additionally, during the screening visit, 1 repetition maximum (1 RM) and VL muscle ultrasound (detailed below) were assessed.

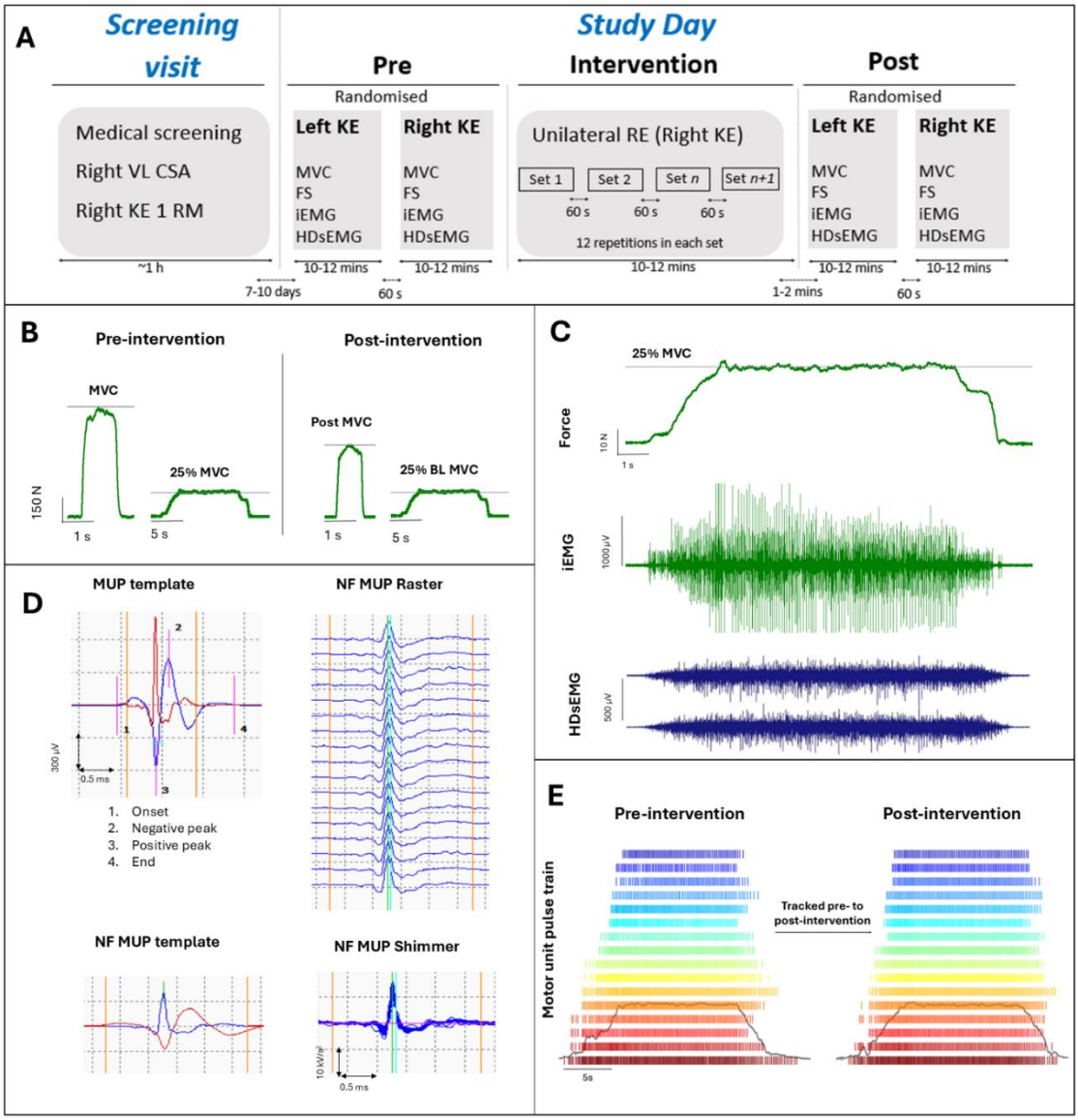


Figure 2.1. Schematic representation of the experimental protocol and timeline (A). Example knee extensor force traces of an older male participant showing MVC and 25% MVC of the exercise leg obtained pre- and post-RE intervention (B). Example force trace (upper) with corresponding intramuscular electromyography (iEMG) trace (middle) and a subset of 2 HDsEMG channels (lower) obtained during a sustained isometric knee extension at 25% of MVC (C). Example iEMG recorded MUP and corresponding near fibre (NF) MUP sampled with iEMG, including consecutive NFMUPs shown as raster and shimmer plots (D). Example HDsEMG recorded MU pulse trains (firing times) of MUs tracked from pre- to post-intervention. The grey line indicates the force trace with a plateau held at 25% MVC (E). *Abbreviations*: *VL; vastus lateralis, KL; knee extensor, CSA; cross-sectional area; 1 RM; One repetition maximum, FS; force steadiness; iEMG; intramuscular electromyography, HDsEMG; High-density surface electromyography, MVC, maximal voluntary contraction; BL, baseline; MUP, motor unit potential; NF, near fibre*

One repetition maximum (1 RM) assessment

The 1 RM is defined as the maximum weight that could be lifted through the prescribed range once only (McNair et al., 2011). A seated leg extension machine was used to measure 1 RM with an initial warm-up of 5-10 low-load repetitions performed on the dominant (right in all) limb. After one minute of rest, participants were asked to perform full knee extension movement with the load at ~80% of estimated 1 RM. After each successful attempt, the weight was increased progressively until a failed attempt occurred (Seo et al., 2012). Each attempt was separated by one minute rest and 1 RM was obtained within five attempts. The 1RM was performed to enable the setting of weight for the acute RE intervention on the study day (75% of 1RM).

Muscle ultrasound recording

The cross-sectional area (CSA) of VL muscle of the exercise limb was measured during the screening visit using an ultrasound probe (LA523 probe, B- mode, frequency range 26- 32 Hz and MyLabTM50 scanner, Esaote, Genoa, Italy) positioned at the anatomical mid-point of VL, which was measured and identified between the greater trochanter and the midline of the patella. A conductive gel was applied to the surface of the probe to improve the fidelity of images. The ultrasound probe was positioned at the VL midpoint and moved in the medial-to-lateral direction to locate the medial and proximal borders of VL where the aponeurosis of VL intersected with the Vastus Intermedius muscle (Guo et al., 2024). Three axial plane images were obtained and the mean area of three images was considered as CSA. Ultrasound images were analysed using ImageJ software (National Institutes of Health, United States) and the average of three ultrasound images was considered as VL CSA of the exercise limb.

Study day assessment

Participants who completed the screening visit and were deemed eligible against the study criteria were invited to the study day assessment. Participants arrived at the laboratory at ~09.00 h (± 1 h) after an overnight fast. The study day experimental procedures included the assessment of maximal voluntary contraction (MVC), force steadiness (FS), iEMG, and HDsEMG measures before and immediately after the unilateral RE (Figure 2.1.A). All described measures were individually performed on both legs and the order of testing was randomised between the legs.

Functional properties

A purpose-built isometric dynamometer (Load cell amplifier; LCA1,12V1A medical PSU, GDM25B12-P1J, Sunpower Electronics, Reading, United Kingdom) was used to measure MVC for knee extension. Participants were seated with their hip and knee flexed at approximately 90° and a seat belt was used to avoid hip or trunk movement during the leg extension. The leg to be tested was securely strapped to a plate connected to a force transducer at the ankle. After a series of moderate-intensity warm-ups, participants were instructed to perform three maximal isometric knee extensions with a 60-second rest between trials. Real-time visual feedback (Spike software v9.06; Cambridge Electronic Design, Cambridge, United Kingdom) and verbal encouragement were provided throughout all attempts. The highest isometric knee extension of the three recorded values was considered the MVC. To quantify the FS, force signals during the plateau phase of a sustained voluntary contraction performed at 25% MVC were low-pass filtered at 20 Hz (fourth-order Butterworth digital filter), and the coefficient of variation (CoV) of force was quantified.

High-density surface electromyography (HDsEMG)

A semi-disposable high-density surface electromyography (HDsEMG) array (64 electrodes, 13 × 5, 8 mm, I.E.D., GR08MM1305, OT Bioelettronica, Inc., Turin, Italy) was positioned over the VL muscle belly with approximate orientation of the muscle fascicles (proximal to distal). Prior to this, the skin was prepared by shaving, applying light abrasion, and cleansing with 70% ethanol. These HDsEMG electrodes were secured using flexible tape and remained in place during the intervention and post-RE assessments. A ground electrode (WS2, OTBioelettronica, Turin, Italy) was positioned around the ankle of the tested leg. HDsEMG signals were acquired in a monopolar configuration, amplified (× 256) with filtering set at 10–500 Hz and

digitally converted at 2000 Hz by a 16-bit wireless amplifier (Sessantaquattro, OTBioelettronica, Turin, Italy) and transferred to a PC for offline analysis.

Intramuscular EMG (iEMG)

The motor point of VL was identified using low-intensity percutaneous electrical stimulations (400V, pulse width 50 μS, current ~10 mA; delivered via a Digitimer DS7A, Welwyn Garden City, United Kingdom). The site of VL, which produced the largest visible twitch from the smallest electrical current, was considered the motor point, and its proper localisation is considered critical for electrode positioning (Botter et al., 2011) .

Intramuscular electromyography (iEMG) recordings were obtained using a disposable concentric needle (model N53153, Teca, Hawthorne, New York, United States) with a recording area of 0.07 mm^2. The grounding electrode was placed over the patella of the tested leg. Subsequently, the surrounding skin at the motor point was prepared by shaving and cleansing using an alcohol adhesive wipe. Participants were instructed to relax their tested leg to enable insertion of the needle into the muscle belly of VL until the appropriate depth of 1.0-2.5 cm was reached. Next, participants were instructed to perform isometric leg extension, and the signal quality was inspected, and the needle was adjusted where necessary. Subsequently, the needle was repositioned by rotating the bevel 180° and slightly retracted by ~5 mm to ensure that different depths of the muscle were captured (Jones et al., 2021). Once the appropriate contraction sequence (sets of 25% of MVC) was completed (minimum of four recordings from distinct perspectives/regions), the needle was withdrawn and disposed of. The EMG signals were recorded at 50 kHz and bandpass filtered at 10 Hz to 10 kHz (CED Micro 1401; Cambridge Electronic Design, Cambridge, United Kingdom) and visualised using

Spike2 (version 9, CED) software to provide real-time visual feedback on a screen positioned in front of the participants.

Resistance exercise (RE) protocol

The acute RE protocol target was set as continuous sets of unilateral leg extension, with each set consisting of 12 repetitions and a 60-second rest period between the sets (Figure 2.1.A). The dominant limb was engaged in RE on a standard leg extension machine (exercise leg), with the contralateral limb functioning as a control (control leg). The exercise intensity was set at the participant's 75% of 1 RM and performed to failure. The criterion for failure was the participant's inability to complete the full range of leg extension despite the investigator's encouragement and was validated by the Borg rating of perceived exertion (RPE) scale corresponding to extremely hard-maximal exertion (19-20/20) (Borg and Kaijser, 2006). The mean number of repetitions completed was 40 (SD; 3.06, range; 33-44), corresponding to 3 sets and 4 additional repetitions.

2.4.3. Data analysis

The HDsEMG signals and corresponding force were selected from contraction number 3 or 4 of the 4 contractions performed. Each force trace was visually inspected and that with the least variation was chosen for analysis. This was analysed offline using the convolution kernel compensation method (Holobar and Zazula, 2007). MU filters and corresponding spike trains estimated with this process (Francic and Holobar, 2021) were visually inspected and optimised using custom software (DEMUSE, MATLAB) and standardised procedures, all performed by a single operator (MP) (Del Vecchio et al., 2020). Only MUs with a pulse-to-noise ratio (PNR) $\geq$ 30 dB were included for further analyses. After editing each MU spike train, MUs were tracked

from pre- to post-intervention via concatenation of signals and application of MU filters from one contraction (within each leg) to the other.

MU firing rate (MUFR) at the recruitment and derecruitment phases (Figure 2.1.E) of isometric knee extension was calculated as the mean instantaneous firing rate (FR) of the first 5 observations of each MU. MUFR during the sustained phase was the mean instantaneous FR throughout this segment. FR variability was considered as the CoV of the inter-spike interval (ISI) during the plateau phase. MU recruitment and derecruitment threshold was defined as the force level corresponding to the first and last observed firing of each MU, respectively. MUs that were tracked from pre- to post-intervention were used to compute a cumulative spike train (CST), which represents the neural drive to the muscle (Martinez-Valdes et al., 2022).

Decomposition of iEMG signals was performed using Decomposition-based quantitative electromyography (DQEMG) software, to extract motor unit potential trains (MUPTs) (Parsaei et al., 2009). This includes preprocessing of signal, signal segmentation and MUP detection, clustering the identified MUPs, and supervised classification of detected MUPs (Stashuk, 2001). Visual inspection of individual MUP templates was performed to ensure cursors were positioned accurately at the onset, endpoint, positive peak, and negative peak of the waveforms (Figure 2.1.D). Where required, cursors were adjusted and repositioned during visual inspection. MUPTs were excluded if they contained MUPs from multiple MUs or fewer than 40 MUPs (Inns et al., 2022).

MUP area, measured in μVms, was defined as the integral of the absolute value of the MUP values between the onset and end markers, multiplied by the sampling time interval (Piasecki et al., 2021b). MUP complexity was assessed using the number of

turns in the MUP template, defined as the number of significant changes in direction within the MUP duration (height > 20 μv) (Piasecki et al., 2021b). The MUP negative peak ratio was calculated based on the absolute value of the 'rise' of the MUP template negative peak (within the 500 μs interval before the negative peak), divided by the 'fall' of the MUP template negative peak (within the 500 μs interval after the negative peak) (Jones et al., 2023). Individual MUP templates were used to calculate a near-fibre MUP (NFM) (Piasecki et al., 2021b). Individual NFMs were visually inspected using DQEMG software, and any that were contaminated from other NFMs were excluded from the analysis. NFM parameters were used to estimate NF jiggle as an estimate of neuromuscular junction transmission instability (Piasecki et al., 2021b).

2.4.4. Statistical analysis

All statistical analysis was conducted using RStudio (Version 4.4.0). To assess differences in MVC, FS, HDsEMG, and iEMG MU variables, separate linear mixed-effects models were constructed with fixed effects of Time and Leg, and their interaction (Leg × Time). The model included random intercepts for each Subject to account for repeated measures. For tracked MU models, MUs were also nested within Subject. All linear-mixed effects models were generated using *lmer* R package (Bates et al., 2015). Where significant main effects were detected, pairwise *post-hoc* tests of the estimated marginal means (EMMs) were performed (Lenth and Lenth, 2018). To assess model fit and estimate the proportion of variance explained, the marginal R^2 (R^2_m) and conditional R^2 (R^2c) are included in each table of model outputs. The R^2_m represents the proportion of variance explained by the fixed effects, and R^2c represents the variance explained by both fixed effects and random effects (Nakagawa and Schielzeth, 2013). Cohen's d effect sizes are reported for all *post-hoc* comparisons. For non-hierarchical models (e.g., MVC and FS), effect sizes were calculated directly

from paired data using the standard deviation of within-subject differences. For hierarchical models in which MUs were nested within subjects, Cohen's d was calculated from EMMs derived from the linear mixed-effects models using *effectsize* R package (Ben-Shachar et al., 2020), accounting for the nested data structure. Delta CST values were compared across limbs with a repeated measures t-test. Pearson correlation test was used to assess the strength, direction, and significance of the relationship between delta MVC and delta FR. The tabulated results are displayed as EMMs, 95% confidence intervals (CI), and *p* values. Statistical significance was accepted at $P < 0.05$.

2.5. Results

2.5.1. Participant characteristics

Participant characteristics are shown in Table 2.1. All participants completed the experimental session without any adverse effects.

Table 2.1. Descriptive characteristics of participants *(n = 13; 5 female)*

Age (years)	74.9 (4.8)
Height (cm)	167.6 (6.8)
Weight (kg)	73.5 (11.7)
BMI (kg/m^2)	26.1 (3.0)
VL CSA (cm^2)	17.1 (4.3)

Data shown as mean (standard deviation). VL CSA obtained from the exercise (dominant) leg only at baseline. *Abbreviations: BMI, body mass index; VL CSA, vastus lateralis cross-sectional area.*

2.5.2. Functional properties

All model outputs, including estimated marginal means (EMM) for functional properties, are shown in Table 2.2. Knee extensor MVC at each timepoint (pre and

post) for the exercise (right) and control (left) legs for 13 participants are shown in Figure 2.2.A. There was a significant leg × time interaction on knee extensor MVC (P = 0.013), and a main effect of time (P = 0.003), with no main effect of leg (P = 0.388). EMMs show a mean decrease in MVC of 14.8% (P < 0.001, d = 1.58) in the exercise leg, and of 6.9% in the control leg (P = 0.003, d = 1.36).

For FS, there was a significant leg × time interaction (P = 0.032), but no effect of leg (P = 0.742) or time (P = 0.062). *Post-hoc* analysis indicated that FS CoV was comparable between the legs at baseline (P = 0.743, d = 0.093), however, following the intervention, FS CoV was higher in the exercise leg compared to the control leg (P = 0.002, d = 0.889) (Figure 2.2.B).

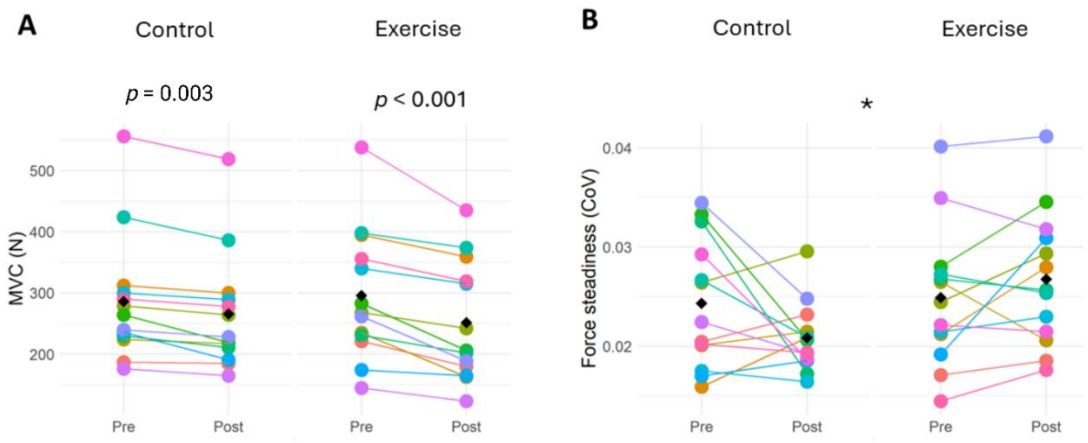


Figure 2.2. Effect of acute unilateral RE on knee extensor MVC (A) and force steadiness (B) in control (left) and exercise (right) legs. Data points are colour-coded for individual participants with the mean of means indicated by black diamonds. *Significant difference between the exercise and control legs at post-intervention. *Abbreviations*: *MVC, maximal voluntary contraction; CoV, Coefficient of variation of force at 25% of MVC*

2.5.3. Motor unit discharge characteristics

From HDsEMG, a total of 616 MUs from 13 participants were sampled in both limbs at both timepoints. Individual MU counts and all model outputs are shown in Table 2.2.

MU firing rate (MUFR) at recruitment showed a significant leg × time interaction (P = 0.001), a significant effect of leg (P = 0.001), but no effect of time (P = 0.182). EMMs showed that, at baseline, MUFR at recruitment was greater in the control leg compared to the exercise leg (P = 0.02, d = 0.171), however post-intervention, it was greater in the exercise leg (P < 0.001, d = 0.369). Moreover, MUFR at recruitment did not differ in the control leg from pre- to post-intervention (P = 0.182, d = 0.105), but for the exercise leg, MUFR at recruitment was significantly greater at post compared to baseline (P < 0.001, d = 0.435) (Table 2.2, Figure 2.3.A).

During the plateau phase, there was a significant leg × time interaction (P < 0.001) and a significant effect of leg (P < 0.001), but there was no main effect of time (P = 0.280). While MUFR at plateau was similar between the legs at baseline (P = 0.477, d = 0.056), following the intervention, FR at plateau was greater for the exercise leg compared to the control leg (P < 0.001, d = 0.741). Additionally, while MUFR at plateau did not change in the control leg across timepoints (P = 0.280, d = 0.091), it was significantly greater following the intervention compared to the baseline for the exercise leg (P < 0.001, d = 0.705) (Table 2.2, Figure 2.3.C).

For MUFR at derecruitment, there was a significant leg × time interaction (P < 0.001) and a significant effect of leg (P < 0.001), but there was no significant effect of time (P = 0.467). EMMs showed that MUFR at derecruitment was comparable between legs at baseline (P = 0.622, d = 0.032), but following the intervention, MUFR at

derecruitment was greater for the exercise leg compared to the control leg ($P < 0.001$, $d = 0.547$). Additionally, although MUFR at derecruitment did not change in the control leg from baseline to post ($P = 0.466$, $d = 0.050$), there was greater MUFR at derecruitment at post compared to baseline for the exercise leg ($P < 0.001$, $d = 0.565$) (Table 2.2, Figure 2.3.E).

There was no significant leg × time interaction for MUFR variability ($P = 0.476$) and no significant effect of leg ($P = 0.607$), or time ($P = 0.619$) (Table 2.2, Figure 2.3.G).

For MU recruitment threshold, there was no significant leg × time interaction ($P = 0.176$), or significant effect of leg ($P = 0.331$) or time ($P = 0.277$) (Table 3.2, Figure 2.4.A). Similarly, for MU derecruitment threshold, there were no significant leg × time interaction ($P = 0.810$), or significant effect of leg ($P = 0.441$), or time ($P = 0.906$) (Table 2.2, Figure 2.4.C).

Table 2.2. Summary of linear regression analyses for functional measures and HDsEMG non-tracked MUs.

	Pre-intervention		Post-intervention		Model fit	Leg	Time	Leg × Time
	Control	Exercise	Control	Exercise				
Functional assessments								
MVC (N)	286 (226 –346)	296 (235 – 356)	266 (205 – 326)	252 (191 – 312)	R^2_m = 0.03 R^2c = 0.98	0.388	0.003 *	0.013*
Force steadiness (CoV)	0.024 (0.020 – 0.028)	0.025 (0.021 – 0.029)	0.021 (0.018 – 0.024)	0.027 (0.024 – 0.030)	R^2_m = 0.11 R^2c = 0.61	0.742	0.062	0.032*
HDsEMG non-tracked MU								
No of MUs	177	178	133	128				
FR recruitment (pps)	6.21 (5.76 – 6.66)	5.89 (5.44 – 6.34)	6.01 (5.55 – 6.48)	6.69 (6.23 – 7.16)	R^2_m = 0.04 R^2c = 0.25	0.001 *	0.182	0.001*
FR plateau (pps)	7.43 (6.75 – 8.11)	7.32 (6.65 – 8.00)	7.26 (6.57 – 7.95)	8.66 (7.97 – 9.35)	R^2_m = 0.09 R^2c = 0.42	0.001 *	0.280	0.001*
FR derecruitment (pps)	5.04 (4.62 – 5.46)	5.09 (4.67 – 5.51)	5.12 (4.69 – 5.55)	6.07 (5.64 – 6.50)	R^2_m = 0.09 R^2c = 0.35	0.001 *	0.467	0.001*
FR variability (%)	0.141 (0.127 – 0.155)	0.124 (0.110 – 0.138)	0.137 (0.121 – 0.153)	0.112 (0.096 – 0.128)	R^2_m = 0.03 R^2c = 0.06	0.607	0.619	0.476
Recruitment threshold (N)	33.3 (24.8 – 41.9)	33.6 (25.1 – 42.2)	35.6 (26.9 – 44.3)	31.8 (23.1 – 40.5)	R^2_m = 0.01 R^2c = 0.34	0.331	0.277	0.176
Derecruitment threshold (N)	30.9 (22.8 – 39.0)	34.5 (26.4 – 42.6)	31.1 (22.9 – 39.3)	35.3 (27.1 – 43.5)	R^2_m = 0.01 R^2c = 0.42	0.441	0.906	0.810

Data presented as estimated marginal means (lower 95% CI, upper 95% CI). Final four columns show model fit and *P* values. *Abbreviations*: *MVC, maximal voluntary contraction; CoV, Coefficient of variation of force at 25% of MVC; FR, firing rate;* R^2_m, marginal R^2 representing variance explained by fixed effects only; R^2c, conditional R^2 representing variance explained by the full model (fixed + random effects). *Statistically significant ($P < 0.05$).

2.5.4. Discharge characteristics of tracked MUs

A total of 374 MUs (61% of all recorded MUs) from 13 participants were successfully tracked from pre- to post-intervention: 179 in the control leg and 195 in the exercised leg. All model outputs for tracked MUs are shown in Table 2.3.

For MUFR at recruitment, there was a significant leg × time interaction ($P < 0.001$) and a significant effect of leg ($P = 0.008$), but there was no effect of time ($P = 0.545$). EMMs analysis indicated that at baseline, MUFR at recruitment was higher in the control leg compared to the exercise leg ($P = 0.008$, $d = 0.265$), whereas post-intervention, MUFR at recruitment was higher in the exercise leg compared to the control leg ($P = 0.003$, $d = 0.303$). Additionally, MUFR at recruitment did not differ in the control leg from pre to post ($P = 0.545$, $d = 0.045$), but for the exercise leg, it was significantly greater at post compared to the baseline ($P < 0.001$, $d = 0.613$) (Table 2.3, Figure 2.3.B).

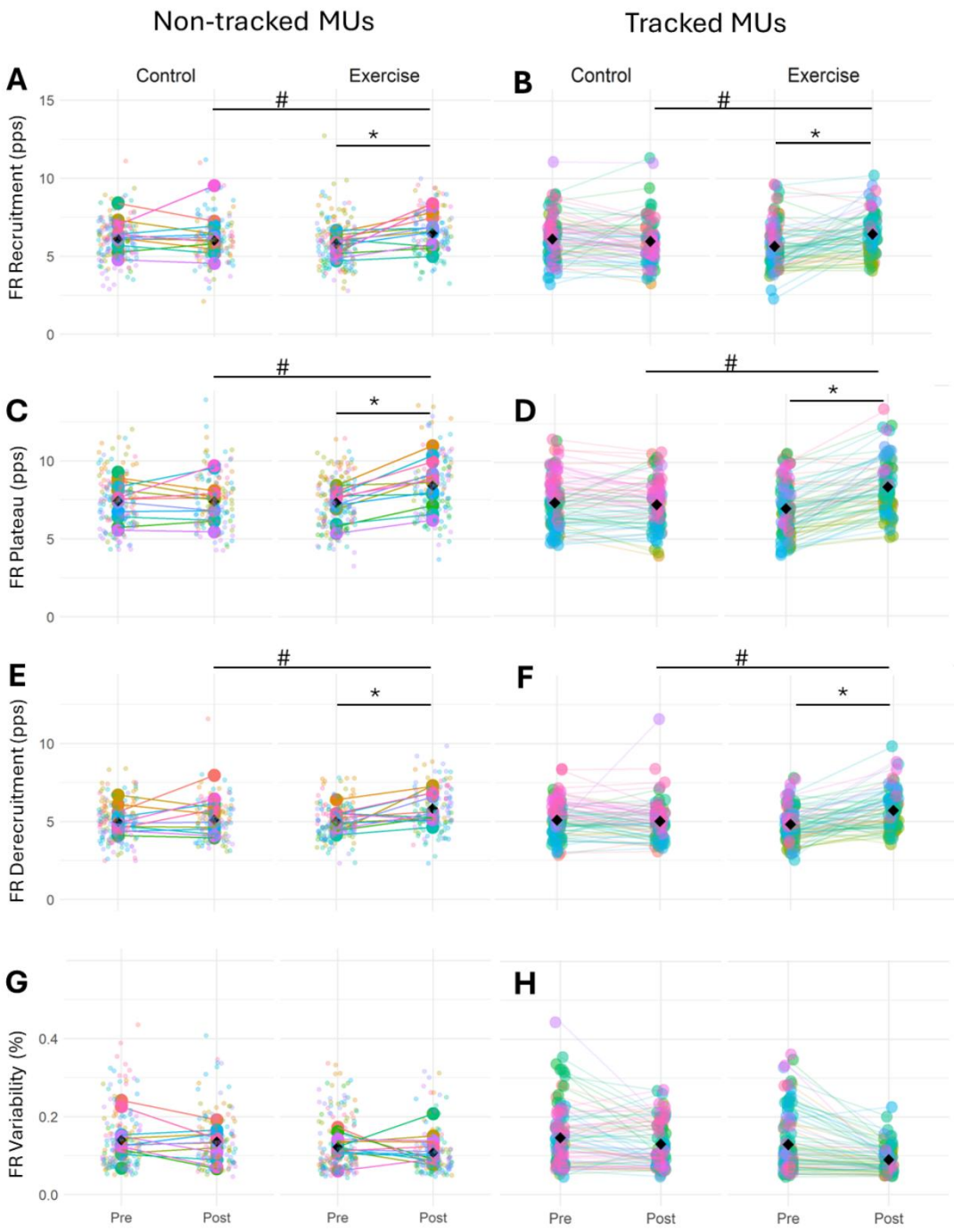


Figure 2.3. Motor unit firing rate (MUFR) at 25% MVC for non-tracked and tracked motor units in the exercise and control legs before and after the unilateral RE intervention. MUFR at the recruitment phase for non-tracked (A) and tracked (B) motor units. MUFR at plateau phase for non-tracked (C) and tracked (D) motor units. MUFR at derecruitment phase for non-tracked (E) and tracked (F) motor units. MUFR variability for non-tracked (G) and tracked (H) motor units during the plateau phase. Data points are colour-coded for individual participants with the mean of means

indicated by black diamonds. Statistical analyses are based on multi-level mixed effects linear models. *Significantly different between pre and post timepoints (P < 0.001). # Significant difference between the exercise and control legs at post intervention (P < 0.001). *Abbreviations: FR, firing rate; MUs, motor units*

There was a significant leg × time interaction for MUFR at the plateau (P < 0.001), but there was no effect of leg (P = 0.257) or time (P = 0.408). EMMs analysis showed that MUFR at the plateau was comparable between the legs at baseline (P = 0.257, d = 0.122), but post-intervention, it was greater in the exercise leg compared to the control leg (P < 0.001, d = 0.818). Moreover, although MUFR at the plateau did not differ in the control leg across timepoints (P = 0.408, d = 0.043), MUFR at the plateau significantly increased from baseline to post in the exercise leg (P < 0.001, d = 0.982) (Table 2.3, Figure 2.3.D).

Considering MUFR at derecruitment, there was a significant leg × time interaction (P < 0.001), but there was no difference between the legs (P = 0.303) or timepoints (P = 0.510). EMMs analysis indicated that MUFR at derecruitment did not differ between the legs at baseline (P = 0.303, d = 0.076), but following the intervention, MUFR at derecruitment was significantly greater for the exercise leg compared to the control leg (P < 0.001, d = 0.588). Additionally, MUFR at derecruitment in the control leg did not change across timepoints (P = 0.576, d = 0.298), but for the exercise leg, MUFR at derecruitment was significantly greater at post compared to the baseline (P < 0.001, d = 0.963) (Table 2.3, Figure 2.3.F).

Considering FR variability, there was no significant interaction between leg × time (P = 0.110) and no significant differences between legs (P = 0.095) nor timepoints (P = 0.152) (Table 2.3, Figure 2.3.H).

For the recruitment threshold, there was no leg × time interaction (P = 0.222), or significant effect of leg (P = 0.688), or time (P = 0.800) (Table 2.3, Figure 2.4.B). Similarly, for derecruitment threshold, there was no leg × time interaction (P = 0.839), or significant effect of leg (P = 0.223), or time (P = 0.839) (Table 2.3, Figure 2.4.D).

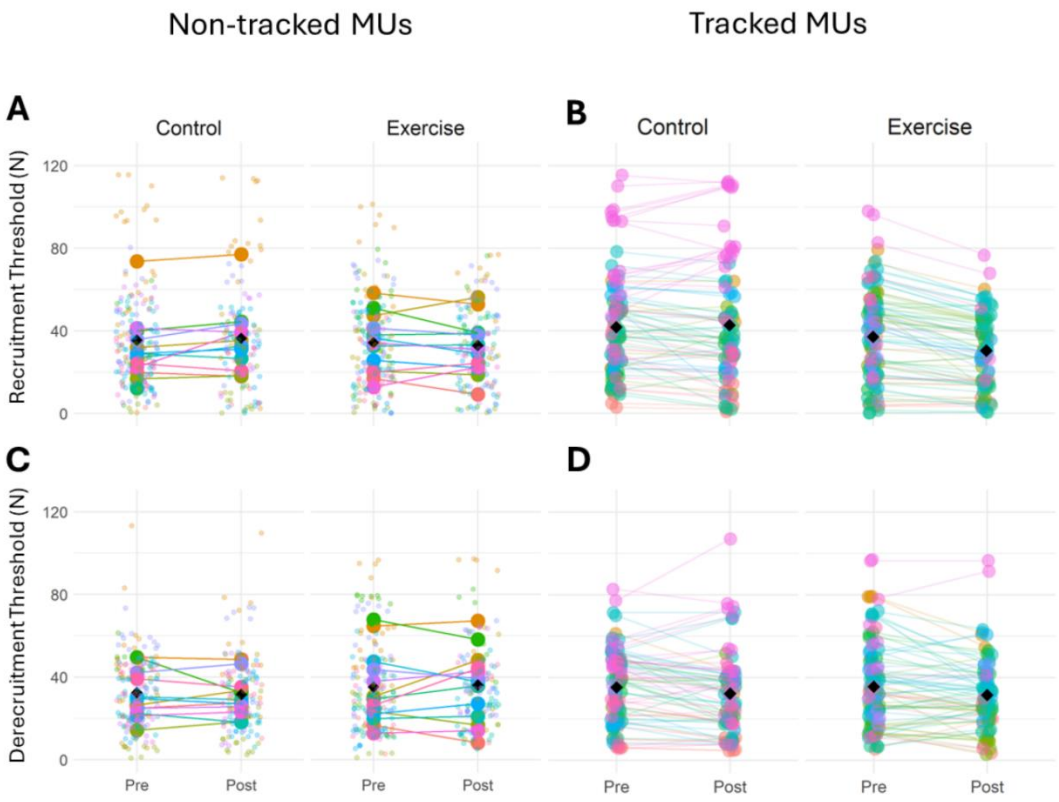


Figure 2.4. Motor unit recruitment and derecruitment thresholds at 25% MVC for non-tracked and tracked motor units in the exercise and control legs before and after the unilateral RE intervention. MU recruitment threshold for non-tracked (A) and tracked (B) motor units. MU derecruitment threshold for non-tracked (C) and tracked (D) motor units. Data points are colour-coded for individual participants, with the mean of means indicated by black diamonds. Statistical analyses are based on multi-level mixed effects linear models

Table 2.3. Summary of linear regression analyses for HDsEMG tracked MUs.

	Pre-intervention		Post-intervention		Model fit	Leg	Time	Leg × Time
	Control	Exercise	Control	Exercise				
FR recruitment (pps)	6.28 (5.66 – 6.90)	5.82 (5.21 – 6.43)	6.17 (5.54 – 6.80)	6.76 (6.13 – 7.39)	$R^2_m = 0.06$ $R^2c = 0.51$	0.008*	0.545	0.001*
FR plateau (pps)	7.33 (6.57 – 8.08)	7.12 (6.37 – 7.86)	7.16 (6.40 – 7.93)	8.69 (7.93 – 9.46)	$R^2_m = 0.14$ $R^2c = 0.68$	0.257	0.408	0.001*
FR derecruitment (pps)	5.14 (4.65 – 5.63)	5.00 (4.52 – 5.48)	5.06 (4.56 – 5.56)	6.05 (5.55 – 6.55)	$R^2_m = 0.14$ $R^2c = 0.73$	0.303	0.576	0.001*
FR Variability (%)	0.148 (0.130 – 0.168)	0.133 (0.115 – 0.152)	0.134 (0.114 – 0.155)	0.097 (0.077 – 0.117)	$R^2_m = 0.08$ $R^2c = 0.49$	0.095	0.152	0.110
Recruitment threshold (N)	39.5 (28.9 – 50.2)	38.5 (28.0 – 49.0)	38.8 (28.1 – 49.6)	33.3 (22.6 – 44.1)	$R^2_m = 0.12$ $R^2c = 0.69$	0.688	0.800	0.222
Derecruitment threshold (N)	34.7 (26.5 – 43.0)	37.3 (29.1 – 45.5)	31.4 (23.0 – 39.9)	34.7 (26.3 – 43.1)	$R^2_m = 0.15$ $R^2c = 0.55$	0.223	0.142	0.839

Data presented as estimated marginal means (lower 95% CI, upper 95% CI). The final four columns show model fit and *P* values. *Abbreviations*: *FR, firing rate;* R^2_m, marginal R^2 representing variance explained by fixed effects only; R^2c, conditional R^2 representing variance explained by the full model (fixed + random effects). *Statistically significant ($P < 0.05$).

2.5.5. Cumulative spike train

MUs that were tracked from pre- to post-intervention were used to compute the cumulative spike train (CST) for each leg at each timepoint. The pre to post difference in the mean of the CST was greater in the exercised than the control leg (P = 0.043), and the same was true of the percentage difference (P = 0.022) (Figure 2.5).

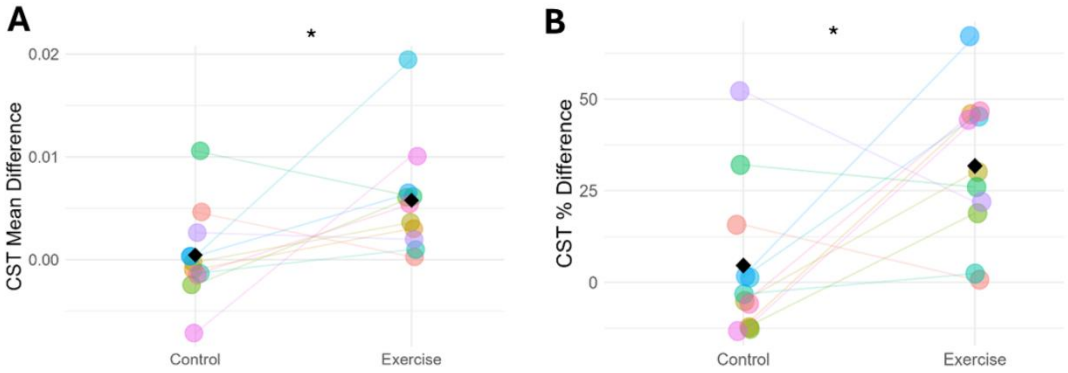


Figure 2.5. The difference from pre- to post-intervention of the cumulative spike train shown as an absolute value (A) and as a percentage (B). Data points are colour-coded for individual participants with means indicated by black diamonds. *Significantly different between the exercise and control legs (P < 0.05). *Abbreviations: CST, cumulative spike train*

2.5.6. Correlation between changes in MUFR and MVC

There was a weak yet statistically significant correlation between delta FR and delta MVC in the exercise leg (r = -0.566, P = 0.043), which was not observed in the control leg (r = -0.330, P = 0.271) (Figure 2.6).

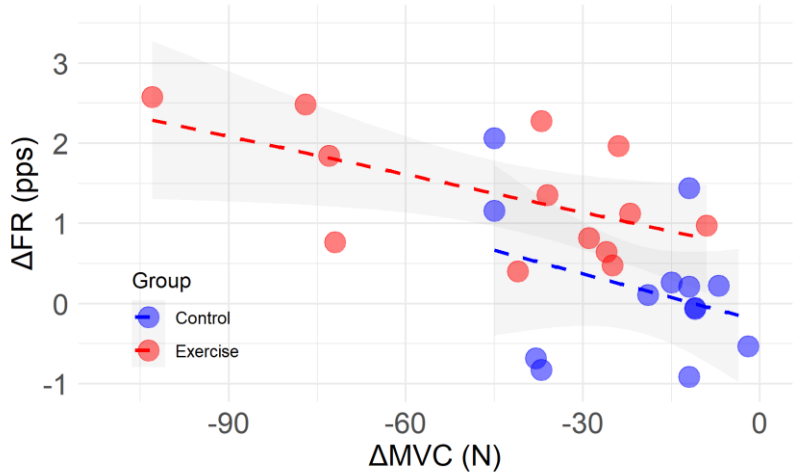


Figure 2.6. Correlation of change in firing rate as a function of change in MVC, from pre- to post-intervention in the control (blue) and exercise (red) legs. *Abbreviations: FR, firing rate; pps, pulses per second; MVC, maximal voluntary contraction*

2.5.7. iEMG-derived motor unit characteristics

A total of 841 MUPs were recorded from both legs at both time points for 11 participants (4 females). All Individual MU counts and model outputs for iEMG data are shown in Table 2.4.

For MUP area, there were no leg × time interaction (P = 0.461), or significant effects of leg (P = 0.119), or time (P = 0.392). Similarly, for MUP turns there were no significant leg × time interaction (P = 0.714) or effects of leg (P = 0.503), or time (P = 0.332). For NF jiggle, there was no leg × time interaction (P = 0.738), or significant effects of leg (P = 0.472), or time (P = 0.673). Additionally, for the negative peak ratio, there were no significant leg × time interaction (P = 0.164), or effects of leg (P = 0.896), or time (P = 0.168) (Table 2.4, Figure 2.7).

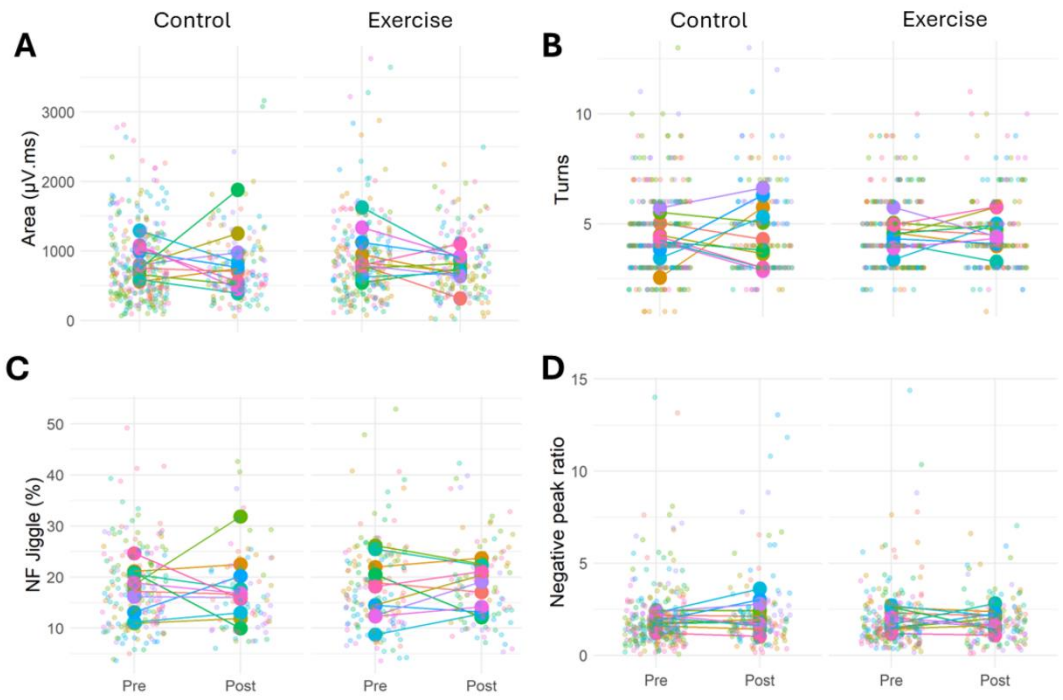


Figure 2.7. Motor unit characteristics assessed using iEMG. Motor unit Area (A), number of turns (B), neuromuscular junction transmission instability (Near fibre jiggle) (C), and negative peak ratio (D) in the exercise and control legs before and after the unilateral RE intervention. Data points are colour-coded for individual participants. Statistical analyses are based on multi-level mixed effects linear models.

Table 2.4. Summary of linear regression analyses for iEMG motor unit properties.

	Pre-intervention		Post-intervention		Model fit	Leg	Time	Leg × Time
	Control	Exercise	Control	Exercise				
No of MUPs	306	238	142	155				
MUP Area (μV.ms)	828 (744 - 913)	901 (812 - 991)	781 (675 - 886)	796 (694 - 899)	R^2_m = 0.01 R^2c = 0.06	0.119	0.392	0.461
MUP Turns	4.42 (4.03 - 4.82)	4.53 (4.12 - 4.93)	4.60 (4.16 - 5.04)	4.61 (4.17 - 5.05)	R^2_m = 0.03 R^2c = 0.12	0.503	0.332	0.714
NF Jiggle (%)	17.4 (14.8 - 19.9)	18.0 (15.4 - 20.6)	17.8 (15.0 - 20.6)	18.0 (15.2 - 20.7)	R^2_m = 0.02 R^2c = 0.21	0.472	0.673	0.738
Negative peak ratio	2.01 (1.72 - 2.29)	1.99 (1.69 - 2.29)	2.24 (1.90 - 2.58)	1.89 (1.55 - 2.22)	R^2_m = 0.04 R^2c = 0.04	0.896	0.168	0.164

Data presented as estimated marginal means [lower 95% CI, upper 95% CI]. Final four columns show model fit and *P* values.

Abbreviations: *MUP, motor unit potential; NF, near fibre;* R^2_m, marginal R^2 representing variance explained by fixed effects only; R^2c, conditional R^2 representing variance explained by the full model (fixed + random effects).

2.6. Discussion

The present study evaluated the effect of acute unilateral knee extensor RE on bilateral neuromuscular function and VL MU features in healthy older adults. The main findings indicate that a single bout of unilateral resistance exercise is sufficient to elicit reductions in maximal knee extensor voluntary force in the exercised and to a lesser extent, in the contralateral non-exercised limb, demonstrating a cross-limb transfer of muscle performance fatigue. However, the majority of markers of individual MU function were altered only in the exercise leg, with MUFR increasing during all phases of a contraction, normalised to 25% of baseline MVC, with no notable adaptation in the non-exercise limb.

The resistance-based exercise used in this study was deliberately selected to induce performance fatigue in VL, which plays a crucial role in daily activities. Resistance exercise-induced reduction in force of ~15% in the exercise limb and ~7% in the non-exercise limb in the current study supports previous findings in young adults (Doix et al., 2013, Halperin et al., 2014, Kawamoto et al., 2014, Martin and Rattey, 2007). Similarly, impaired force accuracy was only apparent in the exercised limb, supportive of previous fatigue-related findings in young adults (Singh et al., 2010).

The decline in contractile function leading to reduced MVC in the exercise leg is undoubtedly partly caused by molecular mechanisms (e.g., reduced sarcoplasmic reticulum Ca^{2+} release, impaired myofibrillar force production, and reduced myofibrillar Ca^{2+} sensitivity (Allen et al., 2008, Debold and Westerblad, 2024)), and the increase in MUFR reported here, which correlated with declines in MVC, likely occurred to compensate for this. Indeed, the loss of force in the exercised leg was 2-

fold greater than the control. However, the mechanisms explaining the loss in the control leg are less clear.

Earlier studies of cross-limb transfer showed voluntary activation of plantar flexors was reduced following eccentric contractions, in the exercised and contralateral non-exercised limb (Marathamuthu et al., 2022), and separately, sustained fatigue protocols impaired voluntary activation bilaterally following unilateral quadriceps exercise (Rattey et al., 2006). Both of these suggest a strong central component of this phenomenon, although unlikely to be mediated by group III/IV afferents (Kennedy et al., 2015). However, we found no alteration of MUFR in the control limb from pre- to post- intervention, albeit when sampled at 25% MVC. Moreover, when estimating neural drive via the CST calculated from the firing times of MUs tracked pre to post, this also showed no alteration in the control limb. This limits the plausibility of the previous hypothesis of diminished neural drive as a causal factor to contralateral decrements. Although sampled in submaximal contractions in the current study, these were normalised to baseline MVC and therefore represent a higher *relative* force value but cannot be extrapolated across the higher recruitment range.

Non-local muscle fatigue may also be mediated by the circulation of metabolic by-products generated within the fatigued muscles (Halperin et al., 2015). The accumulation of metabolites such as potassium, hydrogen, heat-shock proteins, and blood lactate (Behm et al., 2021, Halperin et al., 2015) in the exercised muscle can migrate and exert non-local fatigue effects that impair enzymatic activity, muscle contractile dynamics, and action potential transmission (Cady et al., 1989, Kent-Braun, 1999). Additionally, repeated muscle activation is known to elevate extracellular potassium levels (Sejersted and Sjøgaard, 2000), which may migrate into non-exercise

muscles. This repeated muscle activation alters the electrochemical gradients for potassium, which could contribute to diminished force production (Juel, 1986) and reduced excitability of the muscle, thereby contributing to performance fatigue (Nordsborg et al., 2003).

The current study found no change in MUFR variability at 25% MVC in either leg, which corresponds to previous studies with eccentric and concentric fatigue protocols (Jones et al., 2023). Additionally, we found no changes in MU recruitment and derecruitment thresholds in either leg, including of MUs tracked across the intervention. However, previous studies have shown increased (Farina et al., 2009) and decreased (Contessa et al., 2016) recruitment thresholds. These disparities in findings could be attributed to variations in task, muscle, and age groups considered.

The concurrent use of iEMG enabled a more detailed assessment of peripheral MU characteristics, and we found no RE-induced changes in MU properties in the exercise or contralateral control leg. The MUP negative peak ratio quantifies the relationship between the rise and fall slopes of the negative peak of the MUP template and may be sensitive to metabolite accumulation. In healthy young, this was altered following concentric fatigue protocols (lasting ~1 hour), but not eccentric (Jones et al., 2023). These discrepancies could be primarily attributed to the variations in exercise protocols, particularly exercise duration. The NF jiggle is an indicator of NMJ transmission instability, which shows the variability of temporal dispersion across MU action potential (Piasecki et al., 2021b) and is mainly related to the activity of muscle fibres close to the detection surface of the concentric needle electrode (within ~350 µm) (Stashuk, 1999b). Hence, the contributions of distant fibres to NF MUPs are minimal, which reduces the contamination from other MUs. However, although

eccentric and concentric exercise protocols in healthy young individuals have been shown to increase NF jiggle (Jones et al., 2023), our resistance exercise protocol resulted in no change in NF jiggle in either leg. Again, it is probable these differences are explained by the shorter duration of the RE in the current study.

Limitations

Firstly, the current study focused exclusively on immediate neuromuscular adaptations following a single bout of unilateral resistance exercise-induced performance fatigue. Therefore, these findings may not generalise to other types of intervention, such as those targeting endurance adaptations. Secondly, the current study reported MU data recruited at lower contraction levels (25% MVC), while the exercise intervention was performed at a higher intensity (75 % of 1RM). The MU assessment at higher contraction intensities was restricted by intramuscular needle placement. Therefore, the current study results may not be applicable for MUs with higher recruitment thresholds which are being recruited at higher force levels. Additionally, given the previously reported duration-dependent changes in muscle fibre conduction velocity in VL (Rodriguez-Falces and Place, 2021), post-intervention measures may be influenced by transient recovery which is an inherent limitation of this study design. To minimise potential biases, testing between the legs was randomised at all timepoints. Moreover, this study did not include a healthy young cohort, so it was not possible to infer any age-related alterations in neuromuscular adaptations. Finally, the current study was unable to determine the specific mechanism/s underlying the reduction in force in the control limb. Future studies could include neurophysiological measures such as transcranial magnetic stimulation to further explore the mechanisms underlying the acute effects of cross-limb transfer.

Conclusions

The current study demonstrates that a single bout of unilateral knee extensor resistance exercise performed to fatigue is sufficient to elicit a bilateral reduction in maximal forces with neural adaptations occurring only in the exercise limb. These findings indicate that resistance exercise-induced muscle fatigue results in immediate central and peripheral alterations in the exercise limb. While the exact mechanism accounting for the force decline in the non-exercise limb remains uncertain, it is mainly suggestive of adaptations at systemic and/or central levels. These findings are important in understanding neurophysiological mechanisms following resistance exercise-based performance fatigue and may have potential implications for clinical settings, including those often experienced by older adults (e.g., single-leg fracture, post-injury immobilisation, and stroke).

CHAPTER 3 (Published)

THETA-GAMMA TRANSCRANIAL ALTERNATING CURRENT STIMULATION ENHANCES BALLISTIC MOTOR PERFORMANCE IN HEALTHY YOUNG AND OLDER ADULTS

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Theta-gamma transcranial alternating current stimulation enhances ballistic motor performance in healthy young and older adults

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ABSTRACT

Theta-gamma transcranial alternating current stimulation (TG tACS) over primary motor cortex (M1) can improve ballistic motor performance in young adults, but the effect on older adults is unknown. This study investigated the effects of TG tACS on motor performance and M1 excitability in 18 young and 18 older adults. High-definition TG tACS (6 Hz theta, 75 Hz gamma) or sham tACS was applied over right M1 for 20 min during a ballistic left-thumb abduction motor training task performed in two experimental sessions. Motor performance was quantified as changes in movement acceleration during and up to 60 min after training. Transcranial magnetic stimulation (TMS) was used to assess changes in M1 excitability with motor-evoked potentials (MEP) and short-interval intracortical inhibition (SICI) before and after training. We found that TG tACS increased motor performance compared with sham tACS in young and older adults ($P < 0.001$), with greater effects for young adults ($P = 0.01$). The improved motor performance with TG tACS lasted at least 60 min after training in both age groups. Motor training was accompanied by greater MEP amplitudes with TG tACS compared to sham tACS in young and older adults ($P < 0.001$), but SICI did not vary between tACS sessions ($P = 0.40$). These findings indicate that TG tACS over M1 improves motor performance and alters training-induced changes in M1 excitability in healthy young and older adults. TG tACS may therefore be beneficial to alleviate motor deficits in the ageing population.

1. Introduction

There is extensive literature on the structural, functional, and biochemical changes in the central nervous system that occur with ageing (see Seidler et al., 2010 for review), but our understanding of how these contribute to the decline in motor function commonly observed with advancing age is limited. One factor that is thought to contribute to an age-related decline in motor function is a reduced capacity for the brain to change its neural connections, referred to as plasticity (Mahncke et al., 2006; Pascual-Leone et al., 2011; Semmler et al., 2021). In particular, the plasticity of cortical connections plays a major role in the acquisition of new motor skills (Dayan and Cohen, 2011; Sanes and Donoghue, 2000) and altered plasticity has been implicated in reduced motor function in older adults (Ward, 2006; Zapparoli et al., 2022), along with an impaired ability to relearn motor skills following neurological injury (Laaksonen and Ward, 2022). Therefore, it is essential to develop effective interventions to enhance motor system plasticity and improve motor function to promote healthy ageing and recovery from injury.

Non-invasive brain stimulation (NIBS) over primary motor cortex (M1) has been used to manipulate short-term plasticity and improve motor function (Ziemann and Siebner, 2008), but the effects on older adults have been inconsistent (Semmler et al., 2021). For example, when stimulation is applied prior to the motor task (referred to as priming), there is improvement in visuomotor skill learning in older adults with transcutaneous direct current stimulation (tDCS: Fujiyama et al., 2017) and repetitive paired-pulse transcranial magnetic stimulation (TMS; (Hand et al., 2023), but not with paired associative stimulation (Opie et al., 2019). An alternative approach has been to perform stimulation to manipulate M1 excitability during the performance of the motor tasks (referred to as gating), with some limited success. For example, anodal tDCS over M1 during motor practice in older adults can improve motor

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Contribution to the paper	Conceptualisation; data curation; formal analysis; investigation; methodology; project administration; validation; visualisation; writing—original draft.		
Overall percentage (%)	70%		
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	07/07/2025

Co-author contributions

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

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

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Contribution to the paper	Conceptualisation; data curation; funding acquisition; methodology; project administration; resources; supervision (primary); validation; visualisation; writing—review and editing.		
Signature		Date	07/07/2025

3.2. Abstract

Theta-gamma transcranial alternating current stimulation (theta-gamma tACS) over primary motor cortex (M1) can improve ballistic motor performance in young adults, but the effect on older adults is unknown. This study investigated the effects of theta-gamma tACS on motor performance and M1 excitability in 18 young and 18 older adults. High-definition theta-gamma tACS (6 Hz theta, 75 Hz gamma) or sham tACS was applied over right M1 for 20 minutes during a ballistic left-thumb abduction motor training task performed in two experimental sessions. Motor performance was quantified as changes in movement acceleration during and up to 60 minutes after training. Transcranial magnetic stimulation (TMS) was used to assess changes in M1 excitability with motor-evoked potentials (MEP) and short-interval intracortical inhibition (SICI) before and after training. We found that theta-gamma tACS increased motor performance compared with sham tACS in young and older adults ($P < 0.001$), with greater effects for young adults ($P = 0.01$). The improved motor performance with theta-gamma tACS lasted at least 60 minutes after training in both age groups. Motor training was accompanied by greater MEP amplitudes with theta-gamma tACS compared to sham tACS in young and older adults ($P < 0.001$), but SICI did not vary between tACS sessions ($P = 0.40$). These findings indicate that theta-gamma tACS over M1 improves motor performance and alters training-induced changes in M1 excitability in healthy young and older adults. theta-gamma tACS may therefore be beneficial to alleviate motor deficits in the ageing population.

Keywords: motor cortex, TMS, aging, motor skills.

3.3. Introduction

There is extensive literature on the structural, functional, and biochemical changes in the central nervous system that occur with ageing (see Seidler et al., 2010 for review), but our understanding of how these contribute to the decline in motor function commonly observed with advancing age is limited. One factor that is thought to contribute to an age-related decline in motor function is a reduced capacity for the brain to change its neural connections, referred to as plasticity (Mahncke et al., 2006, Pascual-Leone et al., 2011, Semmler et al., 2021). In particular, the plasticity of cortical connections plays a major role in the acquisition of new motor skills (Dayan and Cohen, 2011, Sanes and Donoghue, 2000) and altered plasticity has been implicated in reduced motor function in older adults (Ward, 2006, Zapparoli et al., 2022), along with an impaired ability to relearn motor skills following neurological injury (Laaksonen and Ward, 2022). Therefore, it is essential to develop effective interventions to enhance motor system plasticity and improve motor function to promote healthy ageing and recovery from injury.

Non-invasive brain stimulation (NIBS) over primary motor cortex (M1) has been used to manipulate short-term plasticity and improve motor function (Ziemann and Siebner, 2008), but the effects on older adults have been inconsistent (Semmler et al., 2021). For example, when stimulation is applied prior to the motor task (referred to as priming), there is improvement in visuomotor skill learning in older adults with transcutaneous direct current stimulation (tDCS: Fujiyama et al., 2017) and repetitive paired-pulse transcranial magnetic stimulation (TMS: Hand et al., 2023), but not with paired associative stimulation (Opie et al., 2019). An alternative approach has been to perform stimulation to manipulate

M1 excitability during the performance of the motor tasks (referred to as gating), with some limited success. For example, anodal tDCS over M1 during motor practice in older adults can improve motor performance for several different speed-accuracy tasks (Goodwill et al., 2013, Zimerman et al., 2013), and motor adaptation tasks (Panouillères et al., 2015), but not for motor sequence learning (Raw et al., 2016), or visuomotor tracking tasks (Mooney et al., 2019). These inconsistent findings suggest that new NIBS approaches are needed to produce more robust effects on motor performance with practice in older adults.

Transcranial alternating current stimulation (tACS) is another NIBS technique that applies small electrical currents to the brain in an oscillatory pattern, which is thought to entrain the membrane potentials of susceptible neurons in the stimulated region at the applied frequency (Krause et al., 2019, Vogeti et al., 2022). Although some effects on motor function have been observed when tACS is applied over M1 at alpha (10 Hz; Antal et al., 2008) and beta (20 Hz; Pollok et al., 2015) frequencies, there is now emerging evidence that tACS in the gamma band (60-90 Hz) modulates M1 plasticity and motor performance. For example, gamma tACS over M1 increases plasticity induced with intermittent theta burst stimulation (iTBS; Guerra et al., 2018), with these effects thought to be driven by changes in M1 GABA (gamma-aminobutyric acid) mediated intracortical inhibition (Guerra et al., 2018, Nowak et al., 2017). Furthermore, gamma tACS over M1 modifies specific aspects of motor behaviour (Joundi et al., 2012, Moisa et al., 2016) and visuomotor skill acquisition (Santarnecchi et al., 2017), but does not influence ballistic motor performance during practice (Bologna et al., 2019). These findings indicate that

gamma tACS alters M1 activity during the stimulation period, and these effects can modify M1 plasticity and the performance of some motor tasks.

Emerging multimodal evidence has shown that high-frequency brain oscillations in the gamma band do not occur in isolation, but are synchronised to low-frequency theta (4-7 Hz) waves during learning (Canolty et al., 2006, Johnson et al., 2017); a process referred to as theta-gamma phase-amplitude coupling (PAC). In particular, theta-gamma PAC has been consistently associated with enhanced memory processing, where gamma oscillations occurring at a particular theta phase are thought to provide a more powerful output by allowing the cortical network to fire in a more tightly coupled manner (see Colgin, 2015 for review). Based on these findings, recent studies have applied tACS in a more physiological way by using waveforms that mimic theta-gamma coupling and examined the effects on different types of learning. For example, theta-gamma tACS applied over the prefrontal cortex has been shown to improve working memory in young (Alekseichuk et al., 2016) and older adults (Reinhart and Nguyen, 2019), and also improves visuomotor learning in older adults (Diedrich et al., 2024). Furthermore, theta-gamma tACS has also been applied over M1, where there is an improvement in motor performance with practice in young adults during a simple ballistic task (Akkad et al., 2021). However, it is not known whether theta-gamma tACS applied over M1 is able to improve motor performance with practice in older adults.

The aim of this study was therefore to examine the effect of theta-gamma tACS on motor performance and M1 excitability in young and older adults. Motor performance was assessed with practice of a ballistic thumb abduction task. This task requires a ballistic movement of the thumb in the abduction direction (rather than the more commonly used

thumb flexion), and results in cortical reorganisation with practice that reflects use-dependent plasticity (Carroll et al., 2008, Muellbacher et al., 2001, Ziemann et al., 2001). Previous research indicates that this ballistic task is a centrally pre-progammed movement that is M1-dependent, where the behavioural improvement is associated with physiological changes in M1 (Muellbacher et al., 2001). Furthermore, this task is analogous to motor skills that emphasise rapid movements with directional accuracy, and this thumb movement is functionally important during palmar arching to facilitate opposition and grasping, which is involved in numerous daily activities (Gracia-Ibáñez et al., 2017). Importantly, the performance of this task is improved with theta-gamma tACS in young adults (Akkad et al., 2021), and there are substantial performance decrements for this task in older adults (Rogasch et al., 2009). Neurophysiological changes in M1 following theta-gamma tACS and motor training were assessed using single and paired-pulse TMS to evaluate alterations in excitatory and inhibitory circuits in the contralateral M1. We hypothesised that theta-gamma tACS would enhance motor performance with practice in young and older adults, and this would be accompanied by a greater increase in M1 excitability. In addition, we also hypothesised that effects of theta-gamma tACS on motor performance would be weaker in older adults.

3.4. Methods

Sample size calculations were performed using simulation-based power estimations on Rstudio (version 2023.12.1 + 402) with '*lme4*' (Bates, 2014) and '*simr*' (Green and MacLeod, 2016) packages. These were based on motor training data obtained from the first five young and older adults (10 participants) and were performed by an unblinded team member that was not involved in data collection. This analysis resulted in a fixed

effects coefficient of -0.304 that was used as an unstandardised effect size of theta-gamma tACS on motor training in young and older adults, which was lowered by 15% (-0.258) to account for biases in power calculations derived from experimental data (Kumle et al., 2021b). Sample size estimations revealed that 24 participants were needed given $\alpha < 0.001$, $\beta > 0.9$ (0.913), which was increased by 50% (36 participants) to ensure robust detection of small–moderate effect sizes.

Eighteen young (8 females; mean age ± SD, 26.3 ± 5.7 years; age range, 18-36 years) and 18 older (12 females; 68.3 ± 5.0 years; age range, 62-78 years) right-handed adults participated in this study. Handedness was assessed by the Edinburgh Handedness Inventory (Oldfield, 1971), and contraindications for TMS were assessed via the TMS safety screening questionnaire (Rossi et al., 2011). Participants were excluded if they had any diagnosed neurological or cardiovascular conditions, a history of concussion or seizure, were pregnant, or were currently on psychoactive medications. Participants who regularly engaged in activities that involve skilled manual dexterity (e.g. musicians) were also excluded. In addition, we assessed participant cognition levels using the Montreal Cognitive Assessment (MoCA) questionnaire (Hobson, 2015), participant physical activity levels using the International Physical Activity Questionnaire- Short form (IPAQ) (Craig et al., 2003), and participant chronotypes using the Morningness-Eveningness questionnaire (MEQr) (Adan and Almirall, 1991). These assessments were included for each participant because previous studies have shown that motor system plasticity or motor behaviour can be influenced by cognitive function (Chou et al., 2022), physical activity levels (Cirillo et al., 2009), and chronotype (Salehinejad et al., 2021). The ethics approval was obtained from the human research ethics committee of the University of

Adelaide and conducted according to the *Declaration of Helsinki*. All participants provided written informed consent prior to the study.

3.4.1. Experimental design

The current study followed a randomised, double-blind, sham-controlled cross-over design. All participants attended two experimental sessions involving the application of high-definition (HD) theta-gamma tACS (theta-gamma tACS) or sham tACS to the right M1 during motor training, with the two sessions separated by at least two weeks to reduce carry-over effects (Bologna et al., 2019). The same experimental procedures were used for both sessions (Figure 3.1) and were performed by the same blinded experimenter to retain consistency between sessions. For each participant, all experimental sessions were conducted between 11 am and 5 pm at approximately the same time of day to minimise the influence of diurnal variations in circulating cortisol on neuroplasticity (Sale et al., 2008).

3.4.2. Experimental procedures

In all sessions, participants were seated on a comfortable chair with their non-dominant (left for all) hand relaxed and rested on a table in front of them. Surface electromyography (EMG) was recorded from the abductor pollicis brevis (APB) muscle in the left hand using two Ag-AgCl electrodes placed ~2 cm apart in a belly-tendon montage on the skin overlying the APB muscle. A third electrode was placed on the radial styloid process of the left hand to ground the electrodes. EMG signals were amplified (300×) and band-pass filtered (20 Hz high pass to 1 kHz low pass) using a CED 1902 signal conditioner (Cambridge Electronic Design, Cambridge, United Kingdom) before being digitised at 2

kHz using a CED 1401 analogue-to-digital converter. Signal noise associated with mains power (50 Hz) was removed using a Humbug mains noise eliminator (Quest Scientific, North Vancouver, Canada). All recorded EMG signals were stored on a computer for offline analysis.

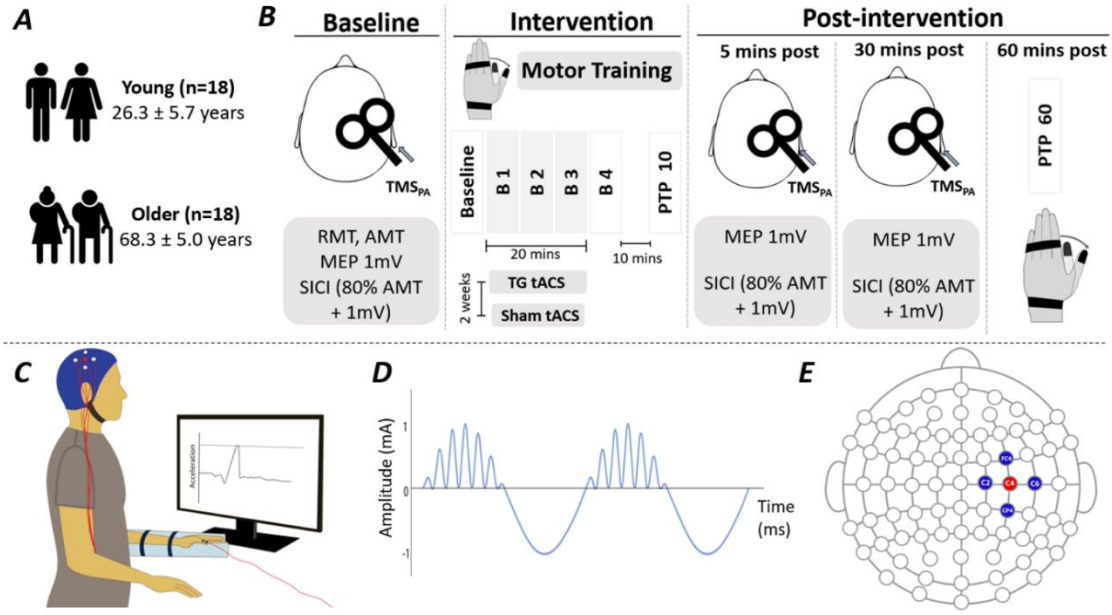


Figure 3.1. Experimental setup. (A) Study participants. (B) Experimental protocol involving baseline, intervention, and post-intervention measures. RMT, resting motor threshold; AMT, active motor threshold; MEP, motor-evoked potential; SICI, short-interval intracortical inhibition (ISI 2 ms, CS at 80% AMT, and TS at 1mV); B 1-4, ballistic motor training blocks 1-4; PTP, post-training performance. (C) Motor training setup; HD tACS on right M1 while performing ballistic thumb abduction task in the left (non-dominant) hand. (D) Theta-gamma transcranial alternating current stimulation (theta-gamma tACS) waveform (sinusoidal 6 Hz theta stimulation at 2 mA peak-to-peak intensity, coupled with bursts of a sinusoidal 75 Hz gamma rhythm amplitude at the positive theta phase). (E) HD tACS electrode placement; anode (red; *C4*) and cathodes (blue; *C2, C6, FC4, CP4*).

Transcranial Magnetic Stimulation (TMS).

The hand area of right M1 was stimulated in all participants using a figure-of-eight branding iron coil (70 mm) connected to two Magstim 200^2 magnetic stimulators via a BiStim module (Magstim, Dyfed, United Kingdom). The coil was held at an angle of 45° to the midsagittal plane to produce a current flow in a posterior-to-anterior (PA) direction perpendicular to the central sulcus. The stimulation was delivered at a rate of 0.2 Hz with a 10% jitter between trials to avoid anticipation of the stimulus. The identification of APB 'hotspot' was determined as the coil position that generated consistent motor-evoked potentials (MEPs) at the lowest stimulation intensity in the contralateral APB muscle. The hotspot was marked using a surgical marker and consistently monitored throughout the experimental session.

Resting motor threshold (RMT) was defined as the smallest stimulus intensity (percentage of maximum stimulation output, % MSO) required to produce a MEP amplitude of ≥ 50 μV in at least 5 out of 10 consecutive trials in the relaxed left APB (Rossini et al., 2015). Following RMT, active motor threshold (AMT) was defined as the lowest stimulus intensity needed to produce a MEP amplitude of ≥ 200 μV in at least 5 out of 10 consecutive trials during a small (~10% of maximum) contraction of left APB (Rogasch et al., 2009). Then, we determined the single-pulse TMS intensity that produced a mean MEP amplitude of ~1.0 mV (range: 0.5 - 1.5 mV) in the resting left APB, when averaged over 20 consecutive trials. The same stimulus intensity was used throughout the experimental session (baseline, 5- and 30-minutes post-training) to assess M1 excitability.

Paired-pulse TMS was used to assess short-interval intracortical inhibition (SICI). This consisted of a sub-threshold conditioning stimulus (CS, 80% AMT) that preceded a suprathreshold test stimulus (TS, 1 mV) by an interstimulus interval (ISI) of 2 ms (Opie and Semmler, 2019). SICI was assessed at all three time points (baseline, 5- and 30-minutes post-training), and each block of SICI consisted of 20 single-pulse and 20 paired-pulse TMS trials delivered in a randomised order, totalling 40 trials. Due to possible changes in M1 excitability following the intervention, we reassessed the baseline single-pulse TMS intensity in a separate TMS block of 20 MEP trials. If the mean MEP amplitude varied from baseline by 30% or greater, the stimulation intensity was readjusted for the SICI block (Nowak et al., 2017), as an intensity producing a larger single-pulse MEP amplitude could induce different levels of intracortical inhibition (Nitsche et al., 2005, Stefan et al., 2002).

theta-gamma tACS intervention

High-definition (HD) theta-gamma tACS was delivered over right M1 via five stimulating HD electrodes (Soterix Medical Inc, New York, United States) arranged in a 4 × 1 HD tACS configuration (4 cathodes and 1 anode) with the anode (active) as the centre electrode (Figure 3.1.E). The electrodes were placed using a stimulating HD cap and connected to an HD-tES stimulator (Soterix Medical MXN-33-HD-tES), which was operated with tACS software on a laptop computer (Soterix Medical, New York, United States). Prior to stimulation, the HD electrodes were attached to the scalp using HD electrolyte gel (Soterix Medical Inc, New York, United States), and the impedance of each electrode was monitored throughout the intervention (< 20 kΩ). Similar to previous work, theta-gamma tACS comprised a sinusoidal 6 Hz theta waveform coupled with a 75 Hz

gamma burst that was amplitude-modulated by the positive peak of the theta wave. This was applied with a peak-to-peak amplitude of 2 mA (Figure 3.1.D). theta-gamma tACS was applied during motor training for 20 minutes (Akkad et al., 2021). During sham tACS, theta-gamma tACS (6/75 Hz) was ramped up to 2 mA over 10s and then immediately ramped down again. Both the experimenter and the participants were blinded to the tACS intervention, which was administered by a second experimenter who was not involved with any other element of data collection or analysis. Following the intervention, participants were asked whether they received real or sham tACS and completed a visual analogue scale (VAS) to assess their perception of pain, intensity, and stimulation localisation.

Motor training

In all experimental sessions, participants performed a ballistic thumb abduction task that involved maximising left thumb acceleration over the training period (Rogasch et al., 2009). The non-dominant (left in all) hand was tested to prevent ceiling effects that might occur in the dominant hand (Akkad et al., 2021). Participants were seated with their left shoulder relaxed and the left elbow flexed to ~ 90°, while the forearm was maintained in a semi-pronated position with the palm facing inwards. A custom-made plastic splint was used to immobilise the left forearm, wrist, and proximal interphalangeal joints, while the left thumb could move freely. A biaxial accelerometer was connected to the distal phalanx of the thumb to record thumb acceleration in the abduction/adduction (x-axis) and flexion/extension (z-axis) planes (Akkad et al., 2021, Rogasch et al., 2009). Acceleration signals were digitised at 2 kHz with a CED interface system and recorded on a computer for offline analysis.

Participants performed the ballistic thumb abduction training at a rate of 0.4 Hz, paced by a metronome. The training consisted of an initial baseline block (block 0, 30 trials), followed by four training blocks: blocks 1-4 (70 trials each with a 30-second break after trial 35), each separated by a 2-minute rest (no thumb movement) period to avoid fatigue (Akkad et al., 2021). theta-gamma tACS or sham tACS was applied during blocks 1-3. Post-training performance was assessed at 10 (PTP 10) and 60 (PTP 60) minutes by performing additional thumb abduction blocks (70 trials each with a 30-second break after trial 35). Participants were continually encouraged to optimise thumb abduction acceleration, while also matching the metronome as accurately as possible (Akkad et al., 2021). To encourage performance improvement, real-time visual feedback of thumb acceleration was presented on a screen in front of the participant. During each break, participants were additionally shown plots of performance over all completed blocks.

Maximal compound muscle action potential (M-waves)

The median nerve was stimulated at the wrist to obtain maximal compound muscle action potential (M-waves) in the left APB using electrical stimulation. A constant current stimulator (DS7A, Digitimer, United Kingdom) was used to apply the stimuli, and the cathode of the stimulating bar electrode was positioned distally (Opie and Semmler, 2019). Each stimulus was a square wave pulse of 0.1 ms duration and the stimulator intensity was set at 120% of that required to elicit a maximal response from APB (Opie and Semmler, 2019). The maximal M-wave (M_{max}) was recorded as the average of five trials recorded at the beginning of each experimental session. One young participant was unable to tolerate the M-wave assessment.

Grooved pegboard task

The grooved pegboard task was used to assess manual dexterity (Reuben et al., 2013). The pegboard contained 25 holes (five-by-five layout), each of which had a 'key' shaped profile with a centre circle and radial groove (oriented in a unique direction). Completion of the task requires pegs with matching profiles to be placed in as many holes as possible over a 30-second period. Participants completed three blocks with the left hand, with a 30-second rest between each block. The average number of pegs that were correctly positioned with the left hand was recorded. Twelve young and 15 older participants completed the pegboard task at the start of the first session.

3.4.3. Data analysis

All EMG data were visually inspected, with any trials having EMG activity exceeding 20 μV peak-to-peak amplitude in the 100 ms prior to TMS excluded from analysis (~7.2% of MEP trials and ~5.5% of SICI trials were removed). The MEP and M-wave amplitude were measured peak-to-peak and expressed in mV. SICI was quantified by expressing the MEP amplitude during each paired-pulse trial as a percentage of the mean single-pulse MEP amplitude within the same block. A SICI value of 0% indicates maximum inhibition, whereas 100% represents no inhibition (Opie and Semmler, 2019).

For the ballistic thumb abduction task, acceleration in the abduction/adduction axis was measured in m/s^2 and was quantified relative to a baseline period 200 ms prior to movement (Rogasch et al., 2009). Trials with a negative value (maximum acceleration < 0 m/s^2) were removed prior to analysis (~0.86% of thumb acceleration performance trials removed). In addition, post-training performance (PTP) was assessed by normalising PTP

10 and PTP 60 acceleration values to the baseline. Finally, we calculated the coefficient of variation (CV) of thumb acceleration at baseline, during training, and at PTP for each participant.

3.4.4. Statistical analysis

Statistical analysis was performed using IBM SPSS (version 28.0) and visualised using Prism (GraphPad software, version 9.0). The distribution of data was visually inspected and assessed using Kolmogorov–Smirnov tests which revealed that the data was non-normally distributed and positively skewed. Therefore, generalised linear mixed models (GLMMs) with Gamma distribution and log link function were used for statistical analyses (Lo and Andrews, 2015). Each model included single-trial data with repeated measures and included random participant intercepts (Barr et al., 2013). The model fit was assessed with the Bayesian Information Criterion (BIC).

Baseline measures

Two-factor GLMMs were used to investigate the effects of treatment session (theta-gamma tACS, sham tACS) and age group (young, older) on baseline RMT, AMT, TS intensities, single-pulse MEP amplitude, SICI, M-wave, and thumb acceleration. Additionally, a two-factor GLMM was used to investigate the effects of treatment session and age group on the CV of thumb acceleration at baseline.

Post-training measures

Two three-factor GLMMs were used to investigate the effects of treatment sessions, age groups, and time (baseline, post 5, post 30) on single-pulse MEP amplitude and SICI. A

single three-factor GLMM for motor training was used to investigate the effects of treatment sessions, age groups, and training blocks (blocks 0-4) on motor skill acquisition. In addition, a three-factor model was used to investigate the effects of treatment sessions, age groups, and PTP blocks (PTP 10, PTP 60) on post-training thumb abduction. Additionally, a three-factor GLMM was used to investigate CV for thumb acceleration for each participant for each training and PTP block. Spearman's correlation test was performed to assess the association between neurophysiological measures and motor performance. All models assessing MEP, SICI, and thumb acceleration also include participant IPAQ, MoCA, and MEQr scores as covariates to control for variations in participant factors. For models assessing thumb acceleration during motor training, the effect of session order (session 1, session 2) was added to assess whether thumb abduction acceleration improved during the second session. For all models, investigations of main effects and interactions were completed using custom contrasts with Bonferroni corrections, and significance was set at $P < 0.05$. Data for all models are presented as estimated marginal means (EMM) with 95% confidence intervals (95% CI), whereas pairwise comparisons are presented as estimated mean differences (EMD) and 95% CI for an unstandardised measure of effect size.

VAS responses

Differences in the perception of theta-gamma tACS and sham tACS were investigated by comparing VAS responses (pain, intensity, and localisation) using paired sample t-tests, with data presented as mean ± SD.

3.5. Results

All participants completed the experimental sessions with no adverse effects. Age-related differences in participant characteristics are shown in Table 3.1. Pegboard test scores varied between age groups ($F_{1,24}$ = 8.5, P = 0.007), with young adults obtaining higher scores than older adults (both $P < 0.01$). Additionally, chronotype (MEQr values) varied between age groups ($F_{1,34}$ = 5.3, P = 0.028), with *post hoc* tests revealing higher MEQr values for older adults compared to young adults (EMD = 1.6, [0.17, 2.94], P = 0.029). There were no differences between other characteristics (all $P > 0.05$).

Table 3.1. Participant characteristics in young and older adults.

Measure	Young	Older	*P* value
Sample size (F, M)	18 (8, 10)	18 (12, 6)	N/A
Age (years)	26.3 [24.3, 28.4]	68.3 [63.2, 73.9]	< 0.001*
Handedness (LQ)	0.80 [0.72, 0.90]	0.91 [0.82, 1.01]	0.109
Pegboard test- L hand	11.8 [10.3, 13.5]	9.1 [8.0, 10.3]	0.007*
Physical activity (IPAQ)	2461 [1726, 3509]	2089 [1465, 2979]	0.512
Cognition (MoCA)	28.5 [27.9, 29.1]	28.8 [28.1, 29.4]	0.526
Chronotype (MEQr)	14.2 [13.3, 15.1]	15.7 [14.7, 16.8]	0.028*

Data presented as EMM [lower 95% CI, upper 95% CI]. LQ, laterality quotient [from -1 (left-handed) to +1 (right-handed)]. IPAQ, International Physical Activity Questionnaire-short form; MoCA, Montreal Cognitive Assessment; MEQr, Morningness-Eveningness questionnaire. Only 26 adults (12 young, 14 older) completed bilateral pegboard tests. *Significantly different between young and older adults.

Baseline neurophysiological measures are shown in Table 3.2. Baseline M-wave responses varied between age groups ($F_{1,65}$ = 8.3, P = 0.005), and *post hoc* comparisons revealed a greater M-wave response in young adults compared to older adults (EMD =

3.1 mV, [0.87, 5.27], P = 0.007). There were no baseline differences for all other neurophysiological measures (all P > 0.08). Furthermore, there was no effect of IPAQ, MoCA, and MEQr on MEP amplitude, SICI, and changes in thumb acceleration throughout both experimental sessions (all P > 0.05). Additionally, CV for the baseline thumb acceleration revealed no significant main effects or interaction between age and tACS condition (all P > 0.09).

Table 3.2. Baseline neurophysiological measures before training in young and older adults.

Measure	Young (n=18)		Older (n=18)	
	TG tACS	Sham tACS	TG tACS	Sham tACS
PA				
RMT (%MSO)	48.6 [44.5, 52.9]	48.2 [44.3, 52.6]	53.9 [49.5, 58.7]	53.1 [48.7 57.8]
AMT (%MSO)	39.9 [36.0, 44.1]	40.9 [37.1, 45.3]	40.4 [36.6, 44.6]	39.8 [36.0, 44.0]
Test TMS intensity (%MSO)	61.0 [55.7, 68.0]	61.2 [55.0, 68.2]	69.7 [62.6, 77.5]	69.4 [62.3, 77.2]
Test MEP amplitude (mV)	1.00 [0.91, 1.11]	1.00 [0.90, 1.10]	0.97 [0.88, 1.67]	0.94 [0.85, 1.04]
SICI (% single pulse)	56.9 [45.9, 70.7]	52.4 [42.3, 64.9]	60.3 [48.7, 74.7]	66.0 [48.7, 74.7]
M-wave (mV) *	9.9 [8.1, 12.1]	10.4 [8.6, 12.7]	7.4 [6.1, 8.9]	6.8 [5.6, 8.3]
Thumb acceleration (m/s^2)	15.5 [12.3, 19.5]	15.8 [12.6, 19.8]	11.8 [9.5, 14.8]	12.8 [10.3,16.0]
Thumb acceleration CV (%)	25.5 [21.8, 30.0]	28.0 [23.9, 32.9]	30.0 [25.6, 35.3]	31.5 [26.9, 37.0]

Data indicated as EMM [lower 95% CI, upper 95% CI]. TG tACS, theta-gamma transcranial alternating current stimulation; RMT, resting motor threshold; MSO, maximal stimulator output; AMT, active motor threshold; MEP, motor-evoked potential; TMS, transcranial magnetic stimulation; SICI, short-interval intracortical inhibition. M-wave data were obtained from 17 young and 18 older adults. *Significantly different between young and older adults (P < 0.05).

3.5.1. Thumb acceleration during motor training

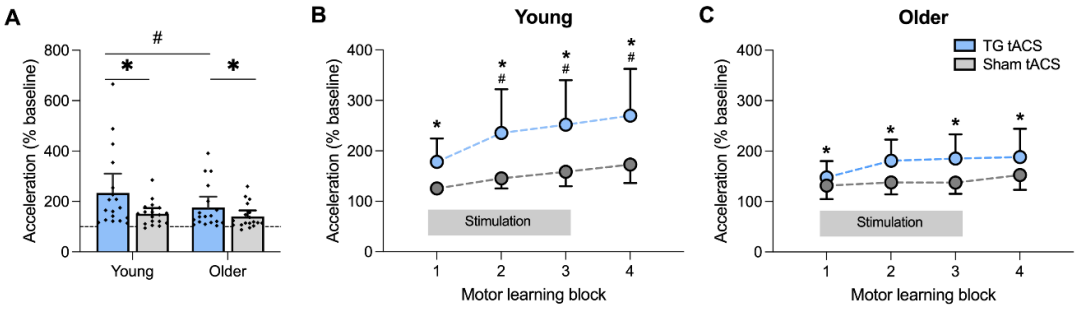


Figure 3.2. Effect of theta-gamma tACS on ballistic motor performance. Data show EMM [± 95% CI] improvement (relative to baseline) in thumb abduction acceleration during motor training (block 1-4). Data show the effect of tACS in different age groups (A), changes in thumb acceleration with training in young adults (B), and changes in thumb acceleration with training in older adults (C). * Significantly different between theta-gamma tACS and sham tACS ($P < 0.001$). # Significantly different compared to older adults ($P < 0.05$).

Changes in thumb acceleration during motor training (blocks 1-4) are shown in Figure 3.2. Thumb acceleration during motor training did not vary between age groups ($F_{1,19973} = 2.3$, $P = 0.13$), but differed between training blocks ($F_{3,19973} = 296$, $P < 0.001$), revealing a progressive increase in thumb acceleration from blocks 1 to 4 (all comparisons $P < 0.001$). Changes in thumb acceleration also varied between tACS sessions ($F_{1,19973} = 2294$, $P < 0.001$), with increased thumb abduction acceleration during the theta-gamma tACS session compared to sham tACS (EMD = 59.2% [56.8, 61.7], $P < 0.001$). Furthermore, there was a two-way interaction between age group and training block ($F_{3,19973} = 47.8$, $P < 0.001$), tACS treatment and training block ($F_{3,19973} = 45.7$, $P < 0.001$), and tACS treatment and age group ($F_{1,19973} = 377$, $P < 0.001$; Fig. 2A). In particular, *post-hoc* analysis for the treatment and age group interaction revealed increased thumb acceleration

following theta-gamma tACS compared to sham tACS for both young (EMD = 83.2% [79.8, 86.7], $P < 0.001$) and older adults (EMD = 35.2% [31.8, 38.7], $P < 0.001$). Moreover, changes in thumb acceleration were increased following theta-gamma tACS in young compared to older adults (EMD = 59.2% [13.8, 104.5], $P = 0.01$). There was also a three-factor interaction between tACS treatment, age group, and training block ($F_{3,19973} = 4.3$, $P = 0.005$; Fig. 2B, 2C). *Post hoc* comparisons showed increased changes in acceleration with theta-gamma tACS compared to sham tACS for young and older adults during blocks 1-4 (all $P < 0.001$). Young adults also showed increased changes in acceleration compared to older adults during the theta-gamma tACS session for blocks 2-4 (all $P < 0.02$), whereas there were no differences between age groups during sham tACS (all $P > 0.38$). Finally, changes in thumb acceleration differed with session order ($F_{1, 19973} = 109$, $P < 0.001$), where there were increased changes in acceleration during the first session compared to the second session (EMD = 13.2% [10.76, 15.73], $P < 0.001$).

During motor training, CV of thumb acceleration differed between age groups ($F_{1,272} = 10.4$, $P = 0.001$), revealing a greater variability of thumb acceleration in older compared to young adults (EMD = 5.9% [2.3, 9.6], $P = 0.002$). The CV for thumb acceleration varied between tACS sessions ($F_{1,272} = 5.8$, $P = 0.017$), with greater variability during the theta-gamma tACS session compared to sham tACS (EMD = 1.7% [0.3, 3.1], $P = 0.017$). The CV for thumb acceleration also differed between training blocks ($F_{3,272} = 7.6$, $P < 0.001$), indicating a general reduction in variability from blocks 1 to 4 ($P < 0.01$). However, there were no significant interactions for CV (all $P > 0.16$).

3.5.2. Post-training motor skill performance

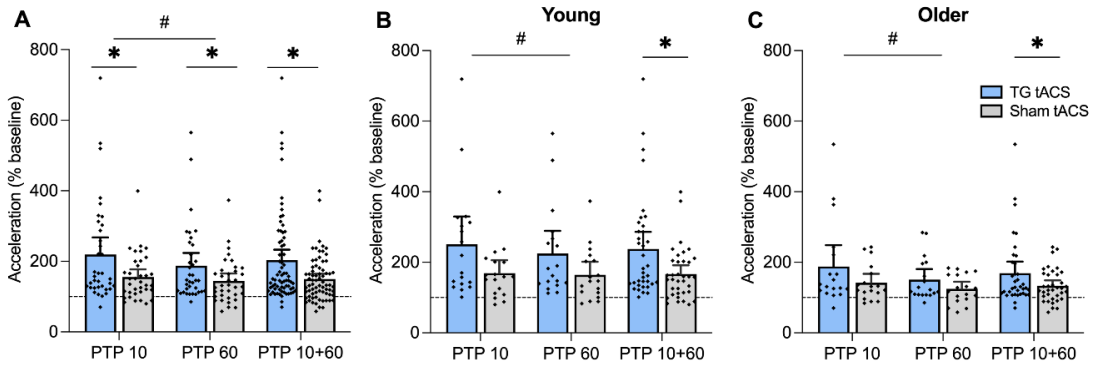


Figure 3.3. Effect of motor training and theta-gamma tACS on thumb acceleration at post-training performance (PTP). Data show EMM [± 95% CI] thumb acceleration (relative to baseline) in all subjects (A), young adults (B), and older adults (C) obtained 10 minutes (PTP 10) and 60 minutes (PTP 60) after training, and with both time points combined (PTP 10+60). # Significant difference between PTP 10 compared to PTP 60 ($P < 0.001$). * Significant difference between theta-gamma tACS and sham tACS ($P < 0.001$).

Changes in thumb acceleration after motor training (PTP) are shown in Figure 3.3. PTP thumb acceleration differed between tACS sessions ($F_{1,10006} = 1076$, $P < 0.001$), where thumb abduction acceleration was greater following theta-gamma tACS compared to sham tACS (EMD = 53.4% [50.3, 56.6], $P < 0.001$). Thumb acceleration performance also varied between age groups ($F_{1,10006} = 5.1$, $P = 0.024$), with increased changes in acceleration in young compared to older adults (EMD = 51.5% [6.7, 96.4], $P = 0.024$). Additionally, changes in PTP thumb acceleration differed between PTP blocks ($F_{1,10006} = 162$, $P < 0.001$), with reduced changes in thumb acceleration during PTP 60 compared to PTP 10 (EMD = 20.7% [17.5, 23.9], $P < 0.001$). There was an interaction between age group and PTP block ($F_{1,10006} = 11.4$, $P < 0.001$) and tACS treatment and PTP blocks ($F_{1,10006} = 40.3$, $P < 0.001$; Fig, 3A), which showed increased changes in PTP thumb

acceleration following theta-gamma tACS compared to sham tACS at PTP 10 (EMD = 63.8% [59.3, 68.3], $P < 0.001$) and PTP 60 (EMD = 43.1% [38.6, 47.6], $P < 0.001$). Additionally, changes in PTP thumb acceleration at PTP 10 was greater compared to PTP 60 for theta-gamma tACS (EMD = 31.1% [26.6, 35.6], $P < 0.001$) and sham tACS (EMD = 10.4% [5.9, 14.9], $P < 0.001$). Furthermore, there was an interaction between age group and PTP block ($F_{1,10006} = 11.4$, $P < 0.001$; Fig. 3B, 3C), where young adults showed increased changes in PTP acceleration at PTP 10 (EMD = 46.0% [1.1, 91.0], $P = 0.045$) and PTP 60 (EMD = 57.0% [12.1, 102.0], $P = 0.013$) compared to older adults. Moreover, changes in PTP thumb acceleration were increased during PTP 10 compared to PTP 60 for both young (EMD = 15.2% [10.7, 19.7], $P < 0.001$) and older (EMD = 26.2% [21.7, 30.8], $P < 0.001$) adults. There were no significant three-way interactions ($P = 0.81$).

CV during post-training acceleration did not vary between tACS conditions ($F_{1,136} = 0.5$, $P = 0.47$), or PTP blocks ($F_{1,136} = 2.8$, $P = 0.10$), but differed between age groups ($F_{1,136} = 25.3$, $P < 0.001$), revealing a greater variability in older compared to young adults (EMD = 8.3% [4.9, 11.6], $P < 0.001$). However, there were no significant interactions for CV during PTP (all $P > 0.42$).

3.5.3. Effect of motor training and theta-gamma tACS on MEP Amplitude

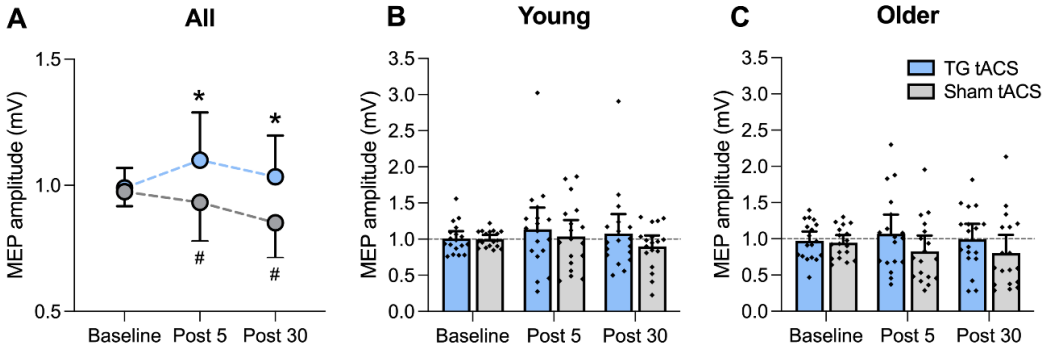


Figure 3.4. **Effect of motor training and theta-gamma tACS on MEP amplitude.** Data show EMM [± 95% CI] test MEP amplitude in all participants (A), young adults (B), and older adults (C). * Significantly different compared to sham tACS ($P < 0.001$). # Significantly different compared to baseline ($P < 0.01$).

MEP amplitude obtained before and after training (at the same TMS intensity) for each tACS session are shown in Figure 3.4. MEP amplitude did not differ between age groups (age effect, $F_{1,3996} = 1.7$, $P = 0.187$), but varied between tACS treatments (treatment effect, $F_{1,3996} = 36.8$, $P < 0.001$), where theta-gamma tACS resulted in increased MEP amplitude compared to sham tACS (EMD = 0.13 mV [0.08, 0.17], $P < 0.001$). In addition, MEP amplitude differed between the timepoints (time effect, $F_{2,3996} = 8.1$, $P < 0.001$), with increased MEP amplitude 5 minutes following the intervention compared to 30 minutes (EMD = 0.06 mV [0.01, 0.12], $P = 0.02$). There was a significant interaction between tACS treatment and timepoint ($F_{2,3996} = 7.0$, $P < 0.001$; Fig. 2A), with *post hoc* analysis showing increased MEP amplitude following theta-gamma tACS compared with sham tACS at post-5 minutes (EMD = 0.16 mV [0.09, 0.23], $P < 0.001$) and post-30 minutes (EMD = 0.19 mV [0.12, 0.25], $P < 0.001$). MEP amplitude following sham tACS was also decreased at post-5 minutes (EMD = 0.10 mV [0.03, 0.18], $P = 0.006$) and post-30 minutes (EMD = 0.18 mV [0.10, 0.26], $P < 0.001$) compared to baseline. There was also an interaction between tACS treatment and age groups ($F_{1,3996} = 7.5$, $P = 0.006$), which revealed increased MEP amplitude following theta-gamma tACS compared to sham in young (EMD = 0.07 mV [0.01, 0.13], $P = 0.02$) and older adults (EMD = 0.17 mV [0.11, 0.23], $P < 0.001$). There were no other interactions (all $P > 0.114$).

3.5.4. Effect of motor training and theta-gamma tACS on SICI

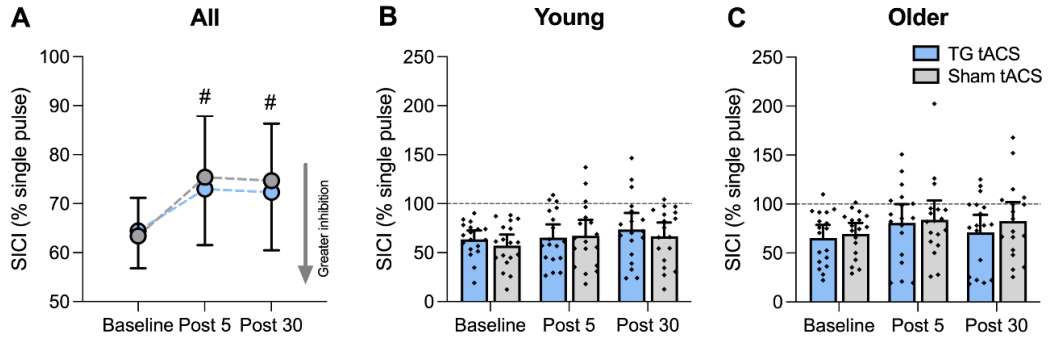


Figure 3.5. **Effect of motor training and theta-gamma tACS on SICI.** Data show EMM [± 95% CI] SICI (% single pulse) in all participants (A), young adults (B), and older adults (C). # Significantly different compared to baseline for theta-gamma tACS and sham tACS combined ($P < 0.05$).

Pre- and post-training SICI for each tACS session are shown in Figure 3.5. SICI did not vary between tACS treatments ($F_{1,4061} = 0.70$, $P = 0.40$) or age groups ($F_{1,4061} = 0.78$, $P = 0.38$), but differed between timepoints ($F_{2,4061} = 7.5$, $P < 0.001$), with *post hoc* comparisons revealing decreased SICI at post-5 minutes (EMD = 8.0% [2.5, 12.9], $P = 0.001$) and post-30 minutes (EMD = 5.8% [1.0, 10.5], $P = 0.01$) relative to baseline (Figure 3.5.A). Moreover, there was a significant interaction between age group and tACS treatment ($F_{1,4061} = 16.2$, $P < 0.001$), with *post hoc* tests showing that for all time points combined (baseline, post 5, post 30), there was less SICI in the theta-gamma tACS session compared with the sham tACS session in young adults (EMD = 5.1% [0.56, 9.72], $P = 0.03$), and there was less SICI for the sham tACS session compared with the theta-gamma tACS session in older adults (EMD = 8.9% [3.5, 14.3], $P = 0.001$). There were no other interactions (all $P > 0.09$). The mean single pulse MEP amplitudes from which SICI was obtained did not differ between tACS treatments ($P = 0.65$), but were influenced by age

group ($F_{1,4067} = 4.4$, $P = 0.04$) and time ($F_{2,4067} = 9.6$, $P < 0.001$). *Post hoc* analyses showed that single-pulse MEP amplitudes (within the SICI block) were greater for young compared with older adults (EMD = 0.13 mV [0.01, 0.26]), and greater post-5 minutes (EMD = 0.06 mV [0.01, 0.12], $P = 0.02$) and post-30 minutes (EMD = 0.11 mV [0.05, 0.17], $P = 0.001$) relative to baseline.

3.5.5. Pain, intensity, localisation (VAS score), and HD tACS blinding of participants

Responses to VAS and blinding questionnaires are presented in Table 3.3. There were no differences in VAS scores for the level of pain, intensity, and HD tACS stimulation localisation between tACS sessions (all $P > 0.10$). During the theta-gamma tACS session, 53% (n = 18) of participants stated the intervention correctly, while 32% (n = 11) stated it incorrectly. In contrast, during the sham tACS session, 24% (n = 8) stated the intervention correctly, 61% (n = 20) of the participants stated it incorrectly, and 15% (n = 5) of participants were unable to differentiate between the two stimulation types in both sessions.

Table 3.3. Comparison of VAS responses (mean ± SD) and HD tACS intervention blinding of participants (n, %) between sessions.

Question	TG tACS	Sham tACS
1. How painful was the stimulation during the HD tACS intervention? (0, not painful at all; 10, very painful)	1.68 ± 2.27	1.15 ± 1.14
2. How intense was the stimulation during the HD tACS intervention? (0, not intense at all; 10, very intense)	3.00 ± 2.83	2.76 ± 2.71
3. How localised were the sensations during HD tACS intervention? (0, highly localised; 10, widespread)	1.24 ± 1.78	1.06 ± 1.34
4. Can you guess the type of stimulation (active or sham) you received today?		

i.	Correct guess (n, %)	18, 52.9%	8, 24.2%
ii.	Incorrect guess (n, %)	11, 32.4%	20, 60.6%
iii.	Unable to differentiate (n, %)	5, 14.7%	5, 15.2%

Data presented as mean ± SD and n, %. VAS, visual analogue scale; HD tACS, high-definition transcranial alternating current stimulation; TG tACS, theta-gamma transcranial alternating current stimulation. The above responses were obtained from 34 adults in the TG tACS session and 33 adults in the sham session.

3.5.6. Correlation between neurophysiological measures and skill acquisition

There were no significant correlations between the change in neurophysiological measures before and after training (MEP amplitudes and SICI) and the change in thumb acceleration performance in young or older adults for both tACS sessions (all $P > 0.05$). Furthermore, there were no significant correlations between baseline neurophysiological measures (RMT, AMT, test TMS intensity, test TMS amplitude, SICI, and M-wave) and the change in motor performance in young or older adults for both tACS sessions (all $P > 0.11$).

3.6. Discussion

It has previously been shown that theta-gamma tACS applied over M1 can improve motor performance of a ballistic thumb abduction task in young adults (Akkad et al., 2021). In this previous study, theta-gamma tACS was shown to be most effective when the gamma oscillation (75 Hz) was applied at the peak of the theta wave (6 Hz), and this finding was replicated in an independent sample from the same study showing that this effect lasted at least one hour (Akkad et al., 2021). Using the same motor task and training protocol, we show a substantial improvement in ballistic motor performance in young participants

with theta-gamma tACS (increase of 134% *vs.* 51% for sham tACS) that was also long-lasting (> 60 mins), indicating that this is a robust effect. In addition, we show for the first time that theta-gamma tACS can improve ballistic motor performance in healthy older adults. During training for the ballistic task in older adults, thumb abduction acceleration increased by 76% after theta-gamma tACS compared with 40% after sham tACS, showing an almost twofold improvement in ballistic thumb abduction acceleration with theta-gamma tACS in older adults. theta-gamma tACS was also accompanied by a long-lasting improvement of task performance at 10 (increase of 88% *vs.* 42% for sham tACS) and 60 mins (increase of 51% *vs.* 25% for sham tACS) after motor training in older adults.

In the previous study by Akkad et al. (2021), a bifocal tACS montage was used to show that theta-gamma tACS over M1 (and parietal cortex, Pz) improved ballistic motor performance in young adults. Although they provide evidence that there is sufficient current over M1 using electrical field simulations (Akkad et al., 2021), there is widespread activation of multiple cortical areas with bifocal tACS, and the possibility exists that the behavioural change seen previously could also have a contribution from the parietal electrode. We therefore selected HD tACS with a 4 × 1 ring montage (one central electrode and four return electrodes) as a more focal way to target M1 and reduce potential activation of other cortical areas (Edwards et al., 2013). Our findings show that theta-gamma tACS using a HD montage is effective at modulating ballistic motor performance in young and older adults, suggesting that an influence of parietal cortex stimulation is unlikely. Furthermore, the possibility exists that different electrode configurations (e.g. two central and three return electrodes) may produce alternative outcomes with HD tACS (Grigutsch et al., 2024), so the electrode configuration used to target a specific cortical

area is likely to be important in determining the beneficial effects of HD tACS, particularly with advancing age (Arias and Heise, 2024).

In contrast to using separate groups in the previous study (Akkad et al., 2021), participants in our protocol performed both sessions in random order that were separated by at least two weeks. We chose to use the same participants for each experimental session in our study to avoid any differences in population characteristics that may confound the interpretation of the effects between sessions. While we found similar baseline performance for each session, covariate analyses identified a significant order effect, where there was greater improvement in thumb abduction acceleration in the first compared with the second session, which was due to a carry-over effect of performance from the first to the second session. This was unexpected, as it has previously been reported that there was no session order effect when this task was performed at least one week apart (Bologna et al., 2019). Nonetheless, our random allocation suggests that session order did not contribute to the greater effects observed with theta-gamma tACS. Furthermore, participants were unable to differentiate between the two tACS conditions, with similar VAS scores for the level of pain, intensity, and stimulation localisation, suggesting successful participant blinding to the stimulation condition. However, future studies should consider using a between-subject experimental design to avoid potential session order effects.

The effects of theta-gamma tACS on ballistic motor performance were reduced in older adults, and this was unrelated to baseline neurophysiological measures or assessments of cognitive function, physical activity levels, or chronotype. However, several factors could contribute to this difference. First, we used the same tACS parameters (intensity,

frequency) in young and older adults, but the optimal parameters may be altered with advancing age. We did not obtain structural magnetic resonance images (MRIs) for our participants, so we were unable to estimate the effective current reaching the cortex, which could be reduced in older adults (when stimulating at the same intensity) due to cortical atrophy (Antal et al., 2017, Freitas et al., 2011). However, it has previously been shown with gamma tACS that the current density reaching M1 was similar in young and older adults when using the same tACS intensity (Guerra et al., 2021). Nonetheless, future studies should consider employing computational modelling with individual MRIs to quantify the effective current reaching the cortex in young and older adults. Furthermore, brain oscillations in sensorimotor areas are altered with advancing age (Ishii et al., 2018), including age-related changes in theta-gamma coupling (Reinhart and Nguyen, 2019, Zhang et al., 2021), so it is possible that the effectiveness of tACS at the frequencies used here (6 Hz theta, 75 Hz gamma) may not be optimal in older adults. Future studies should therefore use electroencephalographic (EEG) measures to explore the effects of personalised tACS frequencies in young and older adults (e.g. Draaisma et al., 2022), but this is challenging in the gamma band as there is no clearly defined EEG peak in many individuals (Leunissen et al., 2022), making optimisation difficult. Second, it is commonly accepted that M1 plasticity declines with advancing age (Freitas et al., 2013, Semmler et al., 2021, Zimerman and Hummel, 2010), with this capacity thought to be a key contributor to the early stages of motor skill acquisition (Classen et al., 1998, Dayan and Cohen, 2011). This reduced plasticity could occur through morphological, neurochemical, and neurophysiological changes in the M1 network in older adults (Seidler et al., 2010), resulting in reduced motor skill acquisition with age (Voelcker-

Rehage, 2008). It is also possible that the maximum capacity for thumb acceleration is reduced in older adults (i.e. lower ceiling effect) due to age-related changes in neuromuscular function, which limits the potential improvement with theta-gamma tACS. For example, the ability to perform ballistic tasks could be reduced in older adults because of a decline in the proportion of muscle occupied by type II fibres (Klein et al., 2003), which are likely to make the biggest contribution to thumb abduction acceleration, particularly at the end of training when acceleration is greatest. Furthermore, higher motor unit firing rates during rapid contractions contribute to an increase in contraction speed (Van Cutsem et al., 1998), and maximum firing rates are reduced in older adults during rapid contractions (Klass et al., 2008), which could limit the performance of ballistic tasks in older adults. Finally, there could be age-related changes in neuromuscular junction efficiency, due to changes in motor axon terminals (thinning, distension and sprouting) and postsynaptic endplates (decreased size, number, length and synaptic folds) (Jang and Van Remmen, 2011).

Gamma oscillations that are phase synchronised to the peak of theta waves (i.e. theta-gamma phase-amplitude coupling; theta-gamma PAC) are known to be an important mechanism for neuronal processing in the hippocampus (Colgin, 2015), where it has been linked with working memory in humans (Alekseichuk et al., 2016, Daume et al., 2024). These observations also extend to the motor system (Canolty et al., 2006), where theta-gamma PAC over motor brain regions has been positively associated with motor learning success (Dürschmid et al., 2014), and in motor recovery following brain-computer interface therapy in chronic stroke patients (Rustamov et al., 2022). As demonstrated previously, theta-gamma tACS over M1 can improve ballistic motor performance in

young adults (Akkad et al., 2021), and we now show that theta-gamma tACS is effective at modulating ballistic movements in healthy older adults. These findings suggest that specific features of M1 oscillations are likely to play a role in ballistic motor performance throughout the healthy lifespan. However, these findings in the motor system are specific to the simple ballistic task used here. This is a widely used experimental task where the explicit goal is to maximise thumb acceleration in the abduction direction, and it results in substantial improvements with practice that are largely dependent on physiological changes in M1 (Muellbacher et al., 2001). Nonetheless, it is currently unknown if theta-gamma tACS can modulate motor performance during more complex visuomotor tasks that more closely represent real-world motor learning requirements, which usually involve coordinated neuronal activity across a distributed network of brain regions involving attentional, memory, visual and motor systems (Dayan and Cohen, 2011). Furthermore, these findings are specific to healthy older adults who demonstrated only minor declines in baseline motor function (Pegboard scores), were moderately active (IPAQ score), and had normal cognitive function (MoCA scores), so the effects of theta-gamma tACS may be different in frail older adults, or older patients with neurological injury. This is supported by recent evidence showing that theta-gamma tACS over M1 during a more complex motor task impaired motor skill acquisition in chronic stroke survivors (Grigutsch et al., 2024). Taken together, these findings suggest that theta-gamma tACS enhances ballistic motor performance in healthy young and older adults, and future studies should explore the effects of theta-gamma tACS on different motor tasks and neurological conditions that have an increased incidence with advancing age.

An improvement in motor performance with the ballistic thumb abduction task is usually accompanied by a post-training increase in MEP amplitude (Muellbacher et al., 2001, Ziemann et al., 2001), which is thought to represent training-dependent plasticity involving LTP-like mechanisms in cortical circuits (Sanes and Donoghue, 2000), although spinal circuits may also be involved in this task (Giesebrecht et al., 2012). In contrast, our data show a reduction in MEP amplitude after motor training (with sham tACS) in young and older adults. This unexpected finding could be due to the extended duration of training undertaken in our study. An increase in MEP amplitude has previously been shown when training is undertaken with this task for shorter durations (Rosenkranz et al., 2007b), and we performed a large number of movement trials that lasted ~25 mins, which could result in a reversal of the expected MEP response due to homeostatic metaplasticity (Hassanzahraee et al., 2020). Furthermore, this effect could be due to the training structure (Lage et al., 2015), where multiple training blocks were performed that were interspersed with rest periods (i.e. interleaved training), as opposed to previous studies showing increased MEPs with less blocks and more repetitive training (Cirillo et al., 2010, Rogasch et al., 2009). Nonetheless, the reduced M1 excitability after motor training was altered with theta-gamma tACS, either by interfering with the neurophysiological processes that generated the MEP depression, or by augmenting training-related MEP facilitation through plasticity-related mechanisms. One way this can occur is through the online entrainment of membrane potentials (with theta-gamma tACS) that interact with the timing of pre- and post-synaptic potentials during voluntary motor activity (Vogeti et al., 2022), resulting in increased neural synchronisation, which is important for inducing synaptic plasticity (Uhlhaas et al., 2010, Womelsdorf et al., 2007).

A reduction in GABAergic inhibition can also play an important role in training-dependent plasticity and motor skill acquisition (Ziemann et al., 2001), and this can be quantified with paired-pulse TMS to assess SICI (Ziemann et al., 1996). Several studies have shown a reduction in SICI after ballistic thumb movements (Liepert et al., 1998, Rosenkranz et al., 2007b), reflecting GABAergic disinhibition in M1 following motor skill acquisition. We found reduced SICI after motor training in young and older adults, which is consistent with these previous findings. However, the magnitude of this change was not different between tACS conditions, suggesting that theta-gamma tACS did not influence training-related GABAergic inhibition. We also showed that SICI was different between tACS sessions in young and older adults (when assessed over all time points), but there was no difference in baseline SICI between tACS sessions or age groups, so this would not affect the change in SICI after training. Importantly, previous studies have shown that gamma tACS over M1 decreases SICI when assessed *during* stimulation (Guerra et al., 2018, Nowak et al., 2017), with this online change shown to be predictive of performance on a subsequent motor learning task (Nowak et al., 2017). However, we were unable to quantify SICI during theta-gamma tACS due to the concurrent performance of the motor task, so it is currently unknown whether theta-gamma tACS (as opposed to gamma tACS alone) modulates SICI. We also could not provide direct evidence of entrainment of brain oscillations with theta-gamma tACS, as electroencephalographic recordings are confounded by large stimulation artefacts (Vogeti et al., 2022). Furthermore, we did not find any correlation between changes in neurophysiology measures (MEP and SICI) and changes in motor behaviour. This is likely to occur because the cortical circuits targeted by TMS may not be the same as those that

are modulated by tACS (Wischnewski et al., 2023), and voluntary movement requires a broader networks of cortical areas that are not specifically targeted by TMS or tACS over M1 (Bestmann and Krakauer, 2015). Therefore, the neurophysiological mechanisms that contribute to improved motor skill acquisition with theta-gamma tACS in healthy young and older adults remain to be determined.

Our experimental approach consisted of a theta-gamma tACS waveform compared with sham tACS, and the possibility exists that the improvement in ballistic motor performance observed here could be due to theta or gamma tACS alone. However, several factors suggest that this explanation is unlikely. First, gamma tACS at 70 Hz applied over M1 does not influence ballistic finger movement and results in poorer retention of ballistic task performance compared with sham tACS (Bologna et al., 2019). Second, Akkad et al. (2021) showed that theta-gamma tACS produced a greater improvement in ballistic motor performance when the gamma oscillation was nested at the peak of the theta wave (theta-gamma Peak), whereas there was no difference in performance when the gamma oscillation was nested at the trough of the theta wave (theta-gamma Trough) compared with sham tACS. This suggests that theta or gamma tACS delivered in both theta-gamma Peak and theta-gamma Trough conditions did not modulate motor performance, but it is the temporal association of the gamma oscillations relative to the peak of the theta wave that is driving this effect. Third, based on previous evidence in the prefrontal cortex, theta-gamma tACS has a greater effect on spatial working memory performance compared with theta tACS alone (Alekseichuk et al., 2016), whereas we are not aware of any evidence showing that theta tACS applied over M1 can modulate motor performance. Taken together, these findings suggest that it is the phase synchronised activity of theta-gamma

tACS that contributes to the improved ballistic motor performance that we have observed in young and older adults. Nonetheless, further studies would be needed to identify the independent effects of theta and gamma tACS alone, along with other frequency coupling conditions, to confirm that theta-gamma coupling is responsible for the improved ballistic motor performance.

3.6.1.Conclusion

In conclusion, we have shown that theta-gamma tACS can enhance motor skill acquisition in healthy young and older adults. These improvements in performance were long-lasting (> 60 mins) and stronger in young compared with older adults. In addition, theta-gamma tACS altered training-induced changes in M1 excitability, but did not influence changes in GABAergic inhibition in both age groups. These findings suggest that theta-gamma tACS represents a promising approach to improve motor function throughout the lifespan in healthy individuals. To better understand the rehabilitation potential of theta-gamma tACS, future research will need to explore the efficacy of different theta-gamma tACS protocols with more complex motor tasks and in neurological conditions that have an increased incidence in the ageing population.

3.7. Corrigendum

Corrigendum to: '*Theta-gamma transcranial alternating current stimulation enhances ballistic motor performance in healthy young and older adults*'

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Regarding our above-referenced publication in *Neurobiology of Aging*, we recently discovered that the theta-gamma (TG)-tACS waveform used in this study was the inverse of the planned stimulation. Specifically, we had intended that the central electrode would apply a gamma burst nested in the theta peak, whereas return electrodes would apply a gamma burst nested in the theta trough (Fig 1A, left). In contrast, due to polarity differences between electrodes, stimulation was inverted, with trough gamma being applied at the central electrode and peak gamma being applied at return electrodes (Fig 1A, right).

Although this does not influence study justification or validity, there are some important changes to interpretation that need to be considered. In particular, this study now provides the first evidence that a trough-gamma TG-tACS waveform can improve acquisition of a ballistic motor task in both young and older adults, which is in contrast to results of a previous study in young adults (Akkad et al., 2021). However, we would like to

additionally highlight that methodological differences between the current and previous studies were not limited to the tACS waveform. Specifically, Akkad and colleagues applied stimulation using a conventional bipolar montage, involving a pair of large (5 x 5 cms) electrode pads placed over motor and parietal cortices (Fig. 1B). In contrast, our study used a high-definition (HD) montage – with a central electrode surrounded by four return electrodes – covering a single motor cortical area of ~ 6 x 6 cms. Stimulation of motor cortex with the conventional montage therefore involved a purely peak-gamma waveform, in addition to trough-gamma activation of parietal cortex. In contrast, our HD montage limited stimulation to a (primarily) motor cortical area of similar size to the Akkad et al. (2021) study. Furthermore, stimulation in this area involved a combination of both trough-gamma (central electrode) and peak-gamma (return electrodes) waveforms. It is therefore possible that peak-gamma stimulation still contributed to the improved motor performance observed by our study, and clarification of this effect in future work will be important.

Although unexpected, we note that functionally-relevant, endogenous oscillatory activity consistent with our stimulation waveform (i.e., phase-amplitude coupling between the trough of a theta wave and gamma amplitude) has been previously observed in motor cortex (Dürschmid et al., 2014). Consequently, there is physiological justification for a modulatory response to this type of waveform, and further investigation of how trough-gamma waveforms influence cortical activity and motor function are needed.

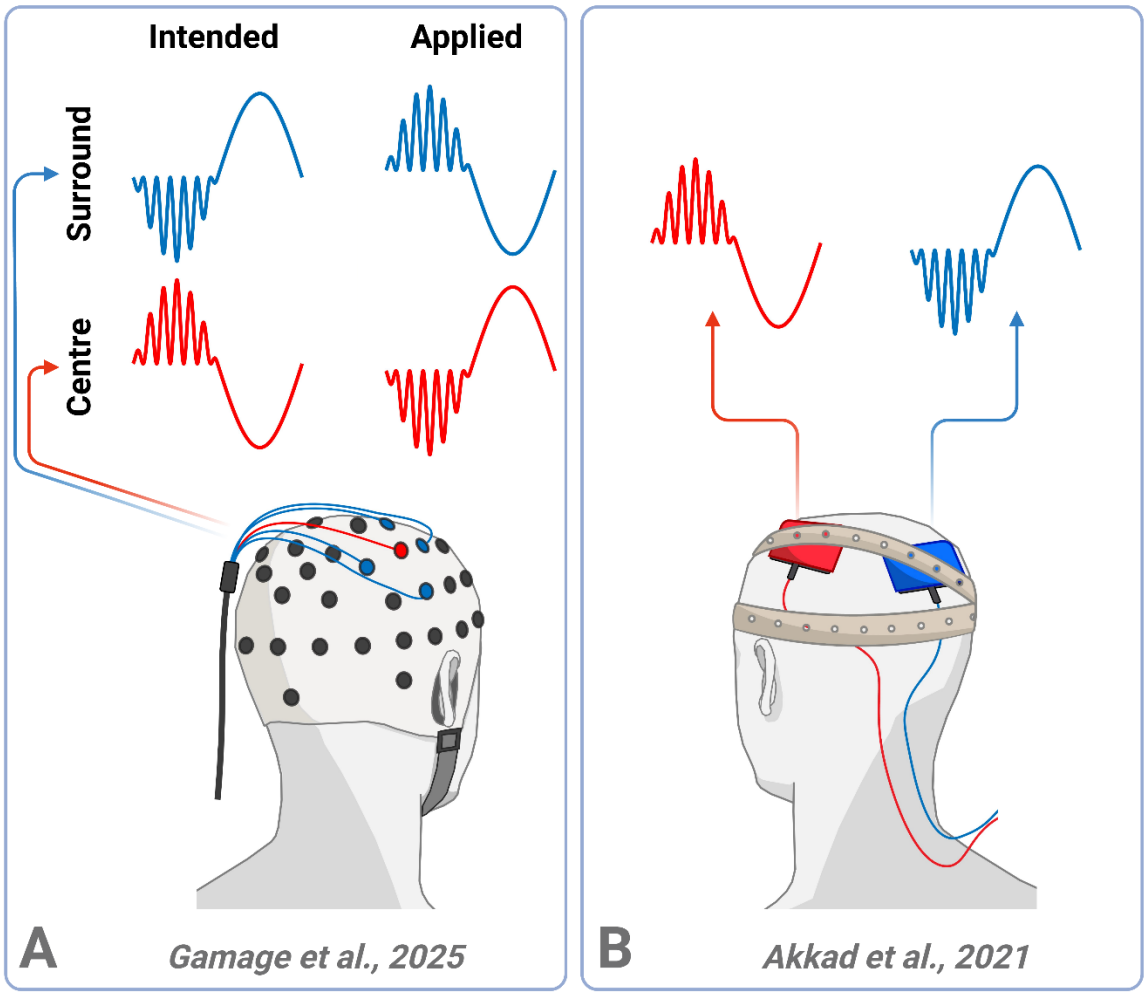


Figure 3.6. Comparison of tACS waveforms. The study by Gamage et al. (2025) utilised a high-definition montage to apply tACS over right primary motor cortex ***(A)***. The intended waveform involved a gamma burst nested in the theta peak (centre electrode) or trough (surround electrodes) *(left)*. In contrast, the applied waveform was the inverse of this *(right)*. Illustration of the conventional tACS montage and waveform used by Akkad et al. (2021) ***(B)***.

CHAPTER 4

TRANSCRANIAL ALTERNATING CURRENT STIMULATION IMPROVES BALLISTIC MOTOR PERFORMANCE IN TRAINED AND UNTRAINED LIMBS OF HEALTHY OLDER ADULTS

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4.1. Statement of Authorship

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Name of principal author (candidate)	Nishadi N H Gamage		
Contribution to the paper	Conceptualisation; data curation; formal analysis; investigation; methodology; project administration; validation; visualisation; writing—original draft.		
Overall percentage (%)	70%		
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	07/07/2025

Co-author contributions

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

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

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Signature		Date	07/07/2025

4.2. Abstract

Transcranial alternating current stimulation (tACS) applied at different frequencies over primary motor cortex (M1) can influence specific aspects of motor function. Here, we examined the effects of theta and combined theta-gamma tACS on ballistic performance in the trained and untrained hands of 60 right-handed healthy older adults (68.9 ± 5.2 years). Participants were allocated to receive high-definition theta-gamma (6 Hz theta, 75 Hz gamma), theta (6 Hz), or sham tACS over right M1 for 20 minutes during ballistic left-thumb training. Behavioural changes were quantified as the changes in performance in trained (left) and untrained (right) hands. Neurophysiological changes in M1 were assessed using transcranial magnetic stimulation, with motor-evoked potentials (MEPs) and short-interval intracortical inhibition (SICI) recorded in both sides before and after training.

Motor performance improved in both hands in theta (trained; 400% vs. untrained; 254%), theta-gamma (trained; 67% vs. untrained; 79%), and sham (trained; 92% vs. untrained; 84%) tACS groups (all $P < 0.001$). However, theta tACS showed a greater improvement compared to theta-gamma and sham tACS for both hands (all $P < 0.02$). The predictive correlation between the trained and untrained gains in motor performance was significant for theta-gamma tACS only ($R^2 = 0.501$, $P < 0.001$). MEP amplitude for trained M1 increased following theta tACS (all $P < 0.027$), but SICI did not vary between groups (all $P > 0.17$).

Our results indicate that unilateral ballistic training concurrently with theta tACS produced the greatest improvement in performance on trained and untrained limbs

compared to theta-gamma and sham tACS, with increased training-induced excitability for theta tACS. Only theta-gamma tACS predicted cross-limb transfer of performance gains. While the exact mechanism of theta and theta-gamma tACS is uncertain, distinct mechanisms may account for the trained limb gains and cross-limb transfer, with a strong cortical involvement.

Keywords: motor cortex, TMS, ageing, motor rehabilitation, brain stimulation

4.3. Introduction

Healthy ageing is accompanied by a progressive deterioration in movement control (Ward and Frackowiak, 2003), which affects functional independence and quality of life. Additionally, the ability to learn and modify movements (Cirillo, 2021, Roig et al., 2014), particularly those involving fine motor skills, also decline during the ageing process (Voelcker-Rehage, 2008). While this behavioural decline is driven by changes in all levels of the motor system (see Hunter et al., 2016 for review), one underlying factor is the changes in the central nervous system (see Seidler et al., 2010 for review). In particular, there is reduced capacity of the brain to modify its neural connections (i.e., neuroplasticity) (Mahncke et al., 2006, Semmler et al., 2021). Therefore, many studies have developed and tested different interventions to boost plasticity and enhance motor performance in older adults, but results are highly variable (see Semmler et al., 2021 for review).

Transcranial alternating current stimulation (tACS) has emerged as an effective method of non-invasive brain stimulation (NIBS) to alter both cognitive and motor behaviour. This technique consists of a small alternating electrical current that is thought to entrain neural activity (Krause et al., 2019, Vöröslakos et al., 2018). When applied over primary motor cortex (M1), tACS at different frequencies can modulate various aspects of motor behaviour (see McNally et al., 2024 for review). For example, tACS at alpha (8-13 Hz) and beta (13-30 Hz) frequencies has been shown to facilitate motor skill acquisition of a serial reaction time task (SRTT) (Antal et al., 2008, Pollok et al., 2015). Gamma tACS (> 30 Hz) can enhance several elements of motor behaviour, including acceleration (Moisa

et al., 2016), force generation (Joundi et al., 2012), and motor learning associated with SRTT (Sugata et al., 2018), but does not improve ballistic tasks (Bologna et al., 2019). Furthermore, the application of different phase-locked frequencies has been used as an alternative approach to mimic the natural oscillations that occur in the cortex (cross-frequency coupling, see Canolty and Knight, 2010 for review), where high-frequency gamma oscillations are nested within phases of low-frequency theta waves (4-7 Hz) (Canolty et al., 2006, Grigutsch et al., 2024, Johnson et al., 2017); a phenomenon termed theta-gamma phase amplitude coupling (PAC). For example, theta-gamma tACS has been shown to modulate cognitive function in young (Alekseichuk et al., 2016) and older adults (Reinhart and Nguyen, 2019) and visuomotor learning in older adults (Diedrich et al., 2024). Additionally, theta-gamma tACS applied over M1 enhances the acceleration of thumb movements in young (Akkad et al., 2021, Gamage et al., 2025c, Grigutsch et al., 2024) and older adults (Gamage et al., 2025c). However, the contribution of the theta oscillation to the improvement in motor performance with theta-gamma tACS is currently unknown, as the effect of theta tACS (alone) on ballistic motor performance has not been assessed (see McNally et al., 2024 for review).

An interesting feature of unilateral training is that it is often accompanied by changes in performance of the untrained limb, a phenomenon known as cross-limb transfer (Gamage et al., 2025b, Manca et al., 2021a). The cross-limb transfer gains vary by task type, with ballistic training producing untrained performance gains ranging from 49-82% in young (Carroll et al., 2008, Hinder et al., 2013) and 6-26% in older adults (Hinder et al., 2013, Hinder et al., 2011), and resistance training eliciting average strength improvements of 18% in young and 15% in older adults (see Green and Gabriel, 2018 for review). This

transfer phenomenon is highly pertinent for older adults due to its efficacy in motor function recovery following stroke (Dehno et al., 2021, Dragert and Zehr, 2013b) and single-limb fractures (Magnus et al., 2013). Although some aspects of cross-limb transfer remain functional throughout the lifespan (Barss et al., 2016), this capacity declines with advancing age (Hinder et al., 2013, Hinder et al., 2011). As the mechanisms associated with cross-limb transfer are thought to include changes at the cortical level (Ruddy and Carson, 2013), studies have explored the use of NIBS as a potential intervention to improve cross-limb transfer in older adults. For example, anodal transcranial direct current stimulation (tDCS) on M1 contralateral to the trained limb enhances cross-limb transfer of grooved pegboard performance (Kaminski et al., 2022) and visuomotor tracking (Goodwill et al., 2015) in older adults, which was otherwise absent following motor training alone (with sham tDCS). Considering the capacity of tACS to modulate neuronal membrane potentials at a specific frequency (Krause et al., 2019), and the established widespread effects of brain oscillations on cortical connectivity, especially at low frequencies (see Bland and Oddie, 2001 for review), tACS may have the potential to modulate cross-limb transfer. However, the effect of tACS on cross-limb transfer in older adults remains unclear.

The current study aimed to assess the effects of tACS over M1 on motor performance and cross-limb transfer in healthy older adults, using theta and combined theta-gamma waveforms. Motor performance and cross-limb transfer were evaluated with a ballistic thumb abduction task, which is commonly used to assess age-related alterations in motor performance (Muellbacher et al., 2001, Rogasch et al., 2009) and can be functionally relevant for opposition and grasping (Gracia-Ibáñez et al., 2017). Additionally, similar

ballistic hand motor tasks have been previously used to assess the cross-limb transfer effect in young and older adults (Carroll et al., 2008, Hinder et al., 2013, Hinder et al., 2011). Training-induced changes in M1 excitability and intracortical inhibition were assessed with transcranial magnetic stimulation (Muellbacher et al., 2001). Since previous studies have shown greater ballistic motor performance with theta-gamma tACS than sham tACS (Gamage et al., 2025c) and greater working memory with theta-gamma tACS than theta tACS (Alekseichuk et al., 2016), we hypothesised that theta-gamma tACS would produce greater motor performance and cross-limb transfer than theta tACS and sham tACS. We also hypothesised that theta tACS would have greater effects compared to sham tACS (Alekseichuk et al., 2016).

4.4. Methods

4.4.1. Participants and ethics approval

Sample size calculations were performed by an unblinded team member using simulation-based power estimations on *RStudio* (version 2023.12.1 + 402) with '*lme4*' (Bates et al., 2015) and '*simr*' (Green and MacLeod, 2016) packages following data collection from 15 participants (5 in each tACS group). This analysis investigated the effects of tACS treatment on ballistic motor training data, which revealed a fixed effects coefficient of −0.306 that was used as an unstandardised effect size of tACS. This value was lowered by 15 % (-0.260) to account for biases in power calculations derived from experimental data (Kumle et al., 2021a). The final sample size estimation revealed that a total of 60 participants were needed, with 20 in each tACS group, given $\alpha < 0.001$, $\beta > 0.9$ (0.913).

Sixty healthy, right-handed older adults (37 females; 68.9 ± 5.2 years; age range, 60-80 years) were recruited to participate in the current study. Exclusion criteria included a history of concussion or seizure, diagnosis of neurological, cardiovascular, or neuropsychiatric conditions, or use of any prescribed medications known to affect cortical excitability (Ziemann et al., 2015). Participants regularly involved in skilled manual dexterity activities (e.g., musicians) were also excluded. Contraindications for transcranial magnetic stimulation (TMS) were assessed using the TMS safety screening questionnaire (Rossi et al., 2011), and handedness was assessed using the Edinburgh Handedness Inventory (Oldfield, 1971). Additionally, since motor system plasticity or motor behaviour can be influenced by physical activity levels (Cirillo et al., 2009), chronotype (Salehinejad et al., 2021), and cognitive function (Chou et al., 2022), we evaluated participant physical activity levels using the International Physical Activity Questionnaire- Short Form (IPAQ) (Craig et al., 2003), participant chronotypes using the Morningness-Eveningness questionnaire (MEQr) (Adan and Almirall, 1991) and participant cognition levels using the Montreal Cognitive Assessment (MoCA) questionnaire (Hobson, 2015). The experimental procedures were approved by the human research ethics committee of the University of Adelaide (*Ref no H-026-2008*), and the study was performed in accordance with the *Declaration of Helsinki.* All participants provided written informed consent prior to the experiment.

4.4.2. Experimental procedures

The present study followed a randomised, double-blind, sham-controlled design. Participants attended one experimental session that lasted ~3 hours and were randomly allocated to receive theta tACS (n =20), theta-gamma tACS (n = 20), or sham tACS (n =

20) during motor training. A between-participant design was used to minimise potential carryover of interventional effects (i.e., responses to training or tACS) (Gamage et al., 2025c). The experimental procedures (Figure 4.1) were performed between 11:00 and 17:00 for all participants, and all experiments were conducted by the same experimenter to retain consistency between sessions. The order of testing between the left and right sides for pegboard, neurophysiological measures, and thumb acceleration testing was randomised and counterbalanced at all timepoints (Hinder et al., 2013, Hinder et al., 2011). All participants and researchers involved in data collection and analyses remained blinded until data analysis was completed.

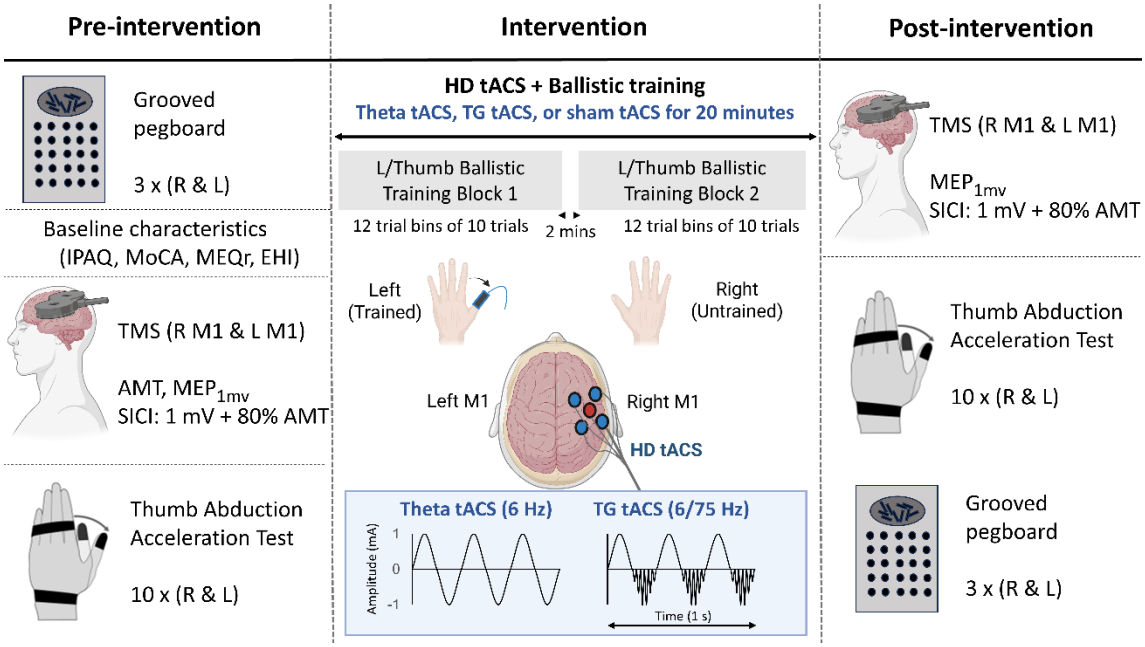


Figure 4.1. Experimental protocol including baseline, intervention, and post-intervention measures. TMS; Transcranial magnetic stimulation; R, right; L, left; M1, primary motor cortex; AMT, active motor threshold; MEP, motor-evoked potential; SICI, short-interval intracortical inhibition (ISI 2 ms, CS at 80% AMT, and TS at 1 mV); IPAQ, International Physical Activity Questionnaire; MoCA, Montreal Cognitive Assessment; MEQr, Morningness-Eveningness questionnaire (chronotype); EHI, Edinburgh

Handedness Inventory. Motor training setup: HD tACS (theta, theta-gamma, or sham tACS) on right M1 while performing a ballistic thumb abduction task in the left (non-dominant) hand. *Figure created using BioRender.com.*

Transcranial magnetic stimulation (TMS) and electromyography

Participants were seated on a comfortable chair with their hands relaxed on a table in front of them. Surface electromyography (EMG) was recorded from bilateral abductor pollicis brevis (APB) muscles: for each muscle, two Ag-AgCl electrodes were placed ~2 cm apart in a belly-tendon montage, with a third electrode positioned on the radial styloid process acting as ground. EMG signals were amplified (300×) and band-pass filtered (20 Hz high pass to 1 kHz low pass) using a CED 1902 signal conditioner (Cambridge Electronic Design, Cambridge, UK) before being digitised at 2 kHz using a CED 1401 analogue-to-digital converter. A Humbug mains noise eliminator (Quest Scientific, North Vancouver, Canada) was used to remove signal noise associated with mains power (50 Hz). All EMG recordings were stored on a computer for offline analysis.

A figure-of-eight branding iron coil (70 mm) connected to two Magstim 200^2 magnetic stimulators via a BiStim module (Magstim, Dyfed, UK) was used to stimulate the hand area of both left and right M1, which were assessed at different times. The TMS coil was positioned at 45° to the midsagittal plane to induce a posterior-to-anterior (PA) current flow perpendicular to the central sulcus, and the stimulation was delivered at a rate of 0.2 Hz with a 10% jitter between trials. Once the APB hotspot of the testing M1 was identified and marked using a surgical marker, it was regularly monitored throughout TMS testing to ensure accurate coil positioning. Active motor threshold (AMT) was defined as the lowest stimulus intensity (percentage of maximum stimulation output; % MSO) required

to generate a motor-evoked potential (MEP) amplitude of ≥ 200 μV in at least 5 out of 10 consecutive trials during a low-intensity voluntary contraction (~10% of the maximum) of APB (Rogasch et al., 2009).

The single-pulse TMS intensity was specified as the lowest intensity that produced a mean MEP amplitude of ~1.0 mV (target range: 0.5 to 1.5 mV, unless 100% MSO was required) during relaxation of the tested APB, when averaged across 15 consecutive trials. This single-pulse TMS intensity obtained at pre-training was used for post-training MEP amplitude assessments. One block of single-pulse TMS was recorded from each side before and after training.

Short-interval intracortical inhibition (SICI) was recorded using paired-pulse TMS with an initial sub-threshold conditioning stimulus (CS; 80% AMT) followed by a suprathreshold test stimulus (TS; 1 mV) with a 2 ms interstimulus interval (ISI) (Opie and Semmler, 2019). One SICI block was recorded from each side before and after training. Each SICI block included 15 single-pulse and 15 paired-pulse TMS trials delivered in a random order. The CS and TS intensities remained constant throughout the experimental session.

Motor task and thumb acceleration testing

Participants performed a ballistic (fast-as-possible) thumb abduction task that requires maximising thumb acceleration over a training period (Rogasch et al., 2009). Biaxial accelerometers were connected to the distal phalanx of both thumbs to record thumb acceleration in the abduction-adduction (x-axis) and flexion-extension (z-axis) planes (Gamage et al., 2025c, Rogasch et al., 2009). Participants were seated with their forearms

placed in a custom-made plastic splint, shoulders relaxed, elbows flexed (~90°), and forearms semi-pronated with palms facing each other. This splint enabled the thumbs to move freely while immobilising the forearms, wrists, and interphalangeal joints of other fingers. All acceleration signals were digitised at 2 kHz with a CED interface system and stored on a computer for offline analysis.

All thumb acceleration movements were performed at 0.5 Hz, paced by a metronome. Ten trials of thumb acceleration in each hand were recorded at pre- and post-training. Thumb abduction training was performed in the non-dominant (left in all) hand in line with a previous study showing the transfer of ballistic performance following non-dominant (left) hand training in older adults (Hinder et al., 2013). Participants were encouraged to maximise thumb acceleration during training (Akkad et al., 2021). Real-time visual feedback of the thumb acceleration was provided on a screen in front of the participants during pre-training, motor training, and post-training, while verbal encouragement was only provided during the training period. Motor training was split into two training blocks of 120 trials each, with a two-minute rest between blocks. Each training block included 12 trial bins (sets) of 10 trials with a 20-second rest in between each trial bin to minimise fatigue. Motor training blocks 1 and 2 included trial bins 1-12 and 13-24, respectively.

Grooved pegboard task

The manual dexterity of participants was assessed using the grooved pegboard task (Reuben et al., 2013) before and after the intervention. The pegboard contained 25 holes (five-by-five layout), each of which had a 'key' shaped profile with a centre circle and radial groove (each oriented differently). Participants were instructed to position as many

pegs into matching holes as possible over 30 seconds. Three trials with a 30-second rest between each trial were performed in each hand, and the average of the trials was recorded.

HD tACS intervention

High-definition (HD) tACS was delivered to right M1 during motor training using five HD electrodes (Soterix Medical Inc, New York, US) in 1 x 4 HD tACS configuration following the international 10-20 system, with the centre electrode positioned at *C4* and four surrounding electrodes placed at *C2*, *C6*, *FC4*, and *CP4* (Figure 4.1). Participants wore a cap with HD electrodes connected to a HD-tES stimulator (Soterix Medical MXN-33-HD-tES) operated with tACS software (Soterix Medical, New York, US) on a laptop. The HD electrodes were attached to the scalp using HD electrolyte gel (Soterix Medical Inc, New York, US), and the impedance was maintained below 20 kΩ.

Participants received either theta-gamma tACS, theta tACS, or sham tACS for 20 minutes during motor training. Theta-gamma tACS included a 6 Hz sinusoid coupled with a 75 Hz gamma burst, with the waveform applied in the central electrode being amplitude-modulated by the negative peak of the theta wave (theta-gamma trough), and peak-to-peak amplitude of 2 mA (Akkad et al., 2021, Gamage et al., 2025c). In contrast, theta tACS comprised a pure 6 Hz sinusoid also applied at 2 mA peak-to-peak amplitude (Figure 4.1). For sham tACS, theta-gamma tACS (6/75 Hz) was ramped up to 2 mA for 10 s and then immediately ramped down again (Gamage et al., 2025c).

VAS (pain, intensity, and localisation) and stimulation blinding

To ensure that experimenters were blinded to the stimulus condition, tACS was administered by an independent experimenter who was not involved in any other element of the project. Furthermore, participant blinding was assessed using a visual analogue scale (VAS) to indicate perception of pain, stimulation localisation and intensity. Additionally, participants were asked to guess the type of stimulation (active or sham) they received.

4.4.3. Data analyses

Motor task and cross-limb transfer of ballistic performance

Thumb acceleration was calculated as the difference between maximum acceleration and the acceleration at 200 ms pre-movement, and expressed in m/s^2 (Rogasch et al., 2009). Trials with acceleration < 0 m/s^2 were removed prior to analysis (~0.39% of thumb acceleration trials removed). The coefficient of variation (CV) of thumb acceleration was calculated for pre-training, during motor training, and post-training for each participant. Ballistic motor performance gain (% improvement) was calculated as the difference between mean post-training and mean pre-training acceleration, expressed as a percentage of mean pre-training acceleration for both trained and untrained hands in each participant (Green and Gabriel, 2018).

TMS measures of MEP and SICI

Each TMS trial was visually inspected, and trials with EMG exceeding 20 μV peak-to-peak amplitude in the 100 ms prior to TMS were excluded from analysis (~4.3% of MEP

trials and ~6.9% of SICI trials were removed). MEP amplitude was measured peak-to-peak and expressed in mV. To quantify SICI, the MEP amplitude for each paired-pulse trial was expressed as a percentage of the average single-pulse MEP amplitude within the same block, where a SICI value of 100% denotes no inhibition, and 0% indicates maximum inhibition (Opie and Semmler, 2019).

4.4.4. Statistical analyses

All statistical analysis was performed using IBM SPSS (version 28.0) and visualised using Prism (GraphPad software, version 9.0). Distribution of data residuals for TMS and behavioural data was visually inspected and assessed using Kolmogorov–Smirnov tests, which revealed non-normal, positively skewed distributions. Therefore, generalised linear mixed models (GLMMs) with Gamma distribution and log link function were used for statistical analyses (Lo and Andrews, 2015). Each model included single-trial data with repeated measures and included random participant intercepts (Barr et al., 2013). Data for all models are presented as estimated marginal means (EMM) with 95% confidence intervals [95% CI], whereas pairwise comparisons are presented as estimated mean differences (EMD) and 95% CI for an unstandardised measure of effect size. For all models, main effects and interactions were examined using custom contrasts with Bonferroni corrections, and significance was set at $P < 0.05$.

Pre-training measures

Two-factor GLMMs were used to assess the effects of tACS treatment (theta tACS, theta-gamma tACS, and sham tACS) and side (trained [left] and untrained [right]) on pre-training TMS measures (AMT, test TMS intensity, test MEP amplitude, and SICI) and

functional measures (thumb acceleration, pegboard score). Additionally, a two-factor GLMM was used to investigate the effects of treatment and side on the CV of thumb acceleration at pre-training.

Post-training measures

Functional measures

A three-factor GLMM was used to assess the effects of tACS treatment, side, and timepoint (pre- and post-training) on ballistic performance of trained and untrained hands. A single two-factor GLMM was used to investigate the effects of tACS treatment and trial bins on ballistic motor performance of the trained left hand. Additionally, a three-factor GLMM was used to investigate the effects of treatment, timepoint, and side on the CV of thumb acceleration for each participant. Moreover, a single-factor GLMM was used to assess the effects of tACS treatment on performance gain for trained and untrained hands, and to evaluate cross-limb transfer of ballistic motor performance. Finally, for pegboard score analysis, a three-factor GLMM was used to assess the effects of tACS treatment, side, and time for trained and untrained hands.

Neurophysiological measures

Two three-factor GLMMs were used to assess the effects of tACS treatment, side, and timepoint on single-pulse MEP amplitude and SICI.

Covariates and correlation analysis

To account for between-subject variance in baseline cognition, physical activity and chronotype, all models assessing MEP, SICI, ballistic motor performance, and pegboard score included MoCA, IPAQ, and MEQr scores as covariates. The *Spearman's correlation test* was used to investigate the association between changes in neurophysiological measures before and after training (MEP amplitudes and SICI) and the change in ballistic motor performance in trained and untrained hands. Additionally, the correlation between pre-training neurophysiological measures (AMT, test TMS intensity, test TMS amplitude, SICI, and pegboard score) and the change in ballistic motor performance in trained and untrained hands was assessed using *Spearman's correlation test.* Additionally, *linear regression analyses* were used to evaluate whether the performance gains in the trained hand could predict the performance gains in the untrained hand for each tACS group.

VAS (pain, intensity, and localisation) and stimulation blinding

Changes in the perception of theta tACS, theta-gamma tACS, and sham tACS were explored by comparing VAS responses for pain, intensity, and localisation using *One-way ANOVA*, with data presented as mean ± SD. Additionally, a *Chi-square test* was used to assess if the stimulation guess proportions differed significantly between the tACS treatment groups, with data presented as n, %.

4.5. Results

All participants completed their sessions without any adverse effects. We were unable to record TMS in six participants due to high TMS threshold (mean pre-training MEP amplitude < 0.1 mV at 100% MSO; *n = 4*) and increased muscle activity at rest (> 0.025 mV pre-training activity; *n = 2*) on either side. Participant characteristics are shown in Table 4.1, where no significant differences were found between tACS stimulation groups for any variable (all $P > 0.05$).

Table 4.1. Participant characteristics.

Measure	Theta tACS	Theta-gamma tACS	Sham tACS	*P* value
Sample size (F, M)	20 [14, 6]	20 [10, 10]	20 [13, 7]	N/A
Age (years)	67.5 [65.3, 69.9]	69.0 [66.7, 71.4]	70.3 [67.9, 72.7]	0.261
Handedness (LQ)	0.90 [0.81, 1.00]	0.91 [0.82, 1.01]	0.80 [0.72, 0.90]	0.209
Physical activity (IPAQ score)	3790 [2555, 5621]	3538 [2386, 5248]	3988 [2689, 5915]	0.911
Cognition (MoCA score)	29.3 [28.7, 29.8]	28.9 [28.4, 29.4]	28.9 [28.4, 29.4]	0.442
Chronotype (MEQr value)	16.1 [15.4, 16.7]	15.7 [15.1, 16.3]	16.2 [15.5, 16.8]	0.571

Data presented as EMM [lower 95% CI, upper 95% CI]. LQ, laterality quotient [from -1 (left-handed) to +1 (right-handed)]. IPAQ, International Physical Activity Questionnaire-short form; MoCA, Montreal Cognitive Assessment; MEQr, Morningness-Eveningness questionnaire.

Pre-training neurophysiological measures are shown in Table 4.2. Pre-training thumb acceleration varied between sessions for the trained (left) hand ($F_{2,57}$ = 6.3, P = 0.003), with greater acceleration for theta-gamma tACS compared to theta tACS (EMD = 3.6 m/s^2 [1.2, 6.0], P = 0.001) and sham tACS (EMD = 2.5 m/s^2 [0.2, 4.9], P = 0.035). Additionally, pre-training thumb acceleration for theta-gamma tACS differed between the trained and untrained hands (EMD = 2.5 m/s^2 [1.7, 3.2], P < 0.001). Furthermore, CV for pre-training thumb acceleration indicated no significant main effects or interactions between the side and tACS treatment (all P > 0.46). There were no pre-training differences for all other neurophysiological measures (all P > 0.05). Additionally, there were no effects of IPAQ, MoCA, and MEQr on thumb acceleration, MEP amplitude, and SICI for all tACS treatments at pre-training (all P > 0.05).

4.5.1. Effects of HD tACS on motor performance during training

Thumb acceleration for the trained hand during motor training (trial bins 1-24) is shown in Figure 4.2. Motor training thumb acceleration differed between tACS groups ($F_{2,14272}$ = 3.7, P = 0.024), with greater acceleration for theta tACS compared to sham tACS (EMD = 4.7 m/s^2 [0.5, 9.0], P = 0.024), but no difference between theta-gamma tACS and theta tACS, or theta-gamma tACS and sham tACS (both P = 0.293). Thumb acceleration also varied between trial bins ($F_{23,14272}$ = 61, P < 0.001), revealing an overall increase in thumb acceleration during motor training. Furthermore, there was a two-way interaction between tACS treatment and trial bins ($F_{46,14272}$ = 6.8, P < 0.001). *Post-hoc* analysis indicated greater thumb acceleration for theta tACS compared to sham tACS for trial bins 9-12 and 14-24 (all comparisons P < 0.038). Additionally, there was greater acceleration for theta

tACS compared to theta-gamma tACS at trial bins 12, 18, and 19 (all comparisons $P < 0.043$). Finally, the CV for thumb acceleration in the trained hand did not vary between tACS treatments ($P = 0.778$), but differed between trial bins ($F_{2,171} = 56$, $P < 0.001$), showing an overall reduction in variability during motor training (all $P < 0.001$). There was no interaction between tACS treatment and trial bins ($P = 0.528$).

Table 4.2. Baseline neurophysiological measures before training.

	Theta tACS		Theta-gamma tACS		Sham tACS	
TMS measures ***(n = 54)***	***Left M1***	***Right M1***	***Left M1***	***Right M1***	***Left M1***	***Right M1***
AMT (%MSO)	47.5 [43.1, 52.4]	43.0 [38.5, 47.9]	44.8 [40.9, 49.2]	46.7 [42.2, 51.9]	41.1 [37.4, 45.2]	42.8 [38.4, 47.6]
Test TMS intensity (%MSO)	72.4 [64.7, 81.1]	76.2 [68.7, 84.4]	75.7 [68.0, 84.2]	74.5 [67.6, 82.1]	68.7 [61.5, 76.6]	66.9 [60.6, 73.9]
Test MEP amplitude (mV)	0.88 [0.74, 1.04]	0.82 [0.71, 0.95]	0.89 [0.76, 1.04]	1.02 [0.90, 1.18]	0.90 [0.76, 1.06]	0.91 [0.79, 1.05]
SICI (% single pulse)	65.6 [52.1, 82.5]	72.8 [59.2, 89.6]	73.4 [59.1, 91.2]	58.5 [48.1, 71.2]	75.4 [60.4, 94.3]	77.2 [63.1, 94.4]
Functional measures ***(n = 60)***	***Left hand***	***Right hand***	***Left hand***	***Right hand***	***Left hand***	***Right hand***
Thumb acceleration (m/s^2)	5.6 [4.4, 6.8]	5.9 [4.7, 7.4]	9.5 # ^ [7.6, 12.0]	6.6 * [5.3, 8.3]	6.7 [5.4, 8.4]	6.7 [5.3, 8.3]
Thumb acceleration CV (%)	26.3 [21.3, 32.5]	25.9 [21.0, 32.0]	24.8 [20.1, 30.6]	24.2 [19.6, 29.9]	24.3 [19.7, 30.1]	29.4 [23.8, 36.3]
Pegboard score	9.4 [8.7, 10.3]	10.8 [9.9, 11.8]	8.5 [7.8, 9.3]	9.9 [9.2, 10.8]	8.9 [8.2, 9.7]	10.4 [9.5, 11.3]

Data indicated as EMM [lower 95% CI, upper 95% CI]. tACS, transcranial alternating current stimulation; MSO, maximal stimulator output; AMT, active motor threshold; MEP, motor-evoked potential; TMS, transcranial magnetic stimulation; SICI, short-interval intracortical inhibition. TMS measures are reported only for 54 participants (33 females) [Theta tACS; 17 (11 females), theta-gamma tACS (10 females); 19, Sham tACS; 18 (12 females)], and functional measures are reported for all 60 participants (37 females). #Significantly different between Theta tACS and theta-gamma tACS ($P = 0.001$); ^Significantly different between theta-gamma tACS and sham tACS ($P = 0.035$). *Significantly different between trained left and untrained right hands for theta-gamma tACS ($P < 0.001$).

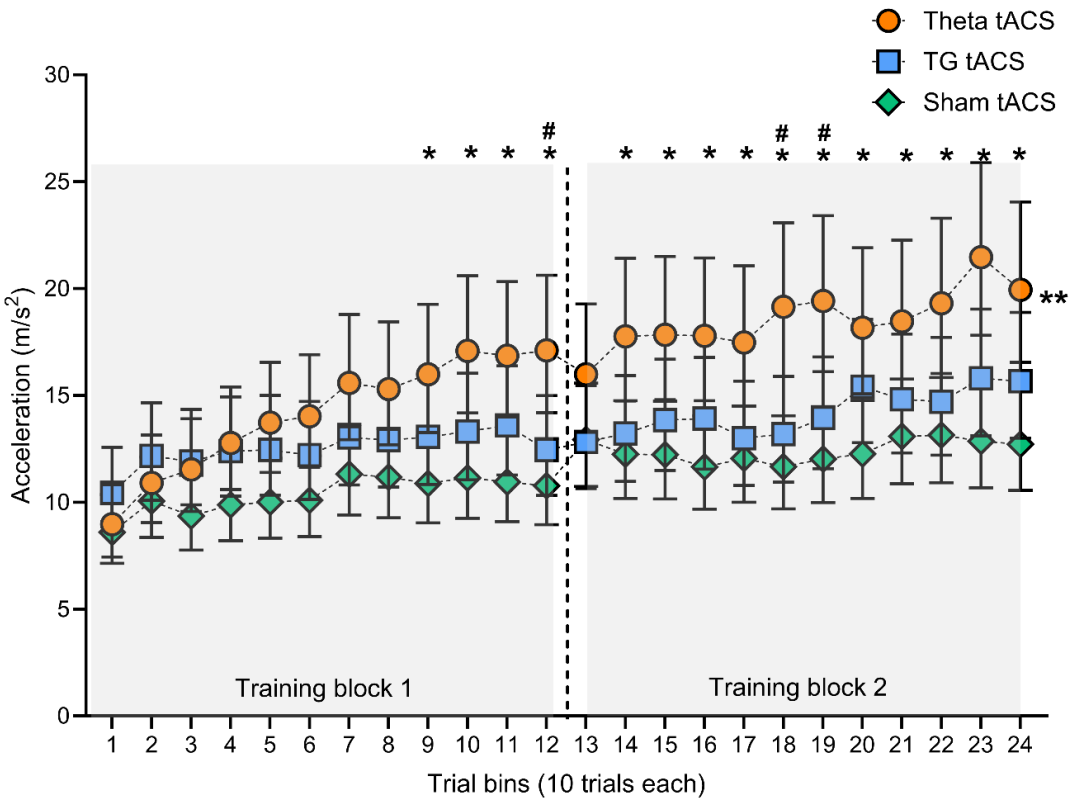


Figure 4.2. Effect of HD tACS during ballistic motor training of the trained left hand. Data show EMM [± 95% CI]. *Significantly different between Theta tACS and sham tACS (all $P < 0.038$). #Significantly different between Theta tACS and theta-gamma tACS (all $P < 0.043$). **Overall, motor training acceleration significantly differed between Theta tACS and sham tACS ($P = 0.024$).

4.5.2. Effects of HD tACS on post-training motor performance (trained and untrained hands)

Changes in ballistic thumb acceleration from pre- to post-training for trained and untrained hands are shown in Figure 4.3A and 4.3B, respectively. Thumb acceleration did not vary between tACS treatments ($F_{2,2371} = 0.7$, $P = 0.483$), but differed between timepoints ($F_{1,2371} = 1783$, $P < 0.001$), revealing a greater thumb acceleration at post-

training compared to pre-training (EMD = 6.6 m/s^2 [5.8, 7.3], $P < 0.001$). Thumb acceleration also varied between sides ($F_{1,2371} = 117$, $P < 0.001$), indicating greater acceleration in the trained compared to the untrained hand (EMD = 1.6 m/s^2 [1.3, 2.0], $P < 0.001$). Furthermore, there was a two-way interaction between tACS treatment and timepoint ($F_{2,2371} = 192$, $P < 0.001$), with *post-hoc* analysis indicating greater thumb acceleration at post-training, compared to pre-training for all tACS groups (all $P < 0.001$). Additionally, at post-training, thumb acceleration was greater following theta tACS compared to theta-gamma tACS (EMD = 6.4 m/s^2 [2.0, 10.9], $P < 0.001$) and sham tACS (EMD = 6.8 m/s^2 [2.2, 11.5], $P < 0.002$). There were also two-way interactions between tACS treatment and side ($F_{2,2371} = 15$, $P < 0.001$), and timepoint and side ($F_{1,2371} = 22$, $P < 0.001$). *Post-hoc* analyses showed greater thumb acceleration at post-training, compared to pre-training for trained and untrained hands (all $P < 0.001$), and thumb acceleration was greater for trained compared to untrained hand for all tACS treatments (all $P < 0.01$).

Importantly, there was a three-way interaction between tACS treatment, timepoint, and side ($F_{2,2371} = 22$, $P < 0.001$), with *post-hoc* analysis revealing greater thumb acceleration at post-training, compared to pre-training, for both trained and untrained hands for all tACS treatments (all comparisons $P < 0.001$). Pre-training thumb acceleration was greater in the trained compared to the untrained hand for theta-gamma tACS (EMD = 2.5 m/s^2 [1.7, 3.2], $P < 0.001$), whereas it was comparable between hands for theta tACS and sham tACS (both $P > 0.112$). Following all tACS treatments, the trained hand had greater acceleration than the untrained hand (all $P < 0.003$). Additionally, thumb acceleration for both trained and untrained hands was greater following theta tACS compared to theta-gamma tACS (trained EMD = 8.6 m/s^2 [3.2, 14.1], $P < 0.001$; untrained EMD = 4.7 m/s^2

[0.6, 8.8], $P = 0.016$), and sham tACS (trained EMD = 9.9 m/s^2 [4.3, 15.5], $P < 0.001$; untrained EMD = 4.4 m/s^2 [0.5, 8.1], $P = 0.022$). Finally, there were no significant main effects or interactions between tACS treatment, sides, and timepoints on CV for thumb acceleration (all $P > 0.19$).

4.5.3. Ballistic motor performance gain (trained and untrained hands)

Performance gain (% improvement) for trained and untrained hands is shown in Figures 4.3C and 4.3D, respectively. Trained hand performance gain differed between tACS treatments ($F_{2,57} = 18$, $P < 0.001$), with *post-hoc* analysis indicating greater performance gain for theta tACS compared to theta-gamma tACS (EMD = 334% [181, 485], $P < 0.001$), and sham tACS (EMD = 309% [167, 451], $P < 0.001$). Similarly, untrained hand performance gain varied between tACS treatments ($F_{2,57} = 4.9$, $P = 0.01$), with *post-hoc* analysis demonstrating greater performance gain for theta tACS compared to theta-gamma tACS (EMD = 175% [17, 333], $P = 0.025$), and sham tACS (EMD = 170% [18, 321], $P = 0.025$). Trained and untrained hand performance gains did not vary between theta-gamma tACS and sham tACS (both $P > 0.686$).

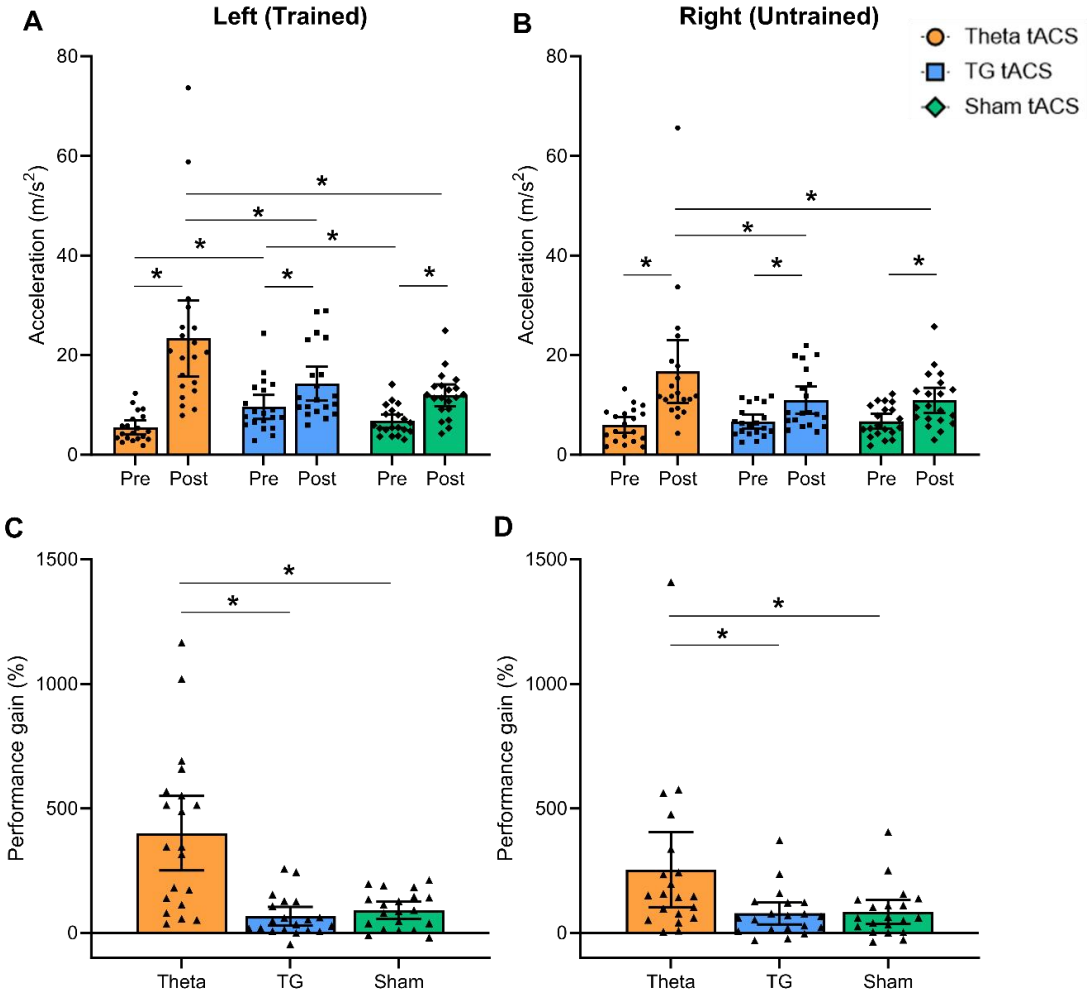


Figure 4.3. Effect of ballistic motor training and HD tACS on ballistic motor performance and performance gain. Data show EMM [± 95% CI] pre- and post-training thumb acceleration performance for trained left (A), and untrained right (B) hands, and performance gain [(post-pre)/pre x 100] for trained left (C), and untrained right (D) hands. *$P < 0.05$.

4.5.4. Effects of motor training and HD tACS on MEP amplitude

Pre- and post-training MEP amplitudes (at the same TMS intensity) for untrained (left) M1 and trained (right) M1 are shown in Figures 4.4A and 4.4B, respectively. MEP amplitude did not differ between tACS protocols ($F_{2,3086} = 0.1$, $P = 0.939$), and timepoint ($F_{1,3086} = 0.7$, $P = 0.412$), but varied between sides ($F_{1,3086} = 5.5$, $P = 0.019$), where trained M1 had greater MEP amplitude compared to untrained M1 (EMD = 0.05 mV [0.01, 0.09], $P = 0.020$). There was a significant interaction between tACS protocols and timepoint ($F_{2,3086} = 15.6$, $P < 0.001$), with *post-hoc* analyses indicating that for both M1 combined (trained and untrained), there was a greater MEP amplitude for theta tACS at post-training, compared to pre-training (EMD = 0.16 mV [0.08, 0.24], $P < 0.001$). However, MEP amplitude was reduced after training for theta-gamma tACS (EMD = 0.13 mV [0.05, 0.20], $P = 0.001$) and sham tACS (EMD = 0.09 mV [0.01, 0.16], $P = 0.028$).

Importantly, there was a three-way interaction between tACS protocols, timepoint, and side ($F_{2,3086} = 7.9$, $P < 0.001$), with *post-hoc* analyses indicating greater post-training MEP amplitude for trained M1 following theta tACS compared to theta-gamma tACS (EMD = 0.33 mV [0.05, 0.61], $P = 0.014$) and sham tACS (EMD = 0.29 mV [0.03, 0.57], $P = 0.027$). Moreover, following theta tACS, MEP amplitude was greater for trained M1 than untrained M1 (EMD = 0.25 mV [0.12, 0.38], $P < 0.001$). Additionally, MEP amplitude for trained M1 was greater at post-training than pre-training for theta tACS (EMD = 0.31 mV [0.18, 0.44], $P < 0.001$), while it was reduced after training for theta-gamma tACS (EMD = 0.21 mV [0.10, 0.35], $P < 0.001$) and sham tACS (EMD = 0.12 mV [0.11, 0.23], $P = 0.031$). There were no other significant interactions (all $P > 0.313$).

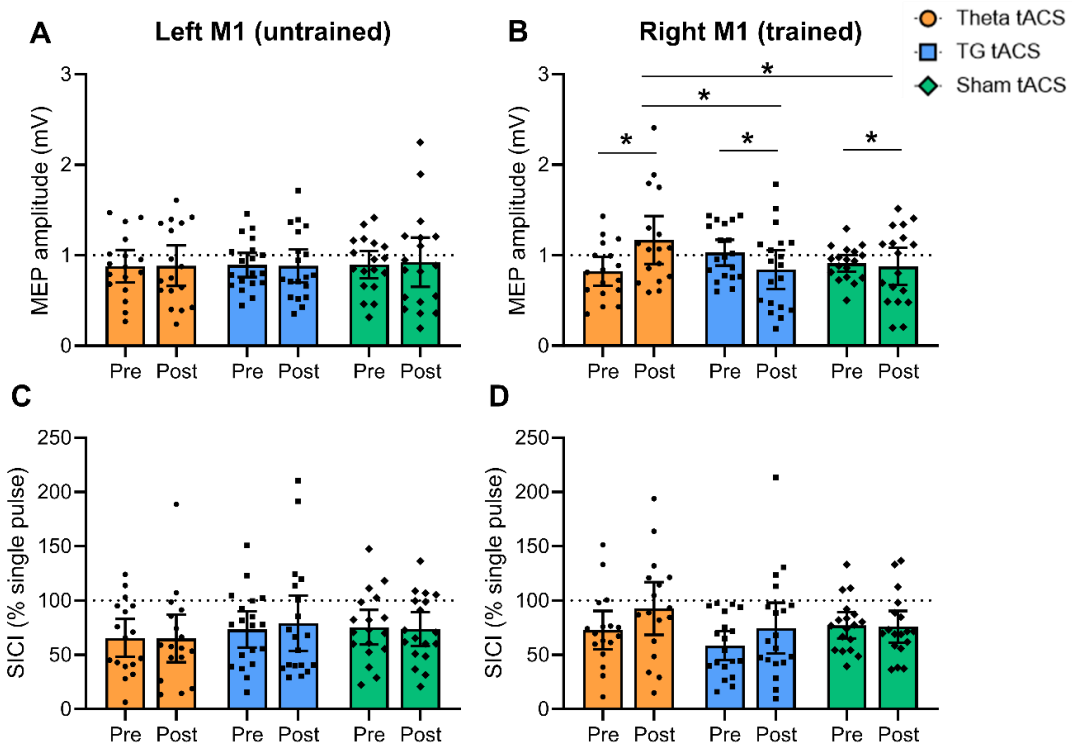


Figure 4.4. Effect of ballistic motor training and HD tACS on TMS measures. Data show EMM [± 95% CI] test MEP amplitude for left M1 (untrained) (A), and right M1 (trained) (B), and SICI (% single pulse) for left M1 (untrained) (C), and right M1 (trained) (D). *$P < 0.05$.

4.5.5. Motor training and HD tACS on SICI

Pre- and post-training SICI for untrained M1 and trained M1 are shown in Figures 4.4C and 4D, respectively. SICI did not vary between tACS protocols ($F_{2,3000} = 0.25$, $P = 0.776$) and timepoint ($F_{2,3000} = 3.4$, $P = 0.067$), but varied between sides, with *post-hoc* comparisons revealing less SICI for trained M1 compared to untrained M1 (EMD = 4.6% [0.16, 9.0], $P = 0.043$). There was a significant interaction between tACS protocols and side ($F_{2,3000} = 13.1$, $P < 0.001$), with *post-hoc* comparisons indicating for all timepoints

combined (pre- and post-training), there was less SICI for trained M1 compared with untrained M1 for theta tACS (EMD = 19.7% [10.9, 28.4], $P < 0.001$). Additionally, there was less SICI for untrained M1 compared with trained M1 for theta-gamma tACS (EMD = 8.2% [0.9, 15.4], $P = 0.026$). However, there were no changes between tACS protocols for trained or untrained M1 (all $P > 0.17$). There were no other significant interactions (all $P > 0.07$).

4.5.6. Effects of motor training and HD tACS on pegboard performance

Pegboard scores did not differ between tACS treatments ($F_{2,225} = 1.4$, $P = 0.244$), but varied between timepoints ($F_{2,225} = 42.8$, $P < 0.001$), with greater pegboard scores at post-training compared to pre-training (EMD = 0.8 [0.5, 1.0], $P < 0.001$). Additionally, pegboard scores differed between sides ($F_{2,225} = 126.1$, $P < 0.001$), where the untrained hand had a greater score compared to the trained hand (EMD = 1.3 [1.1, 1.6], $P < 0.001$). There were no significant interactions between factors (all $P > 0.469$).

4.5.7. Correlation between motor performance and neurophysiological measures

There were no significant correlations between the change in thumb acceleration performance (trained and untrained) and the change in neurophysiological measures before and after training (MEP amplitudes and SICI in either hemisphere) for all tACS groups (all $P > 0.05$). Additionally, there were no significant correlations between pre-training neurophysiological measures (AMT, test TMS intensity, test TMS amplitude, SICI, and pegboard score) and the change in ballistic motor performance in either hand for all tACS groups (all $P > 0.13$). Finally, there were no effects of IPAQ, MoCA, and

MEQr on thumb acceleration, MEP amplitude, and SICI for all tACS treatments at all timepoints (all $P > 0.05$).

4.5.8. Magnitude of untrained performance gains predicted by trained performance gains

As shown in Figure 4.5, the relationship between the trained and untrained performance gains was significant for the theta-gamma tACS group ($R^2 = 0.501$, uncorrected $P < 0.001$), while it was not significant for the other tACS groups (theta tACS; $R^2 = 0.185$, uncorrected $P = 0.058$, sham tACS; $R^2 = 0.154$, uncorrected $P = 0.087$). After correcting for multiple comparisons using the Bonferroni method (adjusted $\alpha = 0.0167$), the relationship remained significant only for the theta-gamma tACS group.

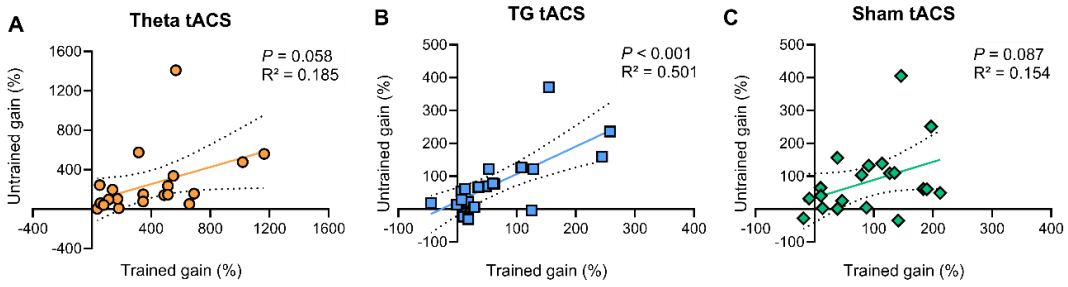


Figure 4.5. Ballistic motor performance gains (%) in the trained left hand against the motor performance gains (%) in the untrained right hand for theta (A), theta-gamma (B), and sham (C) tACS.

4.5.9. Participant HD tACS blinding and VAS score (pain, intensity, and localisation)

Participant responses to VAS and tACS intervention blinding questionnaires are shown in Table 4.3. There were no significant differences in VAS scores for the pain level, stimulation intensity, and HD tACS localisation between tACS treatment (all $P > 0.435$). Following theta tACS, 30% (n = 6) of participants guessed the stimulation correctly, 55% (n = 11) guessed it incorrectly, and 15% (n = 3) were unable to differentiate the stimulation type (active or inactive). Considering the theta-gamma tACS group, 50% (n = 10) of participants guessed the stimulation correctly, 25% (n = 5) had an incorrect guess, while the remaining participants (n = 5) were unable to differentiate the stimulation type. In contrast, 20% (n = 4) of the sham tACS participants guessed the stimulation correctly, with 55% (n = 11) guessing it incorrectly, while 25% (n = 5) were unable to differentiate the stimulation type.

Table 4.3. VAS responses (mean ± SD) and HD tACS intervention blinding of participants (n, %).

Question	Theta tACS	Theta-gamma tACS	Sham tACS
1. How painful was the stimulation during the HD tACS intervention? (0, not painful at all; 10, very painful)	1.10±1.80	0.75±1.33	0.60±0.94
2. How intense was the stimulation during the HD tACS intervention? (0, not intense at all; 10, very intense)	2.05±2.33	1.95±2.04	1.30±1.49
3. How localised were the sensations during HD tACS intervention? (0, highly localised; 10, widespread)	0.45±0.76	0.70±1.08	0.55±0.95
4. Can you guess the type of stimulation (active or sham) you received?			
i. Correct guess (n, %)	6, 30%	10, 50%	4, 20%
ii. Incorrect guess (n, %)	11, 55%	5, 25%	11, 55%
iii. Unable to differentiate (n, %)	3, 15%	5, 25%	5, 25%

Data presented as mean ± SD and n, %. VAS, visual analogue scale; HD tACS, high-definition transcranial alternating current stimulation; tACS, transcranial alternating current stimulation.

4.6.Discussion

The present study aimed to investigate the influence of theta-gamma, theta, and sham tACS on ballistic motor performance and M1 excitability in the trained and untrained hands of healthy older adults. Theta tACS yielded the greatest improvement in both trained left and untrained right hands. However, only theta-gamma tACS predicted the relationship between the trained and untrained ballistic motor performance gains. Additionally, post-training MEP amplitude increased for the trained M1 following theta tACS, but SICI did not vary between tACS groups for trained or untrained M1.

4.6.1.Effects of HD tACS on ballistic motor performance

Cortical oscillatory frequencies such as alpha (8-13 Hz), beta (13-30 Hz), and gamma (>30 Hz) are often associated with motor behaviour, with an increase in gamma power and a reduction in alpha and beta power apparent in sensorimotor regions during motor execution (Davis et al., 2012, Muthukumaraswamy, 2010, Pfurtscheller et al., 2006, Pollok et al., 2014). As a NIBS intervention, tACS is thought to entrain the membrane potentials of neuronal populations in the stimulated region in a frequency-specific manner (Krause et al., 2019, Vöröslakos et al., 2018). The application of tACS over M1 at alpha (Antal et al., 2008), beta (Pollok et al., 2015), and gamma (Joundi et al., 2012, Moisa et al., 2016) frequencies have been shown to modify specific aspects of motor performance (see McNally et al., 2024 for review). Theta rhythm (4-8 Hz) is commonly examined in

the context of human memory (see Herweg et al., 2020 for review), and previous studies have shown that theta tACS can be used to modulate working memory (Alekseichuk et al., 2016, Wolinski et al., 2018), but its effect on motor domains remains uncertain.

We show for the first time that theta tACS produces substantial improvement in ballistic motor performance (trained hand performance gain of 400% *vs.* 92% for sham tACS) in healthy older adults, suggesting that the theta frequency can influence motor behaviour within healthy ageing. Theta oscillations are associated with broadening connectivity and facilitating communication between cortical areas (see Bland and Oddie, 2001 for review), which are processes expected to be involved with the generation, coordination, and modification of movements (Young et al., 2008). For example, previous studies have shown increased theta-band activity during planning, initiation and execution of different motor tasks (Cruikshank et al., 2012, Perfetti et al., 2011, Tombini et al., 2009). The potentiation of performance observed in the current study may therefore reflect a modulatory effect of theta tACS that underlies sensorimotor integration associated with theta-band connectivity. It will be important for future studies to clarify if this effect is specific to older adults, as recent work has identified age-related changes in sensorimotor functional connectivity associated with theta-band oscillations (Yordanova et al., 2024). Nonetheless, we show that theta tACS is an effective tool to boost motor performance in healthy older adults.

In contrast to theta tACS, theta-gamma tACS failed to provide a significant benefit relative to sham (67% gain in trained hand). Emerging evidence suggests that theta-gamma PAC is important for neuronal processing and hippocampal learning (Colgin, 2015, Lopes-Dos-Santos et al., 2018), and is associated with motor learning success

(Dürschmid et al., 2014) and motor recovery in chronic stroke (Rustamov et al., 2022). Earlier tACS studies found that nesting a 75 Hz gamma burst at the peak of a 6 Hz theta wave (theta-gamma peak) enhanced working memory (Alekseichuk et al., 2016) and ballistic motor performance within young adults (Akkad et al., 2021), whereas nesting the gamma burst at the trough (theta-gamma trough) was ineffective. Previous work by our group showed that theta-gamma trough tACS increased motor performance in both young and older adults (Gamage et al., 2025c), but the current study failed to replicate these effects in older adults. While the specific cause of these contradictory findings remains unclear, several factors could contribute. First, there were differences in participant characteristics between the two studies, such as baseline physical activity levels and pre-training ballistic motor performance. Second, there were experimental differences between studies, including methodological design, experimental procedures, and motor training structure. Third, differential effects could also stem from the recognised variability associated with NIBS efficacy (Guerra et al., 2020, López-Alonso et al., 2014). Future studies should therefore consider methods to reduce variability. For example, the use of individualised tACS frequencies and electrode montages (Arias and Heise, 2024), in conjunction with current flow modelling and individual MRIs, will optimise engagement with target brain areas.

4.6.2.Effects of HD tACS on cross-limb transfer

Cross-limb transfer refers to performance gains in the untrained limb following unilateral training. Not only is this effect evident for improvements in strength, but it has also been shown for skill acquisition tasks such as ballistic tasks (see Ruddy and Carson, 2013 for review). In the current study, we demonstrated that training one hand led to an

improvement in ballistic motor performance in the untrained hand, indicating cross-limb transfer of ballistic motor performance in older adults. This improvement in the untrained hand was most pronounced when theta tACS was applied over M1 of the trained hemisphere (254% *vs.* 84% improvement for sham tACS). Although the precise mechanisms of cross-limb transfer are unclear, previous studies have shown that the untrained limb muscles can access the unilateral-training-related adaptations through interhemispheric communication from the associated motor areas (bilateral access hypothesis) (Lee and Carroll, 2007, Ruddy and Carson, 2013), and theta tACS may have played a role in altering the interhemispheric interaction, as shown in previous tACS studies (Lebihan et al., 2025). Nevertheless, the lack of correlation between the trained and untrained hands with theta tACS in the current study may suggest potential contributions from non-cortical locations, such as the spinal cord (Dragert and Zehr, 2011). Furthermore, the theta tACS group may have exhibited better task-specific skill and utilised different thumb kinematic strategies to facilitate the improvement (Rogasch et al., 2009), which were not assessed in the current study.

In contrast with theta tACS, ballistic performance improvements in the trained hand predicted the performance improvement in the untrained hand following theta-gamma tACS. Previous studies with ballistic motor training only (~sham tACS) indicated a strong correlation between the ballistic motor performance gains in the trained and untrained hands for young adults (Hinder et al., 2013, Hinder et al., 2011), suggesting that the extent of untrained limb improvements largely depends on those obtained ipsilaterally (Woldeamanuel et al., 2022). However, this predictive relationship was absent in older adults (Hinder et al., 2013, Hinder et al., 2011), suggesting a decline in the transfer of

motor performance gains with age. Therefore, our findings suggest that theta-gamma tACS may help mediate the transfer of motor performance following unilateral training in older adults, which could be useful in neuromuscular rehabilitation. While the exact mechanism underlying this finding is uncertain, the untrained limb gains with theta-gamma tACS are likely to occur predominantly from cortical sources. Additionally, the precise reason why a predictive correlation between the trained and untrained hand emerged only with theta-gamma tACS and not with theta tACS remains unclear, but it may indicate the involvement of different neural processes that warrant further exploration.

4.6.3.Changes in corticospinal excitability and intracortical inhibition

Previous work has reported an increase in M1 excitability following motor training for skilled tasks (Muellbacher et al., 2001, Ziemann et al., 2001), suggesting use-dependent plasticity arising from LTP-like mechanisms within cortical circuits (Sanes and Donoghue, 2000), although there can be contributions from spinal circuits with ballistic tasks (Giesebrecht et al., 2012). After training, we found an increase in MEP amplitude in the trained M1 for theta tACS, suggesting that theta tACS led to a substantial potentiation of the neuroplastic response to ballistic motor training. In addition, we found a reduction in trained M1 excitability after motor training (with sham tACS), consistent with our previous study (Gamage et al., 2025c). Interestingly, theta-gamma tACS resulted in MEP depression after training, with no difference in post-training MEP amplitudes between theta-gamma and sham tACS. This was unexpected, as we previously found greater MEP amplitudes with theta-gamma tACS compared to sham tACS in older adults (Gamage et al., 2025c). One possible explanation for this divergent finding is the variation in the

training structure (Lage et al., 2015), where the current study involved repetitive practice with fewer training blocks as opposed to the previous study, including interleaved practice with multiple training blocks, each separated by periods of rest (Gamage et al., 2025c). However, these differential effects support previous suggestions that MEPs may not be an ideal indicator of behaviourally-relevant changes in the motor system (Bestmann and Krakauer, 2015).

The modulation of intracortical inhibitory circuits facilitates motor skill acquisition, with a reduction in GABAergic inhibition playing a key role in training-dependent plasticity (Ziemann et al., 2001). We found that SICI was not different between tACS conditions, suggesting that theta-gamma tACS or theta tACS did not affect motor training-related GABAergic inhibition. Similarly, it has been previously shown that training-induced changes in GABAergic inhibition were not modulated by theta-gamma tACS (Gamage et al., 2025c). However, prior studies have shown a reduction in SICI *during* gamma tACS over M1 (i.e., online) (Guerra et al., 2018, Nowak et al., 2017), but this was not assessed in the current study due to the concurrent task performance during the stimulation period. Finally, we did not find any correlation between the neurophysiological measures (changes in MEP and SICI in either hemisphere) and the changes in ballistic motor performance in either limb, consistent with previous studies (Gamage et al., 2025c, Hinder et al., 2013). This is possibly because TMS and tACS involve distinct cortical circuits (Wischnewski et al., 2023), and voluntary movements engage a widespread cortical network beyond M1, which is not specifically targeted by tACS or TMS (Bestmann and Krakauer, 2015). Furthermore, since voluntary movements involve a widespread cortical

activity, future studies would benefit from exploring broader networks using techniques such as TMS + EEG, dual-site TMS or cerebellar inhibition.

4.6.4.Limitations and Further Considerations

It is important to note that the improvement in motor performance with tACS was specific to a simple M1-dependent ballistic motor task (Muellbacher et al., 2001, Rogasch et al., 2009), and the role of theta-gamma and theta tACS during complex motor paradigms warrants further research. We used a pegboard task to assess whether the observed ballistic motor performance gains can be extended to other motor tasks, as the grooved pegboard task assesses manual dexterity, which is particularly applicable in daily tasks (Reuben et al., 2013) and demands coordination in addition to motor abilities (Kaminski et al., 2022). While overall pegboard scores improved after training, they were comparable between the tACS conditions, suggesting that theta-gamma or theta tACS did not improve pegboard performance. Additionally, these results were observed in cognitively unimpaired (MoCA score) healthy older adults who were at least moderately active (IPAQ score), so the effects could vary in frail older adults or patients with neuromuscular deficits. Moreover, since we did not include a young cohort in the current study, it was not possible to infer any age-related changes in cross-limb transfer. In addition, the current study had two extreme scores for motor performance gains, and the results remained unchanged when these data points were excluded. However, these values were not excluded as they were physiologically plausible and indicate genuine motor performance variability between participants.

Furthermore, there is also some concern that concurrent stimulation of peripheral nerves on the scalp may influence behavioural outcomes observed with tACS (Asamoah et al., 2019). However, our participants reported similar VAS scores for pain, intensity, and stimulation localisation, and failed to distinguish between stimulation conditions (active *vs.* sham), indicating that peripheral effects likely had little impact on our findings. Moreover, it was not possible to tailor tACS to an individual's peak frequency, as this study did not include EEG, and future investigations may benefit from utilising personalised tACS approaches (Wansbrough et al., 2024). Finally, future studies should include young and older adults to assess the age-related changes of trained and untrained motor function following unilateral motor training and should investigate the impact of theta and theta-gamma tACS across diverse motor tasks and age-related neurological conditions to clarify exact mechanisms and their clinical implications.

4.6.5.Conclusion

We have shown that theta tACS enhances motor performance in the trained limb of older adults, whereas there was no additional improvement in trained limb performance with theta-gamma tACS (compared with sham tACS). However, theta-gamma tACS resulted in a positive correlation in motor performance gains between the trained and untrained limbs. While the exact mechanism of theta and theta-gamma tACS remains unclear, distinct neural processes may account for the trained limb gains and cross-limb transfer, with a strong cortical involvement. These findings have potential implications for rehabilitation following neurological disorders (e.g., stroke) and orthopaedic conditions (e.g., single-limb fractures), where the unaffected limb can be actively exercised along with non-invasive neuromodulation (e.g., HD tACS), to assist in the motor performance

gains of the affected limb. Future studies should explore the cross-limb transfer effects of theta and theta-gamma tACS with complex motor tasks and in age-related neuromuscular conditions to gain deeper insights into the clinical implications.

CHAPTER 5: GENERAL DISCUSSION

5.1. Overview of aims and summary of key findings

It is well-established that healthy ageing is accompanied by alterations in the neuromuscular system (see Seidler et al., 2010 for review), resulting in age-related loss of muscle function, leading to detrimental outcomes such as mobility disorders and a reduction in general health status (see Wilkinson et al., 2018 for review). Additionally, the capacity of the brain to modify its neural connections also declines with advancing age (Freitas et al., 2013, Semmler et al., 2021, Zimerman and Hummel, 2010). This reduction in neuroplastic potential contributes to declining motor function in older adults (Ward, 2006, Zapparoli et al., 2022), and impairs the ability to relearn motor skills following neurological injury (Laaksonen and Ward, 2022). Therefore, the overarching aim of the current PhD was to investigate how central and peripheral interventions can be used to modulate neuroplasticity and motor function in older adults across three experimental studies involving human participants. These studies resulted in novel findings with potential clinical implications. A summary of the main findings of the experimental chapters is shown in Table 5.1.

Chapter 2 aimed to evaluate the cross-limb transfer effects of acute unilateral resistance exercise on neuromuscular function and MU-level adaptation in healthy older adults. This study demonstrated for the first time that a single bout of unilateral knee extensor resistance exercise performed to fatigue is sufficient to elicit a bilateral reduction in maximal forces in healthy older adults, with central and peripheral adaptations occurring only in the exercised limb. Although the precise mechanism underlying the force decline in the non-exercised limb remains uncertain, it is mainly suggestive of adaptations at systemic and/ or central levels.

Chapter 3 examined the effect of combined theta-gamma tACS over M1 on ballistic motor performance and M1 excitability in healthy young and older adults. This study provided the first evidence that theta-gamma tACS can enhance the acquisition of ballistic motor skills in healthy older adults. These performance improvements were long-lasting (> 60 mins) and stronger in young adults compared with older adults. In addition, theta-gamma tACS altered training-induced changes in M1 excitability, but did not influence changes in GABAergic inhibition in both age groups.

Chapter 4 aimed to assess the effects of tACS over M1 on ballistic motor performance and the cross-limb transfer in healthy older adults, using theta and combined theta-gamma waveforms. This study provided the first evidence that theta tACS enhances ballistic motor performance in the trained and untrained limb of older adults. Importantly, there was no additional improvement in trained or untrained limb performance with theta-gamma tACS compared with sham tACS. However, theta-gamma tACS resulted in a positive correlation in motor performance gains between the trained and untrained limbs, which was not observed with theta tACS. While the exact mechanism of theta and theta-gamma tACS remains unclear, distinct neural processes may account for the trained limb gains and cross-limb transfer, with a strong cortical involvement.

Table 5.1. Summary of key findings for thesis experimental chapters (Chapters 2-4)

Chapter	Intervention	Study design	Participants	Measures	Key results	Explanation
Chapter 2; Published (Gamage et al., 2025b)	Acute unilateral RE (EL: dominant/right leg) sets of 12 reps of knee extension at 75% of 1 RM, to failure	Within-subjects, repeated-measures design	17 older (9 females) 73.8 ± 4.9 years	*Functional assessments* MVC, FS *Neuromuscular assessments* (HDsEMG, iEMG); Features of individual MUs	MVC ↓ in both limbs (EL, -14.8%; CL, -6.9%) FS ↓ in EL MUFR ↑ in EL iEMG measures ↔ in both limbs	A single bout of unilateral RE is sufficient to elicit a bilateral reduction in maximal forces, indicating cross-limb transfer. Neural adaptations were observed only in the EL.
Chapter 3; Published (Gamage et al., 2025c)	TG tACS or sham tACS on M1 during unilateral ballistic motor training (EL: non-dominant/left hand)	Randomised, double-blind, sham-controlled, cross-over design	18 young (8 females) 26.3 ± 5.7 years 18 older (12 females) 68.3 ± 5.0 years	*Functional assessments* Motor performance, PTP *Neurophysiological assessments* (TMS measures); MEP, SICI	Motor performance ↑ for TG tACS in both young & older Greater MEP for TG tACS (than sham) SICI ↔ between tACS protocols	TG tACS improves motor performance, with effects lasting > 60 minutes. TG tACS modulates use-dependent M1 excitability in healthy young and older adults.
Chapter 4; Unpublished	Theta tACS or TG tACS or sham tACS on M1 during unilateral ballistic motor training (EL: non-dominant/left hand)	Randomised, double-blind, sham-controlled design	60 older (37 females) 68.9 ± 5.2 years	*Functional assessments* Motor performance, Cross-limb transfer *Neurophysiological assessments* (TMS measures); MEP, SICI	Theta tACS had the greatest ↑ in motor gains for both limbs TG tACS had a predictive correlation between EL & CL motor gains MEP ↑ for theta tACS in EL SICI ↔ between tACS protocols	Theta tACS enhances bilateral ballistic motor performance, indicating cross-limb transfer. No additional motor improvements with TG tACS (than sham tACS).

↑ increased compared to baseline (unless specified); ↓ decreased compared to baseline (unless specified); ↔ unchanged (between timepoints or protocols)

Abbreviations: RE, resistance exercise; EL, exercise limb; CL, control limb; RM, repetition maximum; MVC, maximum voluntary contraction; FS, force steadiness; HDsEMG, high-density surface electromyography; iEMG, intramuscular electromyography; MU, motor units; MUFR, motor unit firing rate; tACS, transcranial alternating current stimulation; TG, theta-gamma; M1, primary motor cortex; PTP, post-training performance; TMS, transcranial magnetic stimulation; MEP, motor-evoked potential; SICI, short-interval intracortical inhibition.

5.2. Ageing and neuroplasticity

In humans, our understanding of neuroplasticity within motor cortical areas has been greatly facilitated by NIBS techniques (see Semmler et al., 2021 for review). Despite the presence of inconsistent evidence in the literature, it is generally recognised that neuroplastic capacity declines with advancing age (Freitas et al., 2013, Semmler et al., 2021, Zimerman and Hummel, 2010). Numerous neuromodulatory techniques have been applied in an attempt to augment this decline in plasticity with age, including repetitive TMS, paired associative stimulation, and transcranial direct current stimulation (tDCS), but they have produced variable findings (see Semmler et al., 2021 for review). In contrast, the effect of tACS on brain plasticity in older adults has received less investigation. Therefore, tACS was applied with different waveforms (i.e., theta-gamma and theta) to explore their effect on the physiological and functional response to motor training. The findings of Chapters 3 and 4 show for the first time that tACS with both theta-gamma and theta waveforms, when concurrently applied during motor training, can modulate brain excitability in healthy older adults. These findings indicate that older adults retain the capacity for neuroplasticity and can acquire novel motor skills, although sometimes at reduced rates compared with young adults. Taken together, current thesis findings

support that NIBS tools, including tACS, have the potential to modulate cortical excitability and facilitate neuroplasticity in older adults, which highlights the applicability in clinical settings (see Gooijers et al., 2024 for review). Previous studies have shown that tACS has great potential for various post-stroke symptoms, including aphasia and hemispatial neglect induced by stroke (see Ding et al., 2025 for review). However, future studies should investigate the effects of different tACS waveforms (e.g., different combinations of PAC profiles) concurrently applied with different motor tasks (e.g., visuomotor tasks) and also among diverse ageing groups (e.g., frail or affected with neurological conditions) to identify the potential clinical implications of these findings.

Resistance training in general improves muscular force due to a combination of neural plasticity (occurring in the first few weeks of training) and muscle hypertrophy (observed with long-term training) (see Pearcey et al., 2021 for review). Neural adaptations to resistance exercise training may manifest as training-related modifications in MU properties (e.g., MU recruitment and firing rate) (see Folland and Williams, 2007 for review), and research in this area typically focuses on neuromuscular assessment using EMG recordings (Aagaard et al., 2020). Previous research has shown that resistance-training-induced increases in force production are associated with increased MU firing rate (Del Vecchio et al., 2019, Kamen and Knight, 2004), indicating direct evidence of neural adaptations at the motor neuron level. However, most existing studies on MU adaptations to resistance exercise have largely been restricted to young adults and have predominantly included long-term training protocols (see Elgueta-Cancino et al., 2022 for review). Particularly, research on acute MU adaptations to resistance exercise in older adults is limited. Therefore, Chapter 2 aimed to explore the immediate effects of resistance

exercise on MU adaptation in older adults. The findings indicate that the exercise limb exhibited neural adaptations with exercise-induced changes in MU firing characteristics (increased MU firing rate) and modulation in neural drive. These findings indicate that acute neural adaptations to resistance exercise are also evident in older adults, indicating the preserved neural adaptation capacity. However, future studies should incorporate both young and older age groups across varied resistance exercise paradigms with longer duration to more clearly delineate age- and protocol-specific adaptations.

5.3. Ageing and motor function

The majority of older adults demonstrate a general reduction in motor function with advancing age, leading to motor deficits (see Seidler et al., 2010 for review). For instance, compared to young adults, older adults demonstrate greater force variability (Voelcker-Rehage and Alberts, 2005), less accurate movements (Spirduso and Choi, 1993), poor quality of movement (Liao et al., 1997), and slower reaction times (Seidler, 2006). Despite this, older adults retain some motor abilities, most of which are task dependent (see Voelcker-Rehage, 2008 for review). For example, older adults show substantial performance gains ranging from 37-70% (*vs.* 76-84% in young) in the trained hand following ballistic motor training (Hinder et al., 2013, Hinder et al., 2011). Furthermore, resistance training produces strength gains ranging from 28-52% in the trained limb of older adults (*vs.* 28-31% in young) (Bemben and Murphy, 2001, Ehsani et al., 2014).

Previous studies have used several NIBS techniques to modulate motor performance in older adults, yielding mixed results. For example, repetitive TMS in older adults resulted in a ~5% improvement in reaction time (Bashir et al., 2014), and motor training combined

with active tDCS over M1 improved motor performance by ~13-28% in older adults (Goodwill et al., 2015, Goodwill et al., 2013). In contrast, paired associative stimulation had detrimental effects on visuomotor skill acquisition in older adults (Opie et al., 2019), while repetitive TMS had no effects on pinch grip, finger tapping, or 9-hole peg performance in older adults (Bashir et al., 2014). These inconsistent findings indicate the necessity for new NIBS techniques to produce greater effects on motor function in older adults. tACS is emerging as a neuromodulatory tool with great potential to modulate motor function, and previous studies have explored the frequency-dependent effects of tACS on various motor tasks (see McNally et al., 2024 for review). Age-related changes in motor performance were investigated by applying tACS at theta-gamma (Chapters 3 and 4) and theta (Chapter 4) frequencies to explore their role in motor performance in older adults. Ballistic motor performance was used as an indicator of that change. These findings support previous studies suggesting that, despite age-related motor changes, older adults can still acquire new motor skills through training, with improvements occurring at levels that can be comparable to those seen in young adults (see Gooijers et al., 2024 for review). The results also indicated that the concurrent application of tACS on M1 during motor training can facilitate improvements in motor performance in older adults (76% for theta-gamma tACS *vs.* 40% with sham tACS; Chapter 3). Importantly, the observed motor increments in studies included in this thesis (400% for theta tACS *and* 67-76% for theta-gamma tACS) were significantly greater than those reported in previous studies using other NIBS techniques, indicating the greater potential of tACS to enhance motor performance in older adults. These findings can be applied in clinical settings for older adults with pathological conditions affecting a single limb (e.g., stroke or single-

limb fractures), where motor training can be performed on the unaffected limb during HD tACS to facilitate motor recovery and rehabilitation.

Resistance exercise is a common exercise modality that provides various physiological and functional benefits to many populations, including older adults. Resistance training performed over a period of time has been shown to increase strength, endurance, speed, power, balance, and coordination (Suchomel et al., 2018). The acute effects of resistance exercise (performed to failure) result in an immediate decline in force production due to impaired muscle activation and/or contractile function (Enoka and Duchateau, 2016). There is no previous research on the immediate effects of a single bout of unilateral knee extensor fatiguing exercise in older adults. Therefore, Chapter 2 aimed to evaluate the acute neuromuscular adaptations to fatiguing resistance exercise in older adults. Study findings demonstrated that acute resistance exercise results in a modulation in knee extensor strength, which was similar to previous findings in young adults (Doix et al., 2013, Halperin et al., 2014, Kawamoto et al., 2014, Martin and Rattey, 2007). This force reduction is partly due to molecular mechanisms, including reduced sarcoplasmic reticulum Ca^{2+} release, impaired myofibrillar force production, and reduced myofibrillar Ca^{2+} sensitivity (Allen et al., 2008, Debold and Westerblad, 2024). Additionally, there was an increase in the magnitude of force fluctuations following resistance exercise, which was consistent with studies on young adults (Singh et al., 2010). Previous research has shown that the age-related loss of force control capacity depends on the contraction intensity (Pethick and Piasecki, 2022), with greater fluctuations reported at lower contraction intensities, such as 10% MVC (Ranganathan et al., 2001, Tracy and Enoka, 2002). Taken together, these findings suggest that older adults demonstrated

neuromuscular adaptation to acute unilateral resistance exercise to a similar extent as their younger counterparts. Although this study did not have a younger cohort to directly assess the age-related changes, research suggests that, compared to young adults, older individuals demonstrate greater force fluctuations, indicating a loss of force control and poor quality of motor performance (Pethick and Piasecki, 2022). Future studies may benefit from including contractions across a range of intensities to evaluate the changes in maximal strength and force control capacity, given that different functional tasks require varying intensity levels.

5.4. Ageing and cross-limb transfer

The contralateral effects conferred by unilateral training appear to be age- and task-dependent (see Calvert and Carson, 2022 for review). For example, ballistic training protocols typically elicit the largest performance outcomes, with untrained performance gains ranging from 49-82% in young (Carroll et al., 2008, Hinder et al., 2013) and 6-26% in older adults (Hinder et al., 2013, Hinder et al., 2011). In contrast, resistance training produced average contralateral strength improvements of 18% in young and 15% in older adults (see Green and Gabriel, 2018 for review). Despite variations in the magnitude of improvements, skill- and strength-training interventions appear to remain beneficial throughout the lifespan (see Barss et al., 2016 for review).

Multiple factors contribute to the applicability of the cross-limb transfer phenomenon for the ageing population. Firstly, an age-related reduction in neuroplasticity and neuromotor function (Zapparoli et al., 2022) limits the functional recovery of neurological conditions such as stroke (Laaksonen and Ward, 2022). Secondly, age-associated skeletal frailty

leads to an increased risk of falls and fractures among the elderly (Ensrud, 2013, McClung, 2005), with unilateral fractures observed in the majority of affected individuals (Court-Brown et al., 2012). It is commonly accepted that the loss of functional capacity affecting one limb, either following single-limb fractures or hemiparesis due to neurological conditions, can be minimised by actively training the unaffected limb. Two experimental studies (Chapters 2 and 4) were partly motivated by the potential clinical applications of the cross-limb transfer in the ageing population (Altheyab et al., 2024, Farthing and Zehr, 2014, Magnus et al., 2013, Manca et al., 2021a).

I explored the cross-limb transfer phenomenon in terms of interventions targeting the peripheral and central aspects of the neuromuscular system in healthy older adults. These findings align with prior studies that have claimed some aspects of cross-limb transfer remain functional in older adults (see Barss et al., 2016 for review). For example, unilateral ballistic training concurrently performed with tACS resulted in untrained performance gains ranging from 79-254% in healthy older adults (Chapter 4), which was greater than previous studies with ballistic training alone (Hinder et al., 2013, Hinder et al., 2011). Importantly, these results also indicated a predictive relationship between trained and untrained gains, which was otherwise absent in older adults with ballistic training alone (Hinder et al., 2013, Hinder et al., 2011). These findings extend current knowledge by providing the first evidence highlighting the potential of tACS to enhance cross-limb transfer effects in older adults.

Although unilateral strength training performed for a longer duration results in contralateral strength gains (see Green and Gabriel, 2018 for review), immediate effects show a reduction in performance, possibly due to muscle fatigue. Similarly, Chapter 2

findings demonstrated that older adults showed acute, resistance-exercise-induced reductions in strength in the untrained limb (~7%) that were comparable to young adults (Doix et al., 2013, Halperin et al., 2014, Kawamoto et al., 2014, Martin and Rattey, 2007), showing the preserved capacity to transfer muscle performance fatigue (i.e., exercise-induced reduction in performance) into older age. Although these results provide preliminary evidence that older adults exhibit cross-limb transfer following acute resistance interventions, further investigations are needed to strengthen these findings and to identify the mechanisms underlying these observations.

Importantly, it was not possible to infer any direct age-related comparison in the magnitude of cross-limb transfer and neuromuscular adaptations, as neither of these studies included a healthy young cohort. Therefore, future studies may benefit from including both young and older cohorts to examine age-related effects of cross-limb transfer, particularly given that some research has shown a reduction in cross-limb transfer capacity with advancing age (Hinder et al., 2013, Hinder et al., 2011), at least in part due to age-related changes in the neuromuscular system (see Seidler et al., 2010 for review).

5.5. Limitations and future considerations

There are some limitations of the studies undertaken in this thesis that need to be acknowledged. Firstly, all three experimental studies included healthy individuals (young and/or older) without any physical/neurological/cognitive impairments and were at least moderately active, and the effects may vary in frail individuals or patients with neuromuscular impairments (Grigutsch et al., 2024), which could be evaluated in future studies. Secondly, all three Chapters focused exclusively on acute neuromuscular

adaptations (5-60 minutes) following the interventions, and further research is needed to explore the long-term effects of these interventions to assess their clinical applicability.

In Chapter 2, while the exercise intervention was performed at a higher intensity (75% 1RM), MU characteristics were assessed during submaximal contractions (25% MVC); therefore, these findings may not be applicable to MUs with higher recruitment thresholds, which are recruited at higher force levels. In addition, Chapter 2 included the VL muscle, which is one of the knee extensors, and they are considered a stable measure as they represent a majority of the thigh region, which plays a significant role in daily activities and undergoes a significant age-related decline in skeletal mass (Francis et al., 2017). However, these findings may vary in muscles such as biceps brachii, tibialis anterior, gastrocnemius, and intrinsic hand muscles, which could be explored in future studies. Moreover, Chapters 2 and 4 did not include a young cohort, making it impossible to directly compare age-related changes in cross-limb transfer effects. Therefore, future studies may benefit from including both young and older individuals to infer ageing-related modifications.

Considering Chapters 3 and 4, the theta-gamma tACS waveform used in these studies was the inverse of the planned stimulation, which was identified retrospectively. As mentioned in the corrigendum (section 3.7), we had intended that the central electrode would apply a gamma burst nested in the theta peak, whereas return electrodes would apply a gamma burst nested in the theta trough. In contrast, due to polarity differences between electrodes, stimulation was inverted, with trough-gamma being applied at the central electrode and peak-gamma being applied at return electrodes (Gamage et al., 2025a). It is also important to note that the theta-gamma trough stimulation had effects on function in these Chapters,

despite previous research suggesting this waveform is not superior compared to sham stimulation (Akkad et al., 2021). However, future studies should consider exploring how peak-gamma and trough-gamma waveforms influence cortical activity and motor function, particularly in healthy older adults.

Furthermore, the absence of navigation-guided TMS (e.g., neuronavigation) in Chapters 3 and 4, could introduce variability in coil positioning. To minimise its effect, the marks made on the scalp at baseline during the 'TMS hotspot' were continuously monitored throughout the sessions, but future studies may benefit from including navigating-guided TMS methods. Another limitation of Chapters 3 and 4 is that they included a simple, M1-dependent ballistic motor training (Muellbacher et al., 2001, Rogasch et al., 2009) during the tACS stimulation period. Therefore, the role of theta and theta-gamma tACS during complex motor paradigms warrants further research. Additionally, in these chapters, entrainment effects were not measured during tACS (i.e., online), and future studies could measure frequency spectra immediately after stimulation using M/EEG (Bland and Sale, 2019).

The high variability in intra- and inter-individual responses associated with NIBS is a well-known phenomenon (Guerra et al., 2020, López-Alonso et al., 2014). Although in Chapters 3 and 4, I considered some potential confounding factors that could cause variability, such as physical activity level (Cirillo, 2021), chronotype (Korman et al., 2003), and cognition levels (Diedrich et al., 2024), it is possible that other factors, such as genetics, amount of sleep, and emotion, influenced the results (Coombes et al., 2009, Sale and Kuzovina, 2022). Additionally, individual differences in electrical field distribution during transcranial electrical stimulation could contribute to the variability of

aftereffects (Laakso et al., 2019). However, Chapters 3 and 4 did not include structural MRIs for the participants, so it was not possible to estimate the effective current reaching the cortex. Therefore, future studies should consider these potential confounding factors when assessing behavioural and neurophysiological outcomes.

5.6. Learning and development of skills/strengths

This thesis explored the role of several interventions, including acute exercise and motor training with NIBS, to modulate neuroplasticity and enhance motor performance across three human experimental chapters. Numerous laboratory techniques, including HDsEMG, iEMG and NIBS, were utilised to explore changes in central and peripheral components of the neuromuscular system following different interventions.

In Chapter 2, MU adaptations were explored using both iEMG and HDsEMG techniques, which enabled the capture of MUs at different depths (HDsEMG for superficial MUs and iEMG for both superficial and deep MUs) and the tracking of MUs from pre- to post-intervention (using HDsEMG). In addition to evaluating MU adaptations of the exercise (fatigued) leg, this Chapter also assessed the non-exercise (control) leg to explore the cross-limb transfer component. Chapters 3 and 4 included different NIBS techniques such as TMS and HD tACS, producing the first evidence of the role of theta-gamma and theta tACS in modulating M1 plasticity and motor performance in healthy older adults. These were double-blinded studies, where the experimenter and the participant were blinded to the type of stimulation. Additionally, these chapters included a sham-controlled design, allowing a direct comparison between the stimulation conditions. Overall, this thesis yielded novel findings with potential clinical implications for promoting healthy ageing.

5.7. Concluding remarks

The present PhD thesis aims to deepen the understanding of the application of several interventions to modulate neuroplasticity in the context of healthy ageing. I have provided novel evidence on alterations in the central and peripheral neuromuscular system following different interventions, including acute resistance exercise (Chapter 2), and ballistic motor training concurrent with theta-gamma tACS (Chapter 3, 4) and theta tACS (Chapter 4) in healthy older adults. Chapter 2 indicated that acute unilateral knee extensor resistance exercise produces cross-limb transfer effects in healthy older adults, with prominent MU adaptations observed in the exercised limb. Chapter 3 demonstrated that theta-gamma tACS over M1 enhances ballistic motor performance and modulates training-induced changes in M1 excitability in healthy young and older adults. Finally, Chapter 4 showed that theta tACS over M1 improves ballistic motor performance and produces cross-limb transfer effects in healthy older adults. Although this thesis provides unique insights into neuroplasticity and motor performance, further research is needed to understand the precise mechanisms underlying the observed effects.

The current PhD findings may have translational potential for neuromuscular rehabilitation in older populations, particularly affected by neurological conditions (e.g., stroke) and orthopaedic conditions (e.g., single-limb fractures). For example, an unaffected limb can undergo unilateral motor practice, in combination with a non-invasive neuromodulation (e.g., HD tACS), which may serve as a low-cost, feasible approach to promote motor function and modulate neuroplasticity in ageing. Nevertheless, large-scale investigations involving diverse populations and more complex motor tasks are likely

needed to strengthen these findings. Age-related decline in brain and neuromuscular function, along with the continuing increase in the ageing population, emphasises a growing need for such interventions.

APPENDICES

6.1. Conference communications, presentations, and guest lectures arising from this thesis

Title: Transcranial alternating current stimulation improves ballistic motor performance in trained and untrained limbs of older adults (poster) 2025
Florey Postgraduate Conference, University of Adelaide, Australia

Title: Promoting healthy ageing using non-invasive brain stimulation 2024
Three-Minute Thesis (3 MT) Joint PhD competition (1st Prize)
Adelaide-Nottingham Alliance

Title: Unlocking neuroplastic potential: Enhancing motor performance 2024
Faculty of Medicine, University of Colombo, Sri Lanka (Guest lecture)

Title: Enhancing motor learning and motor cortex plasticity through theta-gamma tACS in healthy young and older adults (poster) 2024
Florey Postgraduate Conference, University of Adelaide, Australia

Title: Theta-gamma transcranial alternating current stimulation (TG tACS) improves motor skill acquisition in young and older adults (oral) 2023
Students of Brain Research (SOBR) Symposium, Melbourne, Australia

Title: Theta-gamma tACS increases use-dependent motor cortex plasticity and improves motor skill acquisition in young and older adults (poster) 2023
Australasian Brain Stimulation Society (ABSS) Conference, Sydney, Australia

Title: Enhancing healthy ageing 2023
Three-Minute Thesis (3 MT) Joint PhD competition (Highly commended)
Adelaide-Nottingham Alliance Symposium

Title: Alterations in bilateral neuromuscular properties following a single bout of acute unilateral resistance exercise in healthy older adults (oral) 2022
Sensorimotor control meeting Online

Title: The influence of a single bout of unilateral exercise on bilateral neuromuscular function in healthy older adults (poster) 2022
Biomedical Research Conference, Nottingham, United Kingdom

Title: The influence of a single bout of unilateral exercise on bilateral neuromuscular function in healthy older adults (poster) 2022
EuroPhysiology Conference, Copenhagen, Denmark

Title: Acute unilateral exercise to influence bilateral neuromuscular properties among healthy older adults: a future study (oral) 2022
Biomedical Research Musculoskeletal conference, United Kingdom

Title: Interventions to manipulate neuroplasticity in ageing (oral) 2022
School of Biomedicine PhD introductory seminar (CCSP)-online

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