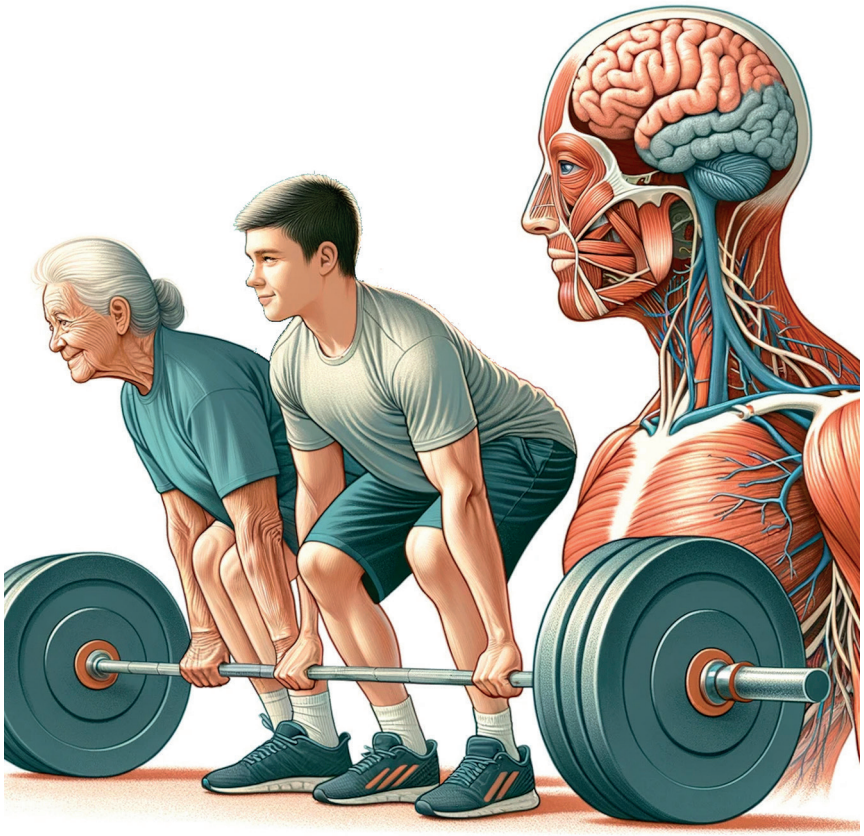
JYU DISSERTATIONS 812

Gonzalo Gomez Guerrero

Assessment of Cortico-spinal Functioning in Young and Older Adults

Effects of Resistance Training and Detraining



UNIVERSITY OF JYVÄSKYLÄ
FACULTY OF SPORT AND
HEALTH SCIENCES

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ABSTRACT

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Assessment of Cortico-spinal Functioning in Young and Older Adults: Effects of Resistance Training and Detraining
Jyväskylä: University of Jyväskylä, 2024, 112 p.
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This dissertation explored the suitability of transcranial magnetic stimulation (TMS) and lumbar stimulation (LS) as tools for assessing cortico-spinal excitability and inhibition. A primary objective was to evaluate the impact of aging and resistance training on these measures of cortico-spinal functioning. In Study I, 22 participants (22–34 years) volunteered for test-retest reliability assessment of TMS-induced motor-evoked potential (MEP), LS-induced lumbar-evoked potential (LEP) and TMS-/LS-silent period (SP). In Study II, 15 (18–42 years) participants volunteered to investigate modulation of LEP during TMS-SP at different contraction intensities. In Study III, 11 young (22–34 years) and 10 older adults (66–80 years) volunteered to assess MEP, LEP and TMS/LS-SP before and after 7 weeks of resistance training and 4 weeks of detraining. The methods demonstrated good-to-excellent test-retest reliability (ICC: 0.816–0.941; CV: 5.5–34.8) for MEP and TMS-SP and moderate-to-good reliability (ICC: 0.520–0.847; CV: 5.4–38.5) was found for LEP and LS-SP. LEP during the TMS-SP was reduced ($p < 0.010$) at 60 ms during 25%, 50% and 75% MVC. LEP at 150 ms was also reduced ($p < 0.010$) during 50% and 75% of MVC. Young were stronger than older adults (~63N·m, $p = 0.006$; ~50kg, $p = 0.002$). Resistance training increased young strength (+18kg, $p < 0.001$), MEP (+7%, $p = 0.023$) and LEP (+17%, $p < 0.001$). Resistance training increased older adults' strength (+13N·m, $p = 0.014$), however, they experienced decreases in MEP (−21%, $p = 0.006$) and LEP (−24%, $p < 0.001$). Therefore, the methods used are reliable to assess cortico-spinal excitability and inhibition. Furthermore, contraction intensities ≥50% of MVC affects spinal excitability within TMS-SP. Despite no changes in TMS-SP and LS-SP after resistance training, a concomitant decrease in MEP and LEP in older adults suggests cortico-spinal adaptations could have a spinal origin. Conversely, physically active young adults showed an increase in MEP and LEP supporting the suggestion that adaptations occurred at the spinal level. Thus, resistance training reversed some of the age-related neural maladaptations in older adults and increased strength. This dissertation confirmed the usefulness of cortico-spinal excitability assessment methods and showed spinal adaptation after a resistance training intervention in both young and older adults.

Keywords: lumbar stimulation, transcranial magnetic stimulation, aging, strength, lower-limbs

TIIVISTELMÄ (ABSTRACT IN FINNISH)

Gomez Guerrero, Gonzalo
Kortiko-spinaalisen Toimintaa Nuorilla ja Iäkkäillä Aikuisilla: Voimaharjoittelun ja Harjoittelutauon Vaikutukset
Jyväskylä: University of Jyväskylä, 2024, 112 s.
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Väitöskirjassa tutkittiin aivokuoren magneettistimulaation (TMS) ja lannerangan sähköstimulaation (LS) soveltuvuutta kortikospinaalisen radan herkkyyden arvioimiseen. Päätavoitteena oli arvioida ikääntymisen ja voimaharjoittelun vaikutuksia kortikospinaalisen radan toimintaan. Tutkittiin TMS-indusoidun motorisen herätepotentiaalin (MEP), LS-indusoidun lannerangan herätepotentiaalin (LEP) sekä molemmilla menetelmillä (TMS ja LS) aiheutetun hiljaisen jakson (SP) luotettavuutta ensimmäisessä osatutkimuksessa. Tähän vaiheeseen osallistui 22 (22–34-v.) henkilöä. Toiseen osatutkimukseen, jossa tutkittiin LEP:n modulaatiota TMS-SP:n aikana lihaksen eri voimatasoilla, osallistui 15 (18–42-v.) henkilöä. Tutkimuksen kolmannessa osassa (11 22–34-v. ja 10 66–80-v. henkilöä) tutkittiin seitsemän viikon voimaharjoittelun vaikutuksia kortikospinaalisen radan herkkyyteen (MEP ja LEP) ja TMS-/LS-SP:hen. MEP:n ja TMS-SP:n luotettavuus ja toistettavuus vaihtelivat hyvästä erinomaiseen ja LEP:n ja LS-SP:n osalta kohtalaisesta hyvään. LEP:n amplitudi pieneni merkitsevästi TMS-SP:n aikana 60 ms kohdalla 25%, 50% ja 75% MVC -voimatasoilla. LEP-amplitudi pieneni myös 150 ms kohdalla merkitsevästi, mutta vain 50% ja 75% MVC-voimatasoilla. Nuoret olivat vahvempia kuin vanhemmat aikuiset (~63Nm, $p = 0.006$; ~50kg, $p = 0.002$). Voimaharjoittelu lisäsi nuorten lihasvoimaa (+18kg, $p < 0.001$), kuten myös MEP- (+7%, $p = 0.023$) ja LEP (+17%, $p < 0.001$) -amplitudia. Voimaharjoittelu lisäsi myös ikääntyneiden lihasvoimaa (+13Nm, $p = 0.014$), mutta heidän kohdallaan MEP- (-21%, $p = 0.006$) ja LEP (-24%, $p < 0.001$) -amplitudit pienenivät. Voidaan todeta, että tutkimuksessa käytetyt menetelmät ovat luotettavia. Lisäksi, lihassupistuksen voimakkuus vaikutti selkäydintason herkkyyteen TMS-SP:ssä. MEP- ja LEP-amplitudin samanaikainen pieneneminen ikääntyneillä sekä kasvaminen nuorilla voimaharjoittelusta viittaisi siihen, että kortikospinaalisen radan muokkautuminen tapahtuisi selkäydintasolla. Näin ollen voimaharjoittelulla voitiin kumota osittain ikääntymiseen liittyvää hermostollista lihastoiminnan heikkenemistä. Tämä väitöskirja vahvisti tutkimuksessa käytettyjen mittausmenetelmien toimivuuden kortikospinaalisen radan herkkyyden arvioinnissa ja osoitti voimaharjoittelun aiheuttaman hermostollisen sopeutumisen tapahtuvan selkäydintasolla sekä nuorilla, että ikääntyneillä aikuisilla.

Asiasanat: lannerangan sähköstimulaatio, aivokuorten magneettistimulaatio, ikääntyminen, voima, alaraajat

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Jyväskylä 29.5.2024
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ORIGINAL PUBLICATIONS AND AUTHOR CONTRIBUTION

This dissertation is based on the following original publications, which will be referred to by their Roman numerals:

I Gomez-Guerrero G, Avela J, Enroth M, Häkkinen E, Ansdell P, Howatson G, Walker S. (2023). Test-retest reliability of cortico-spinal measurements in the rectus femoris at different contraction levels. *Frontiers in Neuroscience*. 2;17:1239982. doi: 10.3389/fnins.2023.1239982.

II Gomez-Guerrero G, Ansdell P, Howatson G, Avela J, Walker S. (2023). Contraction intensity modulates spinal excitability during transcranial magnetic stimulation-evoked silent period in rectus femoris muscle. *European Journal of Applied Physiology*, 124, 1355-1366. doi: 10.1007/s00421-023-05367-1

III Gomez-Guerrero G, Avela J, Jussila I, Pihlajamäki E, Deng FY, Kidgell DJ, Ahtiainen JP, Walker S. (2024) Cortical and spinal responses to short-term resistance training and detraining in young and older adults in rectus femoris muscle. *European Journal of Applied Physiology*. doi: 10.1007/s00421-024-05443-0

Following discussions with supervisors and co-authors, the author of the dissertation drafted the study questions and designs, prepared the data and performed statistical analyses, and took main responsibility for writing the manuscripts. The author actively participated in the data collection in Experiment I and II between 2020-2022 and that data were used in Studies I, II and III.

ABBREVIATIONS

1-RM	One-repetition maximum
aMT	Active motor threshold
ANOVA	Analysis of variance
CI	Confidence interval
CMEP	Cervico-medullar-evoked potential
CNS	Central nervous system
CSA	Cross-sectional area
CV	Coefficient of variance
Double N	Double normalization
EMG	Electromyography
GABA	Gamma-aminobutyric acid
H-reflex	Hoffman-reflex
ICC	Intra-class correlation coefficient
LEP	Lumbar-evoked potential
LMM	Linear mixed model
LS	Lumbar stimulation
LS-SP	Lumbar stimulation-evoked silent period
M1	Primary motor cortex
MDC	Minimal detectable change
MEP	Motor-evoked potential
M-max	Maximal compound action potential
MVC	Maximal voluntary contraction
NMDA	N-methyl-D-aspartate
PNS	Peripheral nervous system
RF	Rectus femoris
RMS	Root mean square
rMT	Resting motor threshold
SD	Standard deviation
SEM	Standard error of measurement
SICI	Short-intracortical inhibition
Single N	Single Normalization
TMEP	Thoracic-evoked potential
TMS	Transcranial magnetic stimulation
TMS-SP	Transcranial magnetic stimulation-evoked silent period
η_p^2	eta-squared

CONTENTS

1 INTRODUCTION

The nervous system, an intricate network of nerves and neuronal cells, serves as the fundamental circuit for transmitting messages between the brain, spinal cord and different body parts. The motor system's function is to transmit information from the brain to the muscle, to produce movement. This function is carried out via the cortico-spinal tract, which connects the brain with the muscle, through the motor-neuron, at the spinal cord. Cortico-spinal and spinal excitability can be assessed using transcranial magnetic stimulation (TMS) and spinal stimulation, respectively (Day et al. 1989a; Ugawa et al. 1991) . TMS generates a magnetic field over the contralateral motor cortex's pyramidal cells, inducing an action potential that results in a motor-evoked potential (MEP) recorded by electromyography (EMG) over the muscle targeted (Barker et al. 1985). Changes in MEP amplitude provide insights into the integrity of the cortico-spinal tract (Day et al. 1989b; Kobayashi and Pascual-Leone 2003). However, it is important to note that MEPs are not able to specifically dissociate changes in excitability occurring at the cortical or spinal level (Taylor 2006). Therefore, understanding both cortical and spinal excitability is crucial for interpreting MEP responses (Inghilleri et al. 1993; Taylor et al. 1996) and possible varied responses to distinct interventions (Butler et al. 2003; McNeil et al. 2009; Taylor 2006).

To gain specific insights into spinal motor-neurons, electrical or magnetic stimulation at the spinal level is necessary (Taylor 2006). Traditional peripheral nerve stimulation has been questioned due to the activation of other inhibitory pathways that may alter the efferent response (i.e. pre-synaptic inhibition) (McNeil et al. 2013). On the other hand, transcutaneous electrical stimulation at the spinal level elicits a monosynaptic response in upper- and lower-limb muscles through activation of cortico-spinal tract neurons (Martin et al. 2008; Petersen et al. 2002; Škarabot et al. 2019b; Taylor et al. 2002; Ugawa et al. 1991). Nevertheless, direct spinal cord stimulation at cervical and thoracic level can be uncomfortable (Brownstein et al. 2020; Martin et al. 2008; Taylor 2006). In contrast, lumbar stimulation, has been validated for use in the lower-limbs (Škarabot et al. 2019b) and it is well tolerated (Brownstein et al. 2020), providing insights into spinal motor-neurons through lumbar-evoked potentials (LEPs).

During voluntary muscle contraction, TMS causes a pause in the ongoing EMG signal, originally known as the 'cortical' silent period. The duration of the cortical silent period offers valuable information about intracortical inhibition (Mills 1988), although recent studies have shown a concomitant inhibition through the whole cortical silent period in the upper-limbs (Yacyshyn et al. 2016). Furthermore, some even question whether spinal inhibition influences the duration of the silent period (Škarabot et al. 2019c). Therefore, in the present dissertation, I will refer to the measure rather than interpret the underlying physiology. As such, it will be referred to as the TMS-evoked silent period (TMS-SP) and Lumbar stimulation-evoked silent period (LS-SP) here. Importantly, there is a lack of reliability studies examining both LEP amplitude and its LS-SP.

Aging is a natural and complex biological process characterized by a gradual decline in physiological functioning, that can impact health and overall wellness (López-Otín et al. 2023). This process affects the nervous system, leading to functional deterioration at both cortical and spinal levels. Neuronal atrophy, particularly within the motor cortex, can impact axonal regeneration potentially reducing motor cortex excitability and decreasing cortical inhibition (Fathi et al. 2010; Oliviero et al. 2006). Spinal motor-neurons, which execute neural commands from the cortex and sensory afferents, are also vulnerable to age-related changes, including neuronal population decline and synaptic input reorganization (Cruz-Sánchez et al. 1998; Tomlinson and Irving 1977). These alterations may contribute to reduced maximal force production, power, and overall physical function as people age (Clark and Taylor 2011).

Resistance training interventions are a safe and robust method to decelerate the age-related decrease in strength and muscle mass and, thus, enhance functional capacity in previously untrained older adults (Siddique et al. 2022). A meta-analysis (Kidgell et al. 2017) reported that resistance training may induce cortico-spinal adaptations in young adults, indicated by both increased MEP amplitude, decreased TMS-SP duration and increases in spinal excitability. However, there is currently a lack of evidence of chronic cortico-spinal adaptation to resistance training interventions in older adults. Consequently, it remains unclear whether resistance training will induce changes at a cortical or spinal level or perhaps involves both (Siddique et al. 2022), and whether certain neural adaptations are specific to young and/or older age.

Therefore, the aim of this dissertation was to assesses cortical and spinal adaptations of a lower-limb muscle to resistance training in young and older adults. Such examinations occurred after the establishment of reliability of the various cortico-spinal measurements included in the dissertation. In addition, this dissertation explores whether spinal excitability is decreased during TMS-SP in a lower-limb muscle.

2 REVIEW OF THE LITERATURE

2.1 Human nervous system

The human nervous system is an intricate network of neuronal cells that enables humans to move, perceive and comprehend the world around them. The nervous system can be anatomically divided into two primary components: the Central Nervous System (CNS) and the Peripheral Nervous System (PNS). Functionally, the nervous system can be divided into autonomic nervous system and somatic nervous system (Kandel et al. 2021, p. 74–93; Noback et al. 2005, p. ix). The somatic nervous system can also be divided into sensory and motor system. The motor system is a biological system that enables humans to move and coordinate their actions, with help from the sensory system. The motor system consists of the brain's motor areas, the spinal cord, and a vast network of motor-neurons and muscles (Kandel et al. 2021, p. 74–93).

2.1.1 Structure of the motor system

The CNS, comprising the brain and spinal cord, serves as the control centre for the body, processing information and coordinating responses. The brain, located in the skull, is responsible for higher functions like cognition, emotion, and consciousness, while the spinal cord, located in the vertebral column, facilitates the transmission of signals between the brain and the rest of the body. The CNS is composed of grey and white matter. The grey matter consists of neuron somas (cell body) and the white matter is composed of neuron axons that have a high concentration of myelin sheets, from which its lipid structure gives the colour (Fields 2010). The PNS, on the other hand, consist of nerves that originate from the brain (cranial nerves) and from the spinal cord (spinal nerves). These peripheral nerves are responsible for transmitting signals from sensory organs and receptors in the body to the CNS and from the CNS to the muscles and glands. (Kandel et al. 2021, p. 74–93; Noback et al. 2005, p. ix)

2.1.1.1 Brain and the motor cortex

The brain is composed of six different structures: cerebellum, medulla oblongata, pons, midbrain (collectively known as the brain stem), diencephalon, which contains thalamus and hypothalamus; and cerebrum (Figure 1-A). The cerebrum is made of two hemispheres, that contain the cerebral cortex, as the outer layer, and three primitive layers that are deeper in the cerebrum, which are the basal ganglia, the hippocampus and amygdala nuclei.

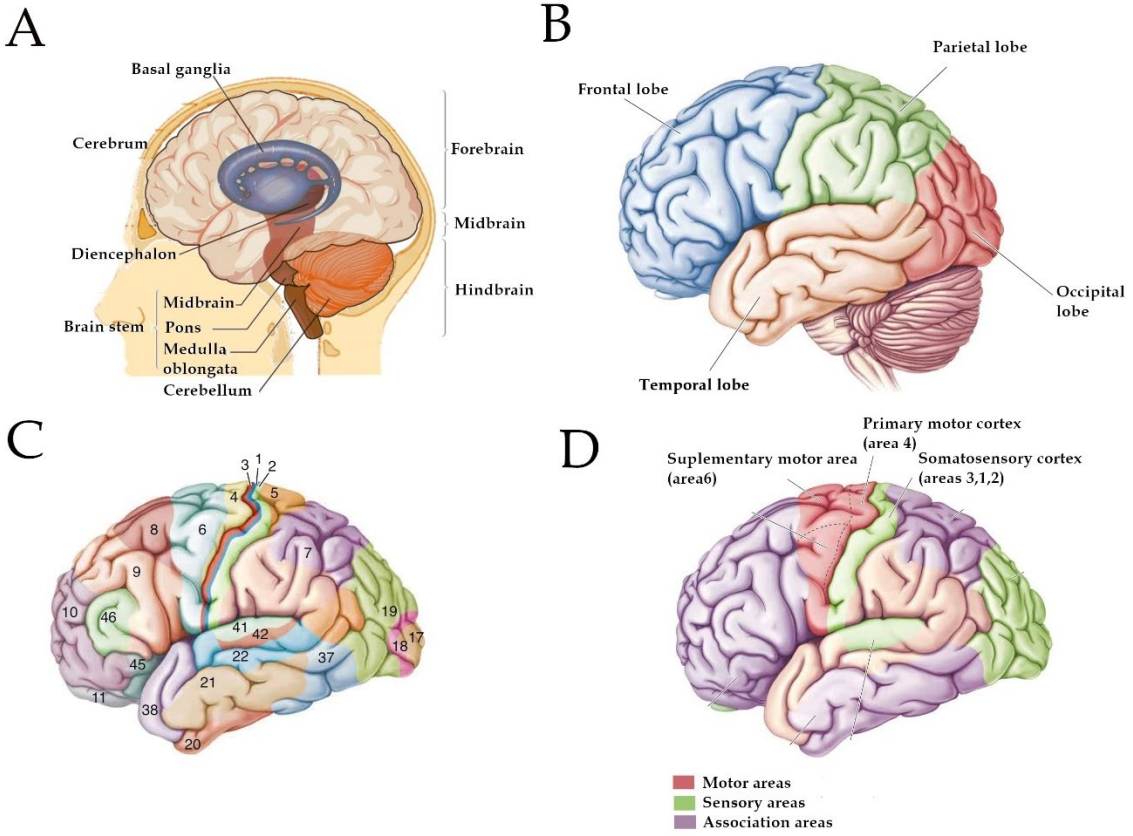

Figure 1 Division of the brain, from a lateral perspective, by: **(A)** different regions, **(B)** different lobes of the brain, **(C)** original classification according to Brodmann's research, and **(D)** Sensory, motor and associative areas of the cortex with some of the areas defined by Brodmann's research. Images adapted from Kandel et al. (2021) **(A)** and Bear et al. (2015) **(B, C, D)**.

Furthermore, the cerebrum hemispheres can be divided into four different lobes: frontal, parietal, occipital and temporal (Figure 1-B). Although, they are all interconnected, the frontal lobe appears to be the area with the greatest interconnectivity (Kandel et al. 2021, p. 74–93). In addition, Brodmann (1909) categorized the cerebral cortex into 52 anatomically and functional areas, based on their cells and the different arrangements of their layers (Figure 1-C) (Amunts and Zilles 2015; Zilles 2018). These organizations help to understand that each lobe is specialized in different functions, and can contain sensory, motor or association areas (Figure 1-B and 1-D).

The primary motor cortex (M1), which is situated in area 4 of Brodman's map, anterior to the central sulcus; is characterized by the low electrical current

intensity needed to elicit a movement of the representation of the region stimulated (Fulton 1935; Penfield and Boldery 1937) (Figure 1-C and 1-D). Furthermore, Penfield and Boldery (1937) stimulated different points anterior and posterior from the central sulcus, leading to the creation of the cortical homunculus map (Figure 2-A). This map is a rudimentary representation of different muscles of the human body in the premotor cortex and M1, however, a recent study using functional magnetic resonance imaging during different batteries of motor and task actions has suggested an interrupted model of M1 organization (Gordon et al. 2023) (Figure 2-B). In contrast to Penfield's continued homunculus map, Gordon's proposal for M1 homunculus map consists of specific areas that are arranged in overlapping circles. The inner parts of these circles intersect from a network for controlling the whole body's actions and responses.

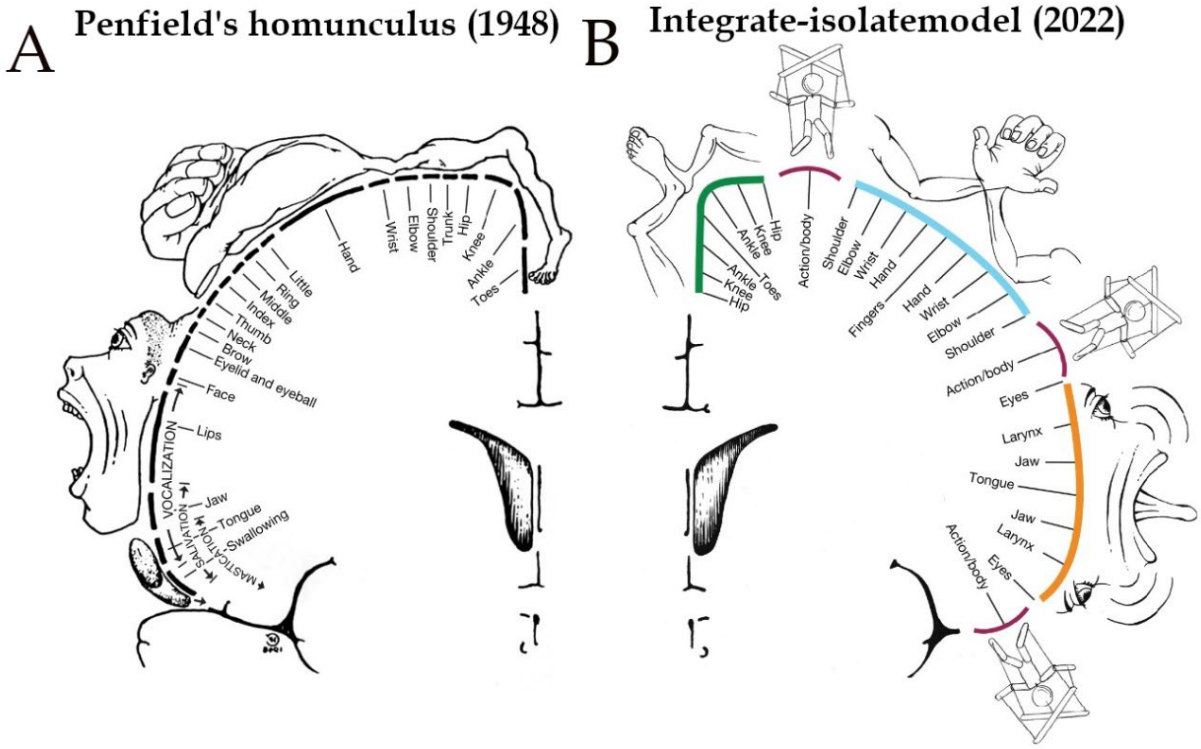

Figure 2 Representation of different muscles in the motor cortex based on **(A)** Penfield's continuous homunculus and **(B)** new integrated-isolated model where functional areas are organised in concentric rings (green: legs; cyan: arms and orange: head), where proximal body parts encircle the more distal parts. In-between those areas there is an integrative network that allows for whole-body control (maroon). This proposal and figure were obtained from Gordon et al. (2023).

M1 is composed of specialized neurons called pyramidal neurons, which are characterized by the pyramidal shape of the cell body and structured into 6 different layers within the grey matter (Amunts and Zilles 2015; Bear et al. 2015).

2.1.1.2 Spinal cord and spinal roots

The spinal cord is a caudal prolongation of the brain steam, that is protected by the vertebral column. It has 5 different sections: cervical (C_1–C_8), thoracic (T_1–T_{12}), lumbar (L_1–L_5), sacral (S_1–S_5) and coccygeal. A pair of nerves project from each vertebra, forming 31 pairs in total. Despite the length of the vertebral column, the spinal cord originates at the first cervical segment, approximately until the first lumbar, followed by a bundle of nerves roots called cauda equina (Figure 3). The spinal cord has two enlargements at the cervical and lumbar level due to the high density of neurons needed to process information in the arm and leg area (Bear et al. 2015, p. 456–457; Noback et al. 2005, p. 129–131; Tan et al. 2023).

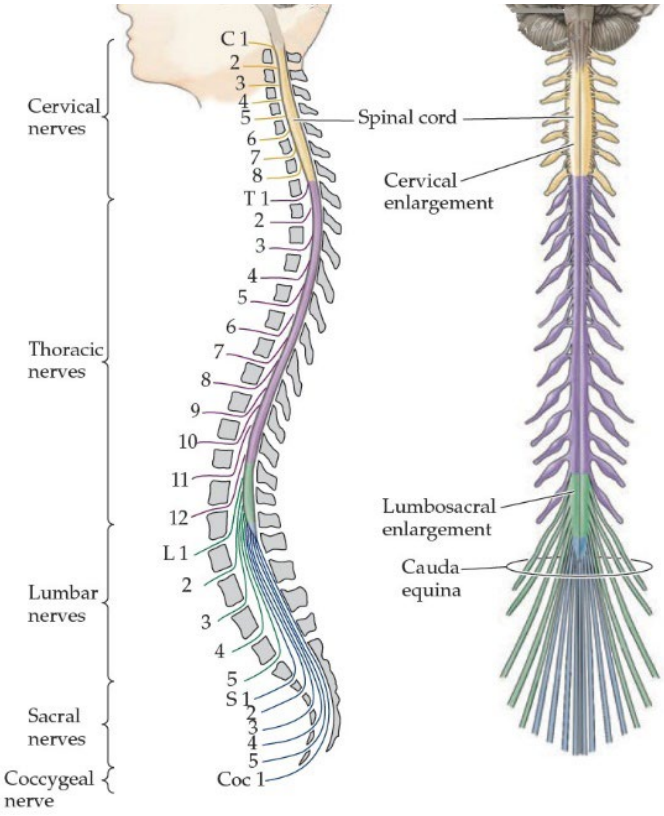

Figure 3 Sections of the vertebral column, spinal cord, and spinal roots. Taken from Purves et al. (2018).

Cross-sectionally, the spinal cord is composed of an external layer of white matter that surrounds grey matter in an H-shape. The white matter can be divided into dorsal (posterior), lateral, and ventral (anterior) columns, which contain axons of tracts linked with specific functions travelling from and to the brain. The grey matter can also be divided into dorsal (posterior) and ventral (anterior) horns. Furthermore, Rexed (1952) observed 10 different laminae, based on the cellular morphology obtained from staining the cat's spinal cord. These organizations have also been observed in other mammals (Figure 4-A) (Buxton and Goodman 1967; Kuypers and Brinkman 1970). The grey matter is composed of the cell body of ascending pathways, situated in the dorsal horn, the α-motor-neuron, located

in the ventral horn and interneurons. Additionally, through the ventral and dorsal horn there is a brunch of spinal nerves, which can be divided into ventral and dorsal roots. While the ventral roots are the axon of the α-motor-neuron, the dorsal roots contain all the sensory neurons, whose cell bodies are in the dorsal root ganglia (Figure 4-B). Sensory neurons can connect directly with the α-motor-neuron or indirectly through interneurons (Enoka 2008, pp. 249–288).

Spinal reflexes are fast responses, that originate from the sensory information carried via the afferent neurons to the spinal cord and generate a motor output, through motor-neurons. Afferent neurons are categorized into four groups (I, II, III and IV), which can be organized into different somatosensory receptors, such as muscle spindles, tendon organs, joint receptors and cutaneous mechanoreceptors, depending on the sensory information they receive (Enoka 2008, pp. 249–288).

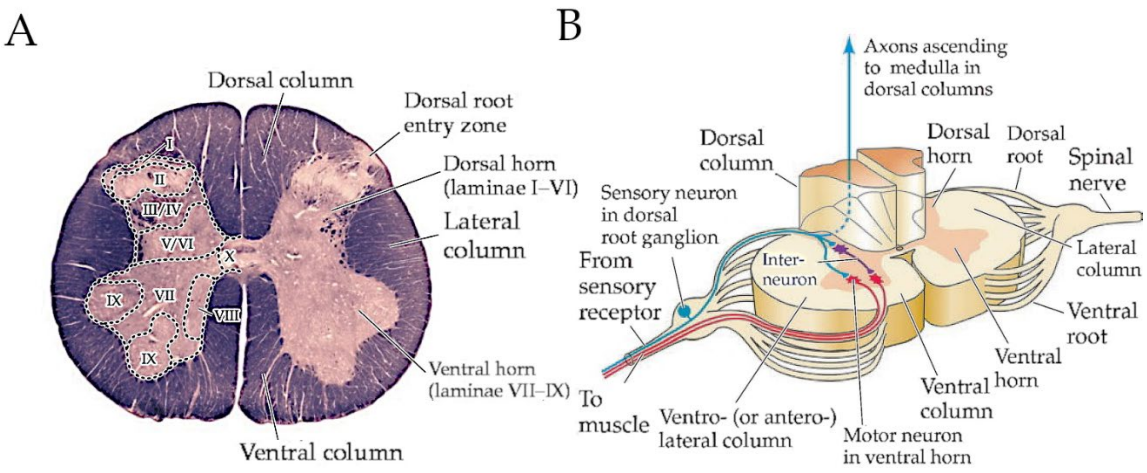

Figure 4 Spinal cord representation as (**A)** internal histology in a photomicrographic section of the lumbar segment of the spinal cord, and (**B)** diagram of the internal structure of the spinal cord**.** Obtained from Purves et al. (2018).

2.1.1.3 Peripheral neuron

Most of the cells in the nervous system are neurons and glia cells. Neurons, depending on their function, can be different in shape, size, or neuronal transmitter, although all of them have the same components: soma, dendrites, axon and post-synaptic terminals (Figure 5). The soma is the genomic and metabolic centre of the neuron. The genes of the cell are contained in the nucleus, and the cell's protein synthesis occurs in the endoplasmic reticulum, which is an extension of the nucleus. The soma branch into several short dendrites, which are tree-like, and a tubular axon. The dendrites, receive the incoming signal from other nerve cells in the post-synaptic terminals. The axon extends for a specific distance from the soma, carrying the signals to targeted neurons. The axon is composed of a tubular body that is insulated by myelin, a sheath of lipid substance that is synthesized by Schwann cells. These myelin sheaths are interrupted at regular intervals, and these intervals are known as the nodes of Ranvier. Finally, some branches sprout to form the pre-synaptic terminal at the

end of the axon (Cajal 1906, Cajal 1894; Purves et al. 2018, p. 2–7). The space between the pre-synaptic and the post-synaptic terminal is called the synaptic cleft, although there are exceptions e.g., when the pre-synaptic terminal connects with other tissue, such as muscle.

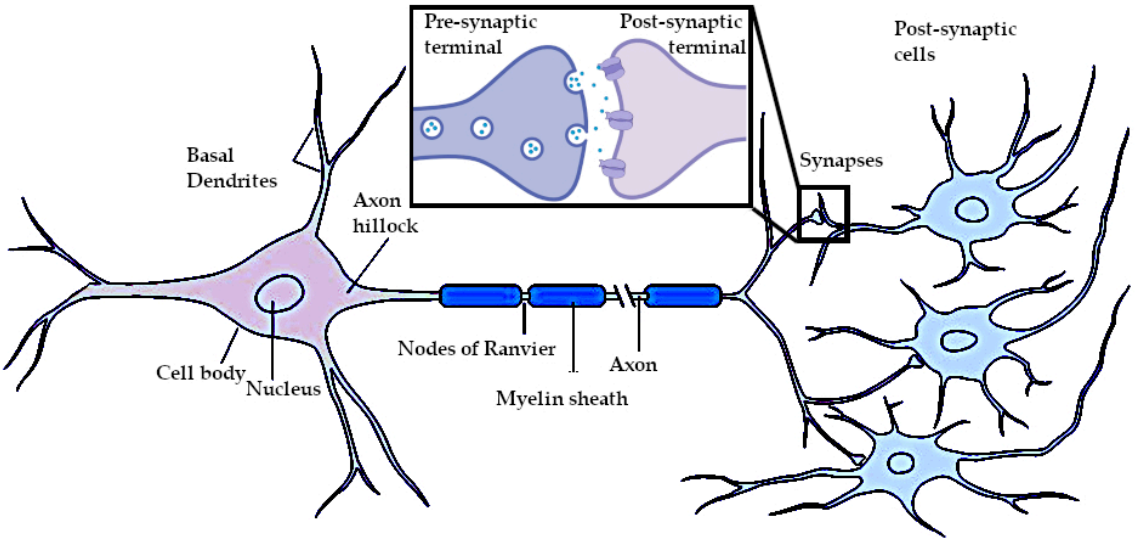

Figure 5 A neuron's structure. Adapted from Kandel et al. (2021).

As an example, the α-motor-neuron is a specialized efferent neuron that transfers the action potential to the muscle at the innervation point, known as neuromuscular junction, to produce movement. A single motor-neuron innervates a series of skeletal muscle fibres, which Sherrington (1925) defined as a motor-unit. The activation threshold of the motor-units is influenced by the diameter of the α-motor-neuron axon and skeletal muscle fibres can be categorized into types depending on the contractile properties and metabolic capacities. Small diameter α-motor-neuron axons innervate Type I fibres often referred to as "slow-twitch" due to their resistance to fatigue and the production of small tension outputs and have a low threshold. Alternatively, large diameter α-motor-neuron axons innervate Type II fibres referred to as "fast-twitch", they are more prone to fatigue but produce large tension outputs and have a higher threshold (Burke 1967; Burke 1968; Burke et al. 1973).

2.1.2 Functioning of the motor system

The functionality of the motor system can be divided into two different systems that are tightly integrated: the somatic nervous system and the autonomic nervous system. The autonomic nervous system, processes sensory feedback and motor control of innervated smooth muscles, cardiac muscle, and glands. The somatic nervous system conveys information that is received from peripheral sensory neurons in the skin, muscle, and joints to the CNS, where the information is processed (in the associated areas of the cerebral cortex and basal ganglia) and issues a response (from the motor cortex and cerebellum), that will be delivered

through the brain stem and spinal cord to the muscle to produce a coherent movement response of the body (Bear et al. 2015, p. 483; Kandel et al. 2021, p. 74-93). The CNS is composed of afferent neurons that carry sensory information (input), efferent neurons that carry the motor information (output), and interneurons that modulate the interaction between afferents and efferent.

Neurons follow the same procedure independently of their location or specialization. Neurons are normally in a resting state, characterized by a resting membrane potential. They receive their input through the pre-synaptic terminal. Depending on the signal generated, this will increase the membrane potential where it needs to rise beyond an electrical threshold to activate the post-synaptic neuron, creating what is known as an action potential. This action potential generation follows the all-or-nothing effect, where there is a response or not. Once the signal has overcome the neuron's threshold, this creates an imbalance on the Na^+-K^+ pump of the membrane of the axon hillock, that will spread along the axon. Therefore, the signal is generated at the axon hillock and propagates along the axon, facilitated by the myelin sheaths, which enables the rapid propagation of electrical impulses. This propagation is resumed at the nodes of Ranvier, which regenerate the action potential.

2.1.2.1 Motor cortex

The function of the motor cortex is to output a response, based on input from sensory feedback that has been processed in other areas of the brain. This information is carried by neurons and interneurons, within the white and grey matter, with different neurotransmitters and receptors that will reach a specific area within the motor cortex. Although, there are many interneurons in the CNS, they can be distinguished by their neurotransmitter, and they will produce either an inhibitory or facilitatory effect to modulate motor output (Larkum et al. 2009; Nistri and Constanti 1979; Sivilotti and Nistri 1991). In the CNS, glutamate and gamma-aminobutyric acid (GABA) neurotransmitters are abundant. Glutamate primarily binds with N-methyl-D-aspartate (NMDA) receptors, while GABA neurotransmitters bind with GABA receptors in the post-synaptic terminals of the next neuron. Repetitive facilitatory or inhibitory effects can lead to modulation of interconnectivity between neurons developing new representation patterns (Hess and Donoghue 1994).

The pyramidal cells situated in layer V of the motor cortex are known as the upper motor-neuron. Through the activation of the axon of these motor-neurons a descending volley down to the spine is produced. The descending volley, known as the efferent signal, will descend to the lower motor-neuron by crossing to the lateral column of the contralateral side at the medulla. Thus, the cortico-spinal tract creates a direct connection with the muscles of the opposite side of the body (Figure 6).

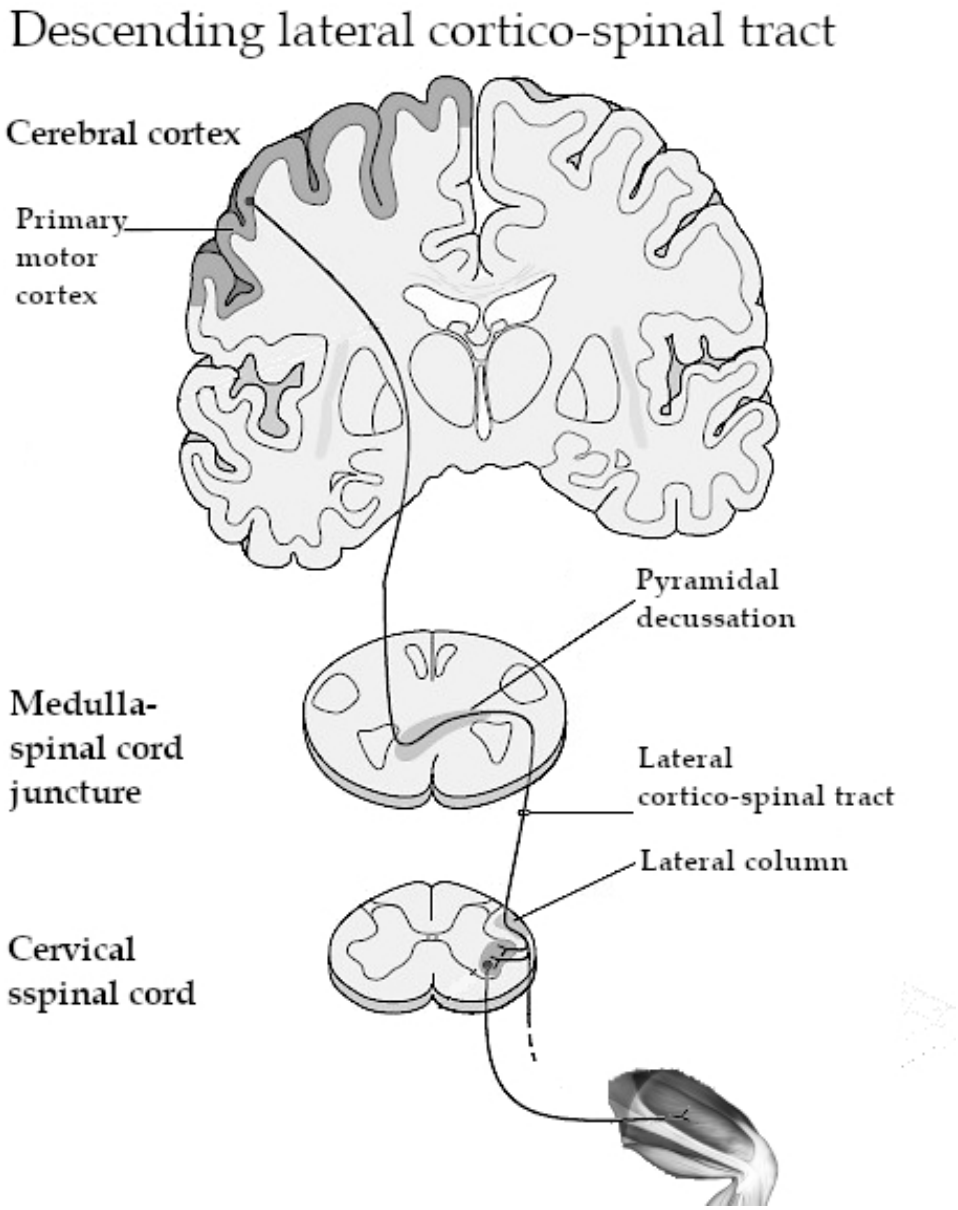

Figure 6 Representation of the cortico-spinal tract and the areas. Adapted from Kandel et al. (2021).

2.1.2.2 Spinal cord and spinal roots

In the spinal cord, afferent neurons can modulate motor-neuron membrane excitability either directly, as in spinal reflexes, or indirectly through interneurons. Such sensory feedback mechanisms can detect changes in the muscle length, tendon stretch, joint angle, etc. Depending on the type of sensory feedback, it can either hyperpolarize (producing an inhibitory effect) or depolarize (producing an excitatory effect) the motor-neurons (Enoka 2008, pp. 249–288).

Muscles spindles, that are within the muscles, contain group Ia afferent and group II afferent neurons. These sensory afferents connect mono-synaptically with the motor-neuron of the same muscles, providing information about the

length of the muscles. On the other hand, Golgi tendon organs contain Ib afferents. They are located in the myotendinous junction and provide tension sensory feedback mono-synaptically to the motor-neuron (Enoka 2008, pp. 249–288).

Moreover, there are many specialized interneurons located in the ventral horn of the spinal cord that are characterized by their ability to control motor outputs, which are task-specific and phase-dependant (Stachowski and Dougherty 2021). Their function is to improve coordination of movements and motor control, depending on agonist muscles (Wilmink and Nichols 2003) antagonist muscles (Calancie et al. 1987; Izumi et al. 2001) and other limb activation (Akay 2020; Zehr and Kido 2001). Some of the most well-studied interneurons are Renshaw cells and Ia inhibitory interneurons (Alvarez et al. 2005). While Renshaw cells modulate recurrent inhibition, by inhibiting the motor-neuron that excites these cells (Hultborn et al. 1979; Renshaw 1946), Ia inhibitory interneurons mediate reciprocal inhibition by hyperpolarising the motor-neurons of the antagonist muscle (Eccles et al. 1962; Hultborn et al. 1971). Furthermore, Ia excitatory interneurons, can produce a facilitatory effect on the motor-neuron. These mechanisms are essential to create smooth and coordinated muscular activity.

2.1.2.3 Peripheral neuron

Two of the main functions of the peripheral neurons is to transmit sensory information and initiate a motor response. For that, neurons use neurotransmitters. Once the neurotransmitters are in the synaptic cleft, they will bind with the specific receptors in the pre-synaptic terminal of the adjacent neuron. GABA neurotransmitters will bind with $GABA_A$ or $GABA_B$ receptors of the post-synaptic neuron, causing hyperpolarization of the neuron's membrane and producing an inhibitory-post-synaptic potential (IPSP), cancelling the transmission of an action potential (Browery and Smart, 2006).

On the other hand, glutamate neurotransmitters, will bind with NMDA receptors of the post-synaptic neuron, depolarizing the neuron's membrane and generating an excitatory-post-synaptic potential (EPSP). This will generate an action potential in the post-synaptic neuron (Browery and Smart, 2006; Mori and Mishina, 1995). If post-synaptic potentiation happens over time, the neuron will respond by generating more neurotransmitter receptors, which will reduce the threshold needed to depolarize the post-synaptic neuron (Schulz and Fitzgibbons, 1997). These plastic changes can occur at cortical and spinal level.

Nevertheless, at the motor-unit end plate, the neurotransmitter Acetylcholine (Ach) will bind with Ach receptors in the muscle membrane, to produce muscle contraction by recruiting Type I fibres for slow and controlled movements or Type II fibres for fast and powerful movements.

2.2 Evoked potentials elicited at the cortex and spine

An evoked potential is an electrical response to a specific stimulus, recorded by electromyography (EMG), in a conductive tissue (i.e., muscle, brain, spinal cord) (Pagni et al. 1988). Evoked potentials can be induced at the cortical level by TMS (Barker et al. 1985) or at the spinal level by transcutaneous electrical or magnetic stimulation (Ugawa et al. 1995a).

2.2.1 Transcranial magnetic stimulation

Transcranial Magnetic Stimulation (TMS) was introduced by Barker et al. (1985) as a more tolerable alternative to transcranial electrical stimulation in humans, that was developed by Merton and Morton (1980). Transcranial electrical stimulation uses an anode and a cathode over the skull of the target area to elicit an action potential. When the action potential is recorded at the cervical level, a complex descending volley can be observed, with an early component due to direct stimulation of the cortico-spinal neurons, known as the D-wave, and later wave-forms, known as I-waves, produced by activating neurons trans-synaptically (Boyd et al. 1986; Burke et al. 1990; Day et al. 1987; Day et al. 1989a). However, the lack of conductivity of the skull and the high threshold of certain motor areas, made the electrical stimulation method uncomfortable due to the high-intensity of the stimulator (Gualtierotti and Paterson 1954). On the other hand, TMS was based on Faraday's (1839) theory in which an electrical current through a wire coil generates a magnetic field. This field, applied to the skull, will elicit a secondary conductor, in the neurons that are in the line of the magnetic field (Hasey 1999). Therefore, TMS is a non-invasive technique that, when applied to the motor area will elicit an action potential in the pyramidal cells that connect with the cortico-spinal tract activating the cortico-spinal neurons of the area that is activated, producing a MEP in the EMG of the muscle targeted (Figure 7) and cause a muscle twitch (Barker et al. 1985). When this action potential was recorded at the cervical level, it was observed that the main component of the descending volley were I-waves, suggesting that pyramidal cells of the M1 are activated trans-synaptically (Kaneko et al. 1996; Nakamura et al. 1996).

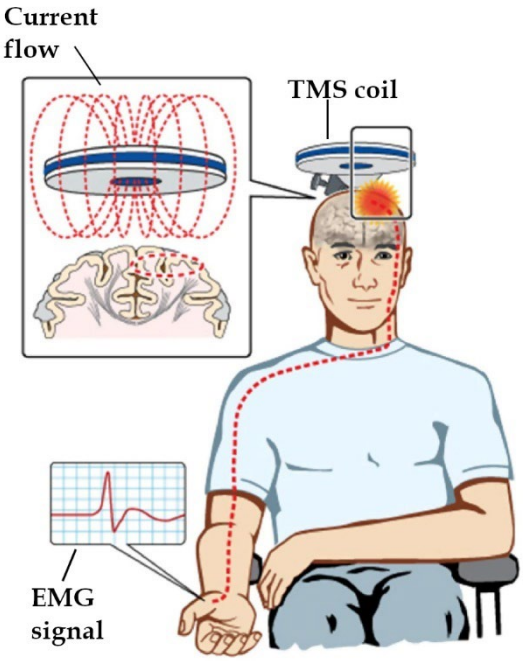

Figure 7 Transcranial Magnetic Stimulation eliciting a motor-evoked potential in the hand muscles. Obtained from Vucic et al. (2013).

Initially, a single round coil was used to elicit a MEP response in the upper-limbs. However, the coil was not focal and made it very difficult to target a specific area, therefore Ueno et al. (1988) developed the figure-of-eight coil, which consists of 2 coils creating a magnetic field in opposite directions that will create a higher current density on the target regions (Ueno et al. 1988). Despite the focality and the possibility to use the figure-of-eight coil to elicit responses in both upper- and lower-limbs, there was a trade-off between depth and focality (Lu and Ueno 2017). In addition, the deeper the pyramidal cell of the target muscle, the higher the intensity needed to elicit an action potential. Therefore, the double-cone coil, which is a figure-of-eight coil but with the coils bent 90–120^{o} from the axis, can target deeper areas in the brain (Lontis et al. 2006; Roth et al. 2002).

It is important to understand that the current direction in the coil will generate a current in the opposite direction beneath the coil (Rotenberg et al. 2016, pp. 80). If the coil is oriented Posterior-Anteriorly it will induce Anterior-Posterior current along the brain tissue, whereas a coil-oriented Anterior-Posteriorly will induce a Posterior-Anterior current along the brain tissue. The orientation is very important because, depending on the induced current, it can generate an action potential in interneurons instead of pyramidal cells or can activate neurons at the soma, instead of the axon hillock. These activations could increase (hyperpolarize) or decrease (depolarize) the membrane threshold of the neuron (Ueno et al. 1990, pp. 29–47; Day et al. 1989a; Di Lazzaro et al. 2001). A Posterior-Anterior current flow from the back to the front of the head activates neurons of the cortico-spinal tract mono-synaptically, producing large and consistent MEPs (Burke et al. 1993; Day et al. 1989b; Di Lazzaro et al. 2001). On the other hand, Anterior-Posterior current flow from the front of the head to the

back, activates cortico-spinal neurons poly-synaptically, which tend to produce smaller and more variable MEPs (Di Lazzaro et al. 2001; Hannah and Rothwell 2017). Therefore, Posterior-Anterior current is more sensitive to detect changes in cortico-spinal excitability since Anterior-Posterior may be influenced by excitation of other brain regions (Di Lazzaro et al. 2004; Hannah and Rothwell 2017).

2.2.1.1 Placements

When Penfield and Boldery (1937) stimulated the motor cortex (cerebellum area 4) they realized that there was a twitch response by the muscle associated to that area. Therefore, a similar protocol is used to locate the "hotspot" of a target muscle with TMS. The "hotspot" of the target muscle has been defined as the location of the scalp where the TMS consistently produces the largest MEP amplitude in the EMG (Ahdab et al. 2016; Reijonen et al. 2020). The minimum amount of stimulator output intensity needed to elicit a MEP >50 μV at least 50% of the time in a resting condition is known as the resting motor threshold (rMT) (Di Lazzaro et al. 2004; Rossini et al. 1994). These criteria can be applied in an active condition, when MEPs are >100 μV over 50% of the time, and this is known as the active motor threshold (aMT) (Di Lazzaro et al. 2004; Tokimura et al. 1996; Ziemann et al. 1998). rMT and aMT are considered a method to individualize the intensity of stimulation (Rossini et al. 1994) and also act as a measure of cortico-spinal excitability (Pascual-Leone et al. 1995; Wassermann 2002).

2.2.1.2 Stimulation paradigms

TMS is a versatile tool that can measure different aspects of the nervous system, depending on the settings of the stimulator output. The stimulator output can be set to deliver a single or a double stimulation giving two types of stimulation paradigms: single-pulse and paired-pulse. Single-pulse paradigms employ distinct, individually tailored pulses targeted at a specific area of the cortex. On the other hand, paired-pulse paradigms use two distinct pulses that are administered in a closely timed sequence. Each pulse can be targeted at the same cortical area or at different areas, and they can be used to evaluate their functional interconnection (Rotenberg et al. 2016, pp. 8).

Motor-evoked potentials. When single-pulse stimulation is applied over the motor cortex, a response in the EMG of the contralateral muscle is induced followed by a small twitch. The response is known as a MEP that can be elicited by electrical (Merton and Morton 1980) or magnetic stimulation (Barker et al. 1985). MEPs provide a valid but indirect measure of cortico-spinal excitability, by analysing the changes of the amplitude and/or area of the response (Magistris et al. 1998). However, the positioning of the EMG electrodes in the muscle can alter the responses, especially when EMG electrodes are replaced from one testing session to another (Hermens et al. 1999; Wong and Ng 2006). Therefore, to adjust for any possible change of any stimulation above the motorneuron, MEPs amplitude have been compared to the maximum compound action potential (M-max) as a method of normalization (Gandevia et al. 1999; Taylor et

al. 1999; Taylor et al. 2000). In addition, MEPs can also be influenced by stimulator output, observed as increases in MEP amplitude with increases in stimulator output intensity (Groppa et al. 2012; Temesi et al. 2014) and/or voluntary activation, such as increased MEP amplitude with increases in voluntary contraction (Groppa et al. 2012; Taylor et al. 1997; Ugawa et al. 1995b). Therefore, a double normalization was suggested to take into account muscle voluntary activity by comparing not only to the M-max, but also to the muscle EMG root mean square (RMS) previous to the stimulation (Sidhu et al. 2013; Škarabot et al. 2019).

Furthermore, in a relaxed condition when stimulator output is increased above the motor threshold, single motor-units are recruited in an orderly manner (Hess et al. 1986) in a similar way as the motor-unit orderly recruitment principles established by Henneman (1957). Therefore, the higher the intensity, the higher threshold motor-units will be recruited. However, a MEP plateau has been observed, at high stimulation intensities, when compared to the maximum compound action potential (M-max). This could be due to desynchronization of motor-neurons and/or inhibitory processes at the cortical and/or spinal level (Magistris et al. 1998). One important factor is the high-intensity needed to elicit a response in some muscles when in a relaxed condition, increasing the possibility of stimulating other areas at the same time.

On the other hand, increasing voluntary activation will reduce the motor threshold, due to the ongoing activity, not only at the spinal but also at the cortical level. Furthermore, when voluntary activation is increased, MEP amplitude increases when stimulator output intensity is kept constant. Thus, reducing intensity of the stimulator output and increasing focality of stimulation (Di Lazzaro et al. 2004). Nevertheless, Temesi et al. (2014) have shown that the plateau can be reached earlier with increasing voluntary activation.

Although many factors can affect the amplitude of the MEP, once the methodology has been established, an increase in MEPs size is recognized as an increase in cortico-spinal excitability and a decrease in MEP size will be interpreted as a decrease in cortico-spinal excitability.

TMS-evoked SP. When a MEP is elicited during muscle voluntary contraction, there is an interruption of the EMG background activity, which was originally known as the cortical silent period (Day et al. 1989b; Mills 1988), although recent evidence has shown ongoing spinal inhibition during the silent period (Yacyshyn et al. 2016). Therefore, it has been questioned whether this may influence the length of the silent period (Škarabot et al. 2019c). Thus, in the present dissertation, I will refer to the measure rather than interpret the underlying physiology. As such, it will be referred to as the TMS-evoked and LS-evoked SP here. Some authors suggested that the length of TMS-SP provides information about cortical inhibition (Inghilleri et al. 1993; Triggs et al. 1993). This was supported by a study by Ziemann et al. (1996) in which the authors found that a specific drug targeting inhibitory receptors ($GABA_B$) elongated the TMS-SP compared to the control condition, while peripheral nerve stimulation-evoked silent period did not change. In addition, Inghilleri et al. (1993), observed a

reduced MEP amplitude when re-evoked at different time points (100, 150 and 200 ms), using a paired-pulse paradigm, over a ~200 ms TMS-SP. However, a concomitant inhibition was observed at spinal and cortical levels from the first 50–100 ms during the same ~200 ms TMS-SP (Fuhr et al. 1991; Inghilleri et al. 1993). The authors concluded that the first half of the TMS-SP was spinal, and the latter part was cortical in origin.

Reduced spinal excitability is possibly due to motor-neuron afterhyperpolarization and/or recurrent inhibition via Renshaw cells, as well as Ia interneuron unloading through reciprocal inhibition (Fuhr et al. 1991; Mills 1988; Ziemann et al. 1993). Interestingly, a recent study showed reduced spinal excitability up to 150 ms in the upper-limbs after TMS, which was argued to be attributed to an increase in Golgi tendon organs and muscle spindle unloading (Yacyshyn et al. 2016). One methodological consideration is that traditional Hoffman-reflex (H-reflex) methodology used in previous studies (Fuhr et al. 1991; Ziemann et al. 1993) limits the assessment of modified spinal excitability < 100 ms, as the measure reflects modified pre-synaptic inhibition.

Additionally, TMS-SP length may be affected by voluntary activation (Cantello et al. 1992), stimulator output intensity (Säisänen et al. 2008) and/or TMS-SP analysis method used (Damron et al. 2008; Säisänen et al. 2008). A study led by Säisänen et al. (2008) used different voluntary contractions, stimulator outputs adjusted to the motor threshold. Stimulator output had a significant effect on the TMS-SP length, as opposed to the voluntary contraction, which did not show any effect. However, increases in voluntary torque production increases the tension of the tendon and, consequently, increases golgi tendon organ activity (Houk et al. 1970). In addition, muscle relaxation rate following TMS is greater with greater torque, which could activate muscle spindles as the sarcomeres lengthen (Vernillo et al. 2022). As such, afferent feedback mechanisms may be modified by increased torque level and potentially influence spinal excitability during TMS-SP.

In addition, the importance to standardize the analysis used to determine the length of the silent period has been reported by several authors (Damron et al. 2008; Säisänen et al. 2008; Škarabot et al. 2019c; Vernillo et al. 2022). Damron et al. (2008) reported the reliability of different visual and automatic mathematical analysis to calculate the length of the TMS-SP. The start points for both visual and mathematical were at the stimulator output, MEP onset and MEP offset, while the end point was when the EMG background returned to voluntary EMG signal. Although all methods showed excellent reliability (> 0.90 intraclass correlation coefficient (ICC)), visual analysis from the stimulator output showed the lowest coefficient of variation, independent of the number of stimulations (Damron et al. 2008).

2.2.2 Spinal stimulation

Spinal stimulation has been used to understand the modulation of spinal excitability, within the cortico-spinal tract, that TMS cannot detect (Taylor and Gandevia 2004; Ugawa et al. 1991). Spinal stimulation consists of eliciting a

magnetic or electrical current through the spinal cord, which targets the axons of cortico-spinal tract neurons depolarizing the motor-neuron mono-synaptically (Martin et al. 2008; Škarabot et al. 2019b; Taylor et al. 2002). Although other structures, such as the rubrospinal tract, reticulospinal tract and interneurons could be activated with spinal stimulation (Brownstein et al. 2020; Taylor et al. 2002), TMS paired with spinal stimulation has shown to activate the same cortico-spinal neurons as TMS (Martin et al. 2008; Škarabot et al. 2019b; Taylor et al. 2002).

2.2.2.1 Electrode placement

Transcutaneous spinal stimulation has been validated by pairing spinal stimulation either before or after TMS, using interstimulus intervals up to 16 ms, in 1 or 2 ms steps, at the cervical (Taylor et al. 2002), thoracic (Martin et al. 2008) and lumbar (Škarabot et al. 2019b) segments of the spine. All authors reported that an occlusion of MEP amplitude was observed when spinal stimulation was delivered prior to the TMS arrival to the motor-neuron. On the other hand, when spinal stimulation was delivered at or after the TMS arrival at the motor-neuron, MEP amplitude doubled. Thus, the authors discussed that the occlusion in MEP amplitude was due to the antidromic volley sent by spinal stimulation within the same tract, and MEP amplitude increase was due to the already excited state of the motor-neuron after the TMS volley passed through.

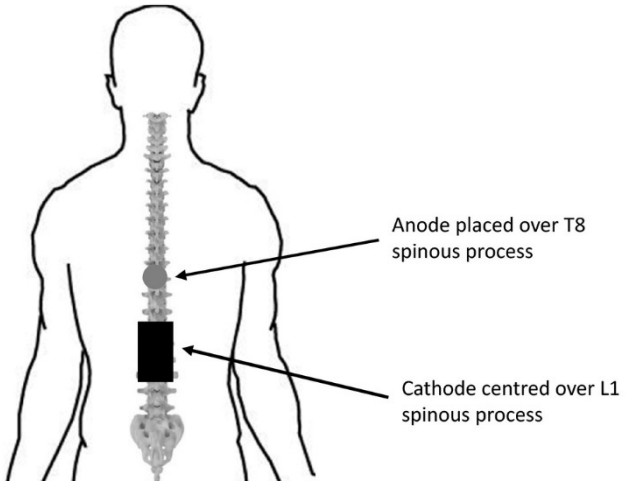

Figure 8 Anatomical placement of lumbar stimulation electrodes along the vertebral column. Image obtained from Škarabot et al. (2019b).

Stimulating at the cervical level (C_1–C_3) can elicit cervico-medullar-evoked potentials (CMEP) in upper-limbs (Petersen et al. 2002; Taylor et al. 2002) and lower-limbs (Ugawa et al. 1991), although CMEPs in lower-limbs were elicited in less than 50% of the participants and the electrical intensity needed to elicit a response was at least 10% higher than in the upper-limbs (Ugawa et al. 1991). Therefore, stimulation at thoracic and lumbar segments of the spine were implemented. Even though thoracic stimulation (T_1–T_8) was able to produce a

thoracic-evoked potential (TMEPs) in both upper- and lower-limbs, the discomfort was greater than from lumbar stimulation (LS) when targeting the lower-limbs, to elicit a lumbar-evoked potential (LEP) (Brownstein et al. 2020). Placements for lumbar stimulation are originally positioned by anatomical palpation, locating L_1 to position the centre of the cathode and T_8 for the centre of the anode (Figure 8).

Nevertheless, as previously mentioned, other structures like rubrospinal or reticulospinal tracts, as well as interneurons can be activated simultaneously. In addition, neurons in the dorsal (Hofstoetter et al. 2008; Hofstoetter et al. 2018) or ventral roots (Petersen et al. 2002; Taylor et al. 2002; Taylor and Gandevia 2004) can be activated, due to the thickness of their axon and their position with respect to the electrodes. Specifically, for LS, the electrodes are placed anatomically very close to the cauda equina. Therefore, different tests were suggested by Škarabot et al. (2019b) to check that dorsal and ventral roots are not activated.

Anatomically, dorsal roots are located in the posterior part of the spinal cord (Figure 4-B). In addition, afferent neurons have a lower threshold than the efferent neurons, which makes them more sensitive to electrical stimulation. Therefore, when a paired pulse separated by 50 ms is applied to the spinal cord, there may be a decrease in the second response when compared to the first response (Hofstoetter et al. 2008; Roy et al. 2012), which indicates an inhibitory effect from the posterior root-muscle reflex (Hofstoetter et al. 2008). On the other hand, if the second response is of similar amplitude to the first, there is an activation of the efferent pathway only (Roy et al. 2012).

In addition, ventral roots can also be activated, which will activate the motor-neuron post-synaptically instead of trans-synaptically, and this would obscure all inhibitory and/or excitatory events affecting the motor-neuron. One sign of ventral activation can be tested by the latency of the response. If the latency decreases when increasing the stimulation intensity, the stimulation is no longer pre-synaptic due to the few milliseconds that it takes for the signal to cross the synapse. According to Petersen et al. (2002), a decrease > 1.5 ms while increasing stimulator output intensity will indicate that the stimulation is post-synaptic and the stimulus is activating the ventral roots. Furthermore, during voluntary contraction, motor-neuron excitability is increased when compared to the relaxed condition, increasing LEP amplitude (Ugawa et al. 1995b). Similarly, spinal response amplitude, when stimulated pre-synaptically, should increase while the latency remains constant (Škarabot et al. 2019b; Taylor et al. 2002).

Thus, the electrode placement is very important to determine whether the motor-neuron is activated pre-synaptically at the cortico-spinal tract or whether other roots or nerves that are not of interest are activated. These tests are useful to determine the optimal position of the electrodes, by indicating whether the electrodes should remain in the original position or moved caudally along the spinal cord.

2.2.2.2 Stimulation paradigms

Paradigms for transcutaneous stimulation of the spinal cord are similar to the ones used in TMS: single-pulse and paired-pulse. Single-pulse stimulation elicits a single action potential that results in a response in the EMG and, depending on the section of the spine, are known as CMEP, TMEP or LEP. These responses represent the integrity of the spinal motor-neurons. Decreases in the amplitude indicate a reduced excitability and increases in the amplitude indicate increases in excitability (Ansdell et al. 2020; Weavil et al. 2016).

In addition, transcutaneous spinal stimulation can be paired with electrical stimulation or TMS. Paired electrical stimulation can be used to understand changes in root activation and has been used to evaluate reflex activity in spinal cord injury (Hofstoetter et al. 2018). Furthermore, paired TMS-spinal stimulation has been used to investigate possible inhibitory and excitatory mechanism affecting spinal excitability (Brownstein et al. 2020; Yacyshyn et al. 2016). However, LEP reliability from either single- or paired-pulse stimulation has not been tested.

2.3 Aging

Aging is a time-related dysfunction process, where damage is accumulated over time, leading to gradual degeneration and loss of function at molecular, cellular and organism levels (Kyriazis 2020). This leads to a gradual decrease in physical and mental capacity, increasing the risk of disease and death. Specifically, the drastic decline of strength has been correlated with impairments of functional capacity, fall risk and motor control (Clark and Taylor 2011). These changes are associated with structural and functional changes of the nervous system and skeletal muscle.

2.3.1 Structural deterioration within the nervous system

Structural adaptations to aging have been observed within the CNS and the PNS. Studies analysing brain magnetic resonance imaging data have shown that the brain volume decreases with age (Raz et al. 2005; Salat et al. 2004). Grey and white matter volume decrease in the cerebrum, and specifically in the motor cortex region. This decrease involves a reduction in the number of pyramidal cells, glial cells, and neural demyelination (Marner et al. 2003; Pakkenberg and Gundersen 1997). Furthermore, a decrease of glutamate (Kaiser et al. 2005) and GABA neurotransmitters has been observed in the motor cortex (Gao et al. 2013).

In the spinal cord, similar changes have been observed. Zhou et al. (1996) reported a decrease in grey and white matter area of the lumbar segment, specifically the first lumbar vertebra (L_1). The few studies investigating the population of motor-neurons at the lumbar segment of the spinal cord have reported a reduction in number of motor-neurons (Kawamura et al. 1977;

Tomlinson and Irving 1977). Specifically, after the age of 60 there was a decrease of about 50% of motor-neurons observed (Tomlinson and Irving 1977). In addition, neural demyelination of ventral fibres in the aging spinal cord (Piekarz et al. 2020) and increases in axonal thickness of the afferent neurons occurs.

2.3.2 Functional deterioration within the nervous system

At the cortical level, different functional changes can be observed as a consequence of the structural changes. Resting motor threshold (Tecchio et al. 2008; Young-Bernier et al. 2012) and aMT (Hassanlouei et al. 2017; Rozand et al. 2019) are significantly higher in older adults, when compared to younger counterparts. Furthermore, lower MEP amplitudes have been observed in older adults in the hand muscles (First Dorsal Interosseous), but not in the quadriceps muscles (Vastus lateralis), when compared to young adults (Rozand et al. 2019), demonstrating different responses between upper- and lower-limbs. However, even though Rozand et al. (2019) did not show statistical significance between young and older adults in the vastus lateralis muscle, MEP amplitudes of young adults were constantly higher from 100–140% aMT while contracting at 10% of MVC. Interestingly, changes in intracortical networks with aging have been discussed in a review by Clark and Taylor (2011) as the factor for reductions in motor performance due to the reduced ability to modulate motor networks. In support, a meta-analysis has reported that short-intracortical inhibition (SICI) was decreased in older adults, but no significant changes in TMS-SP were reported (Bhandari et al. 2016). Therefore, changes in MEP amplitude might be due to the adaptation of facilitatory and inhibitory pathways with age.

At the spinal level, a decrease in H-reflex (Kido et al. 2004; Scaglioni et al. 2002; Scaglioni et al. 2003) and tendon jerk (Bryndum and Marquardsen 1964; Milne and Williamson 1972) were observed in the older adults. Those results showed that aging affects both efferent and afferent signalling by reducing motor output, not only through the decrease in motor-neurons, but also by impaired modulation of excitatory and inhibitory spinal reflexes (Aagaard et al. 2010; Geertsen et al. 2017).

2.3.3 Structural and functional deterioration within the muscular system

Age-related loss of muscle mass is known as sarcopenia, with recent definitions emphasizing functional decline, such as loss in strength, power, and mobility (Cruz-Jentoft et al. 2019; Rosenberg 1997). Magnetic resonance imaging and dual-energy X-ray absorptiometry studies have shown that inactivity during aging decreases muscle volume, strength and power and increases fat mass (Bazzocchi et al. 2013; Farrow et al. 2021). One factor for the decrease of muscle mass is the decrease in synthesis rate of myosin heavy chains, which oversee remodelling muscle contractile protein (Balagopal et al. 1997).

Furthermore, the decrease in motor-neurons and muscle size will produce structural changes at the neuromuscular junction, which occur before the loss of fast motor-units. In terms of structure, both the area of the nerve terminal and the

count of post-synaptic folds decrease, causing a functional decline in the post-synaptic response of the neuromuscular junction. This means that the speed of motor nerve conduction slows and reduces the size of the M-max amplitude and area (Kurokawa et al. 1999).

2.4 Resistance training

Resistance training is a form of physical activity that involves the contraction of skeletal muscle fibres against an external load or one's own body weight. When resistance training is undertaken over a period of several weeks it has the potential to improve maximum strength and power and increase muscle mass that can help to improve athletic performance or perform activities of daily living (ACSM 2009; Fragala et al. 2019; Stone et al. 2002). Resistance training can use isoinertial, isokinetic and/or isometric contractions. Isoinertial contractions involve eccentric (increasing the joint angle) and concentric (decreasing the joint angle) actions, which change the muscle length. On the other hand, isometric contractions consist of keeping the same joint angle during the entire action.

Independently of the type of contraction to improve strength, power or increase muscle mass, resistance training should follow the main principles of exercise: e.g., progressive overload, specificity, and reversibility (ACSM, 2009).

- Progressive overload consists of utilizing the right stress, increasing the demand of the musculoskeletal and nervous system that will induce adaptations.
- Specificity dictates that the body will undergo specific adaptations depending on the training programme.
- Reversibility refers to the progressive loss of the adaptations produced by the training, once the training is stopped.

Therefore, different resistance programmes can achieve different goals through modifying certain variables such as the intensity (load), volume (training frequency, number sets and repetitions), velocity of the movement and/or rest time. According to the general guidelines of the American College of Sports Medicine (2009), the main goals of resistance training can be:

- Maximum strength: programme frequency should have 2–6 sessions per week, in which 2–6 sets of 1–5 repetitions are performed 80–100% 1-repetition maximum (1-RM)
- Hypertrophy: programme frequency should have 2–6 sessions per week, in which 2–6 sets of 8–12 repetitions are performed 60–85% 1-RM
- Power: programme frequency should have 2–6 sessions per week, in which 2–6 sets of 1–6 repetitions are performed < 60% 1-RM

As an example, Campos et al. (2002) selected untrained men into 4 different groups: control, low- (4 sets of 3–5 repetitions, intermediate- (3 sets of 9–11 repetitions) and high-repetition (2 sets of 20–28 repetitions) groups. All groups performed leg press, squats, and knee extension for 20 training sessions over 8 weeks. While the low-repetition group showed the greatest improvements in 1-

RM in all exercises, the intermediate group showed the greatest increase in fibre cross sectional area, and the high group showed the greatest improvement in a repetition-to-failure test. In addition, a review by Fry (2004) discussed that using loads > 60% 1-RM induces greater gains in maximum strength than low loads < 60% 1-RM, which has been subsequently supported by two meta-analyses: one in young adults (Schoenfeld et al. 2017) and another in older adults (Csapo and Alegre 2016). Alterations in maximum strength can manifest in two primary ways: through neural adaptations or morphological transformations (Moritani and deVries 1979; Sale 1988).

2.4.1 Neural adaptations

Neural adaptations reflect the changes that occur in the nervous system after the repeated exposure to a certain stimulus. Neural adaptations take place within the immediate weeks after starting resistance training (Goodwill et al. 2012; Holtermann et al. 2007; Moritani and deVries 1979; Weier et al. 2012), and can be at the cortical (Goodwill et al. 2012; Weier et al. 2012) or spinal level (Holtermann et al. 2007). Despite limited experimental evidence, recent meta-analyses have indicated adaptations in both cortico-spinal excitability and inhibition (Kidgell et al. 2017; Siddique et al. 2020).

2.4.1.1 Cortico-spinal excitability

Changes in cortico-spinal excitability has been studied in younger adults, where MEP amplitude has increased (Goodwill et al. 2012; Leung et al. 2017; Weier et al. 2012), decreased (Jensen et al. 2005) or showed no change (Christie and Kamen 2014; Manca et al. 2016). Nevertheless, independently of the heterogeneity of the responses within small sample studies, a meta-analysis by Kidgell et al. (2017) has shown that short-term (9–12 training session) of resistance training increases cortico-spinal excitability in young adults. Specifically, Weier et al. (2012) reported increased MEP amplitude at 110–140% aMT in m.rectus femoris (RF) within a recruitment curve (90–140% aMT) following 12 sessions of heavy-squat training (4 sets, 6–8 repetitions, at 80% 1-RM) in healthy young adults. On the other hand, physically active older adults have shown a lowered MEP amplitude compared to less physically active older adults (Hunter et al. 2016), although no significant differences were found after 6 sessions of resistance training in older adults (Christie and Kamen 2014), which is the only study reporting MEP in older adults after resistance training.

A single resistance training session led to an acute increase of spinal excitability, but no changes were observed after 12 resistance training sessions of 4 sets of 6–8 repetitions of squats at 80% 1-RM with 5 min rest in young adults (Ansdell et al. 2020). In contrast, spinal excitability has increased after 9–38 training sessions when assessed by peripheral nerve stimulation (H-reflex), during maximal (Aagaard et al. 2002) or submaximal contractions (20% and 60% of MVC (Holtermann et al. 2007); and 10% of MVC (Vila-Chã et al. 2012)) in younger adults. Conversely, in older adults, H-reflex amplitude was not changed

after 6–9 training sessions, when measured at rest and at 10% of the MVC (Christie and Kamen 2014; Unhjem et al. 2021). Therefore, the number of training sessions might be an important methodological issue in this age-related difference in adaptability.

2.4.1.2 Cortical and spinal inhibition

Similar to MEP amplitude changes, some studies have reported a decrease in TMS-SP length after six sessions (Christie and Kamen 2014) and nine sessions (Coombs et al. 2016) of resistance training suggesting reduced cortical inhibition. However, no changes were reported after twelve training sessions in another study (Kidgell et al. 2011). Despite between-study heterogeneity, various meta-analyses have reported an overall decrease in TMS-SP after resistance training in young adults (Kidgell et al. 2017; Siddique et al. 2020). Furthermore, six training sessions of resistance training also resulted in a decreased TMS-SP in older adults, without any changes in H-reflex (Christie and Kamen 2014). It could be argued that cortical inhibition, specifically $GABA_B$ functioning, is reduced in older adults, possibly contributing to the increased maximum strength. However, considering the effect of pre-synaptic inhibitory process on H-reflex and the recent findings of spinal inhibition throughout most of the TMS-SP in the upper-limbs, using spinal stimulation, as well as the lack of investigation of spinal excitability during the TMS-SP in the lower-limbs, spinal inhibition might have a concomitant effect during the TMS-SP. In addition, to my knowledge, there is no current investigation that has reported spinal inhibition after resistance training.

2.4.2 Morphological adaptations

Morphological changes occur in response to resistance training, which reflect the body's ability to adapt at structural levels. In young adults, resistance training produces several changes in the morphology of the muscle. Early studies have proposed that changes in the muscle's morphology may take place after 8 weeks (Garfinkel and Cafarelli 1992; Sale 1988) of resistance training in the form of increased muscle cross-sectional area (CSA) (Folland and Williams 2007). This phenomena is known as muscle hypertrophy, which consists of the growth of the whole tissue, by increasing the number of sarcomeres in parallel of already existing myofibrils (Haun et al. 2019; Russell et al. 2000). Although changes in muscle CSA (Damas et al. 2016; DeFreitas et al. 2011; Walker and Häkkinen 2014), and skeletal muscle mass (Walker and Häkkinen 2014) have been reported after 8–10 weeks of resistance training, others have reported increased CSA as early as 1 week (DeFreitas et al. 2011), however, those early changes might be due to muscle swelling (Damas et al. 2015). It seems that 4 weeks (Stock et al. 2017) or at least 8–12 sessions are needed to observe at least 3–4% change in muscle morphology in young adults (Damas et al. 2018). Furthermore, older adults also increased CSA after 10 week of resistance training, although the increase was smaller in magnitude (-3%) when compared to the younger adults (Walker and Häkkinen 2014). In addition, Walker and Häkkinen (2014) did not observe

changes in skeletal muscle mass for the older adults, as opposed to the younger adults.

3 PURPOSE OF THE STUDY

Recognizing the important role of lower-limb functionality in ambulation (Landin et al. 2016), a predictor of disability and mortality (Guralnik et al. 1995; Millington et al. 1992), it is imperative to develop methodologies that target lower-limb function in older adults. These methodologies should facilitate the study of spinal excitability through single- or paired-pulse stimulation paradigms. Recent evidence indicates a prolonged spinal inhibition in the upper-limbs during the TMS-SP (Yacyshyn et al. 2016). However, the influence of contraction intensity on spinal inhibition within this period in the lower-limbs remains unexplored, despite its utility in inferring cortical inhibition. While MEP, TMS-SP, and LEP have been validated (Di Lazzaro and Rothwell 2014) their reliability at different submaximal contraction intensities has not been examined. Submaximal contraction intensities can increase specificity of the intensity used during testing and intervention protocols, and it is important to understand the potential magnitude of error before performing repeated measure studies. Resistance training has been demonstrated to modulate cortico-spinal and spinal excitability in young adults, yet its effects in older adults remain unclear. Therefore, the aims of this dissertation were to:

- determine test-retest reliability of motor-evoked potentials and lumbar-evoked potentials during different contraction intensities in m.rectus femoris in a wide range of ages (Study I)
  Hypothesis: Good-to-excellent reliability in motor-evoked potential and lumbar-evoked potentials is expected at both low- and high-level contraction intensities. Furthermore, high-level contraction intensities are expected to exhibit better reliability than low-level contraction intensities (Brownstein et al. 2018; Temesi et al. 2017).
- examine the effect of stimulator output and contraction intensity on motor-evoked potentials in m.rectus femoris (Study I)
  Hypothesis: MEP amplitude will increase as the stimulator output intensity increases at each contraction level. Additionally, it is expected that MEP amplitude will increase with higher contraction intensity at

the same stimulator output intensity (Groppa et al. 2012; Taylor et al. 1997; Ugawa et al. 1995b).

- evaluate spinal excitability, via spinal stimulation, during the transcranial magnetic stimulation-evoked silent period in m.rectus femoris during different contraction intensities (Study II)
  Hypothesis: Decreased spinal excitability at different time-points of the transcranial magnetic stimulation-evoked silent period (Yacyshyn et al. 2016) and that the decrease in spinal excitability will be accentuated by the increase in contraction intensity (Houk et al. 1970; Vernillo et al. 2022).
- assess cortico-spinal and spinal adaptations to a short-term resistance training and detraining intervention in young and older adults in m.rectus femoris (Study III).
  Hypothesis: There is an expected difference between young and older adults in strength, skeletal muscle mass and cortical and spinal excitability at baseline (Hunter et al. 2016; Walker and Häkkinen 2014). Furthermore, resistance training will increase maximum strength in both groups. In addition, cortico-spinal excitability adaptations will occur in young and older adults after resistance training as well as decreases in inhibition (Goodwill et al. 2012; Leung et al. 2017; Weier et al. 2012). Finally, it is expected that all variables return to baseline after the detraining period in both young and older adults.

4 METHODS

4.1 Participants and ethical statement

For Studies I–III healthy active young adults between 18-35 years old were recruited. In Study I and III healthy active older adults between 65–80 years old were also recruited. All included participants were free from neurological illness and musculoskeletal injury in the lower-limbs for the last 6 months, were not taking any medications known to affect the nervous system and had no contraindications to TMS, which was assessed via a health questionnaire (modified from Rossi et al. (2009)).

Data from Studies I–III is presented in Table 1. In Study I and III, twenty-seven adults (14 female) volunteered where five participants were removed during the offline analysis due to possible activation of ventral roots (see Lumbar-evoked potentials). Therefore, the data presented in Table 1 are representative of the twenty-two (12 female) volunteers fulfilling all study requirements (Study I). In Study III, one more participant was excluded, because they missed > 1 training session during the intervention. Therefore, the data presented in Table 1 are representative of the 21 (11 young adults (6 female) and 10 older adults (6 female)) volunteers fulfilling all study requirements. In Study II, twenty-two healthy adults (8 female) volunteered. Here, seven participants were not considered due to possible activation of ventral roots (see Lumbar-evoked potentials). Therefore, the data presented in Table 1 are representative of the 15 (4 female) volunteers fulfilling all study requirements.

Before testing, all participants were fully informed of the procedures and possible risks, and each participant provided written inform consent. All participants were informed that they could withdraw from the study at any timepoint and at will. All Studies were approved by the Ethical committee of the University of Jyväskylä (Study I and III: 857/13.00.04.00/2021 and Study II: 10.01.2020) and was conducted with accordance with the *Declaration of Helsinki* (2013) except for the registration of the data in a database.

Table 1 Mean (± SD) of anthropometric, peripheral nerve stimulation, lumbar stimulation and transcranial magnetic stimulation parameters from the participants in Study I, II and III.

		Study I	Study II	Study III	
				Young adults	Older adults
Participants (%female)		22 (55%)	15 (27%)	11 (55%)	10 (60%)
Height (m)		1.71 ± 0.10	1.75 ± 0.8	1.74 ± 0.10	1.66 ± 0.06
Age (years)		47 ± 23	30 ± 5	27 ± 5	71 ± 4
M-max (mV)		1.91 ± 0.88	1.70 ± 0.58	2.65 ± 1.25	1.23 ± 0.50
M-max stimulator output (mA)		229 ± 79	257 ± 151	227 ± 22	239 ± 28
LEP stimulation intensity (mA)	**25% M-max**	240 ± 98	-	262 ± 93	200 ± 77
	50% M-max	274 ± 104	308 ± 108	-	-
aMT (%)		35 ± 9	-	31 ± 6	40 ± 11
TMS stimulator output (%)	**25% of MVC**	-	66 ± 16	-	-
	50% of MVC	-	64 ± 12	-	-
	75% of MVC	-	65 ± 14	-	-

M-max = maximal compound action potential, LEP = lumbar-evoked potentials, aMT = Active motor threshold, TMS = Transcranial magnetic stimulation.

4.2 Experimental set-up

In all Studies, participants were asked to abstain from consuming caffeine within the 12 hours leading up to the examination, and refrain from engaging in strenuous physical activities 48 hours preceding each testing session. All responses were assessed in m.rectus femoris (RF). Participants were seated in a custom-built electromechanical dynamometer with a calibrated load cell (Faculty of Sport and Health Sciences, University of Jyväskylä, Finland) with the hip and knee flexed to 90° and the shin strapped with a non-elastic restraint ~2 cm superior to the ankle malleoli (Figure 9). The voltage signal originating from the load cell was calibrated and converted into torque (N·m). All measures were performed on the right (i.e., dominant) leg assessed by self-report of which foot they primarily kick a ball (van Melick et al. 2017).

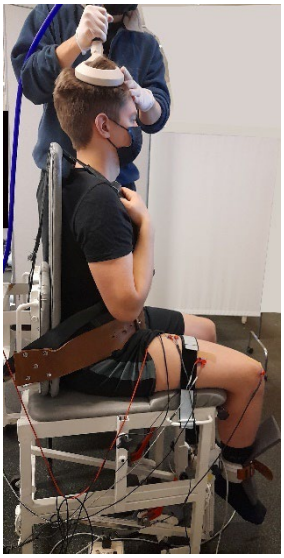

Figure 9 Set up of the Participant position in all Studies and testing sessions. The TMS coil was placed above the M1 leg representation. EMG was placed over RF and vastus lateralis, and the ground electrode was placed on the patella. The torso and leg were strapped to avoid any undesired hip flexion, hip abduction or adduction.

Once the participant was secured to the dynamometer, M-max was assessed in a relaxed condition (see Peripheral nerve stimulation). Two maximal voluntary contraction (MVC) trials of ~5 s was performed 60 s apart. Prior to the MVC, two contractions of ~5 s at ~50% and ~80% of estimated MVC were performed as a warm-up. To perform MVC, participants were instructed to push "as hard and as fast as possible". Verbal encouragement and visual feedback were provided to motivate participants to produce maximal effort. In all Studies, torque was sampled at 1000 Hz, amplified by a custom-built amplifier (ForAmps 1, v1.2, University of Jyväskylä, Finland) and converted by a 16-bit A/D board (CED Power1401-3, Cambridge Electronics Design, Cambridge, UK) in combination with Spike2 software (version 6.10, Cambridge Electronic Design, Cambridge, UK). In addition, both TMS and LS stimulation protocols were standardized by controlling participants' limb position (Mogk et al. 2014) strapping the leg and maintaining their hands over the straps (Figure 9). During active condition, participants were asked to contract to a submaximal torque displayed in the screen, making the task the focus of attention (Lefebvre et al. 2004). Similarly, during relaxed condition, participants were asked to focus on the force trace displayed in the screen, which was the focus of attention during the active condition, and count down from 100, while stimuli were delivered with a relaxed muscle (Lefebvre et al. 2004). Furthermore, every set of stimuli, independently of whether the muscle was relaxed or active, were given after a >30 s quiet period, and would always start with same instructions: relaxed " the set will start now"; active: "go to the line" and "relax", so the participants alertness would be exactly the same (Lefebvre et al. 2004). Finally, EMG was visually controlled when stimulations were delivered during relaxed condition, to avoid any muscle activity that could influence the MEP amplitude (Škarabot et al. 2019a).

In the lumbar stimulation (LS) testing sessions of Study I, all sessions of Study II and the LS testing sessions of the control period in Study III, placement of the LS electrodes was assessed to avoid activating spinal nerve roots. In all sessions, LS was adjusted to the required intensity (25% (Study I and III) and 50% (Study I and II) of M-max). When using TMS, aMT was assessed in Study I and III and TMS stimulator output intensity was standardized to obtain a TMS-SP of ~200 ms in Study II.

4.2.1 Familiarization session

In all Studies, the first session was a familiarization session, where the participants were introduced to all instructions and stimulations that were given during the testing sessions. Furthermore, this session was used for preliminary assessment of the LS electrode placement and aMT for Study I and III and TMS stimulator output intensity for Study II.

4.2.2 Experiment I (Study I and III)

In Experiment I participants visited the laboratory on five different testing periods and one familiarization session (Figure 10-A). Testing periods were defined as control testing (Con), pre-training testing (Pre), mid-training testing (Mid), post-training testing (Post) and detraining testing (De) (Figure 10-A). Every testing period was structured the same: A LS session, a TMS session and a one-repetition maximum (1-RM) session conducted within a 7-day period. Sessions for each participant were consistently scheduled at the same time of the day (± 2 hours), and there was a 48- to 72-hour interval between LS, TMS and 1-RM (Figure 10-B).

In Study I, data from LS and TMS session from Con and Pre were used to assess test-retest reliability of the methods. Four testing sessions included two different protocols: two sessions were dedicated to LS and the other two to TMS stimulation. One session of each stimulation method was performed 10–14 days prior to the second one. For each participant, sessions were performed at the same time of day (± 2 hours). The TMS test session was performed at least 48 hours after the LS test session.

In Study III, all testing periods were used to assess the effect of resistance training and detraining on cortico-spinal excitability and cortical and spinal inhibition.

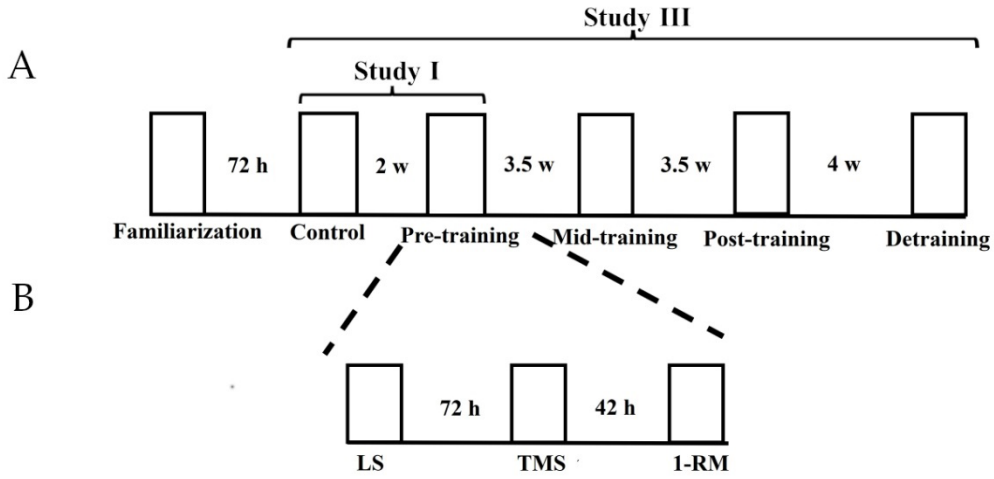

Figure 10 Experiment I timeline, for which data for Study I and Study III were obtained **(A)** and the order of the six different testing periods. The time between testing sessions refers to the total time between one test period to the next. **(B)** An example of the testing sessions within each testing period. The time between the sessions is the minimum amount of time between each test. h = hours; w = weeks; LS = lumbar stimulation; TMS = transcranial magnetic stimulation; 1-RM = one-repetition maximum

In each testing session, visual feedback was provided to the participants to produce the required submaximal torque and then a single TMS or LS stimulus was delivered manually. Contractions at 20% and 60% of MVC were held for 5–8 s. Sets of ten stimulations were given per condition (LS: 25% (Study I and III) and 50% (Study I) M-max; TMS: 120%, 140% and 160% aMT) and per contraction intensity as a single block, giving a total of 40 LS (Study I) or 20 LS (Study III) (Figure 11-A) and 60 TMS stimulations (Figure 11-B). To avoid fatigue, 30 s and 45 s rest was given between contractions during 20% and 60% of MVC, respectively, and 60 s and 180 s rest was given between the sets of 10 contractions.

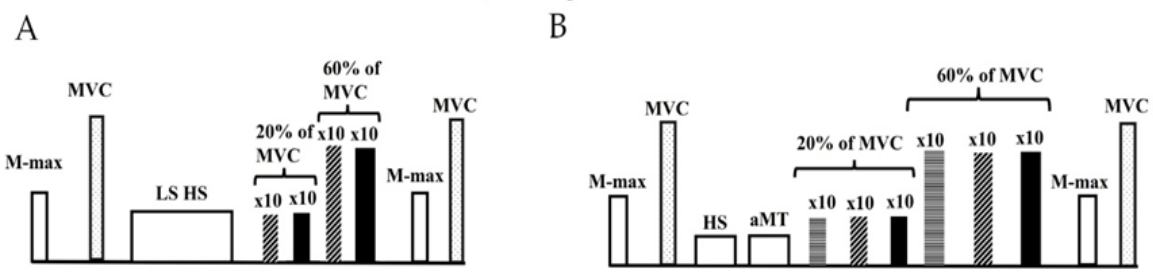

Figure 11 Testing sessions in Experiment I for Study I and III **(A)** lumbar session low-intensity (diagonal stripes) (Study I and III) and high-intensity (filled bars) stimulation (Study III), and **(B)** TMS stimulation using 120% aMT (horizontal stripes), 140% aMT (diagonal stripes) and 160% aMT (filled bars) (Study II and III). M-max: maximal compound action potential, MVC: Maximal voluntary contraction, LS: lumbar stimulation HS, hotspot; aMT: active motor threshold.

4.2.3 Experiment II (Study II)

Experiment II included a familiarization session and one testing session. During the testing session, unconditioned and conditioned LEPs were delivered during

the same voluntary contraction. Unconditioned LEP consisted of a single stimulation delivered at the lumbar level. Conditioned LEPs consisted of a paired stimulation of TMS and LS separated by predetermined and randomly ordered time delays (60, 90, 120 and 150 ms). Participants were instructed to contract to, and briefly hold, one of the three different contraction intensities (25, 50 and 75% of MVC) in a randomized order. Once the participant reached the required level, an unconditioned LEP was delivered followed by a conditioned LEP at one of the different time delays (Figure 12). The contractions were held for 5–8 s and stimuli were delivered 2–3 s apart. Sets of five unconditioned and conditioned LEPs were given per time delay and per torque level as a single block, giving a total of 60 unconditioned and conditioned stimuli. To avoid fatigue, 30, 45 and 60 s rest was given between contractions at 25%, 50% and 75% of MVC, respectively, and 60, 120 and 180 s rest was given between the sets of 5 contractions.

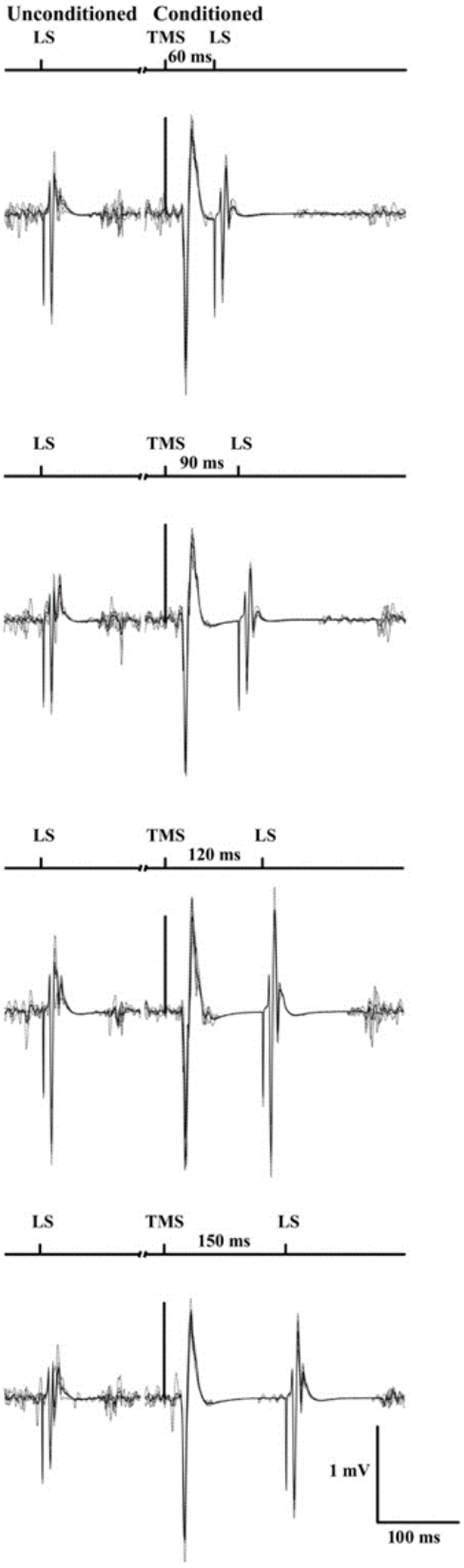

Figure 12 One participant's mean (solid) and individual (dashed) responses from 5 trials that represent one set of unconditioned and conditioned lumbar stimulation at different time delays taken from 25% MVC trials in Study II. *LS* = lumbar stimulation, *TMS* = transcranial magnetic stimulation.

4.3 Bipolar surface electromyography and torque

In all Studies, muscle activity was recorded using adhesive Ag/AgCl electrodes (3 × 2 cm, BlueSensor N, Ambu, Penang, Malaysia) from RF according to SENIAM guidelines (Hermens et al. 1999). Skin was shaved, abraded with sandpaper, and wiped with alcohol before positioning the electrodes in a bipolar arrangement with a 2 cm centre-to-centre distance. Impedance was set < 2 kΩ, and the ground electrode was positioned on the patella. EMG electrode positions were marked with a permanent marker over the skin, photographs were taken and the distance from the iliac crest to the middle of the electrode pair was recorded (Study I and III). Additionally, during the training intervention (Study III), the marks were redrawn by the research assistant after every training session. EMG data were sampled online at 3000 Hz, amplified (1000×) and bandpass filtered (16–1000 Hz; Neurolog System, Digitimer Ltd, UK) using CED Power1401-3 (Cambridge Electronic Design Ltd, Cambridge, UK).

4.4 Peripheral nerve stimulation

In all Studies, percutaneous electrical stimulation of the femoral nerve in a resting condition was performed to elicit M-max in RF (1 ms pulse duration; Digitimer DS7AH, Hertfordshire, UK). Electrodes (3.2 cm cathode/anode arrangement; Polar Neurostimulation Electrodes, Espoo, Finland) were placed 2 cm apart and placed at each side of the femoral nerve, located by palpation and identification of the femoral artery (Walker et al. 2016). M-max was elicited by gradually increasing stimulator output intensity until the EMG response plateaued. To ensure supramaximality, 150% stimulation intensity was used (Table 1).

4.5 Lumbar stimulation

Transcutaneous LS was used to elicit LEPs with a constant-current stimulator (1 ms pulse duration; Digitimer DS7AH, Hertfordshire, UK) via self-adhesive electrodes (Polar Neurostimulation Electrodes, Espoo, Finland). The cathode (5×10 cm) was centred over the first lumbar vertebra (L_1) and the anode (circular shape; 3.2 cm diameter) was placed on the midline of the vertebral column ~5 cm above the top edge of the cathode as described by Škarabot et al. (2019b).

Stimulator output was adjusted to elicit LEPs of 25% (Study I and III) or 50% (Study I and II) of M-max in a resting condition. In each testing session, intensities were calculated for all Studies, the order was randomized during Study I and kept constant during Study II and III.

Potential activation of ventral roots was assessed by examining the onset latency of the LEP with an increasing stimulation intensity (Petersen et al. 2002)

until the maximal intensity used in the Study (Figure 13-A) and also tracking LEP amplitude during increasing voluntary contractions (Taylor et al. 2002) (Figure 13-C). Onset latency would be expected to shorten when increasing stimulation intensity and LEP amplitude would have remained consistent during higher contraction intensities should the ventral roots be activated.

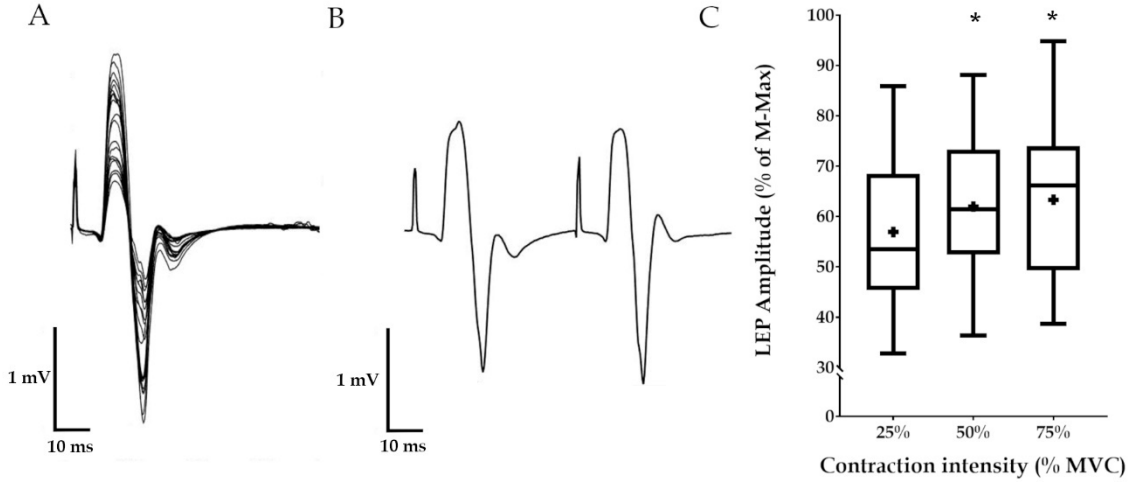

Figure 13 Representation of the three different tests used before the data collection to localize the hotspot of LS. Data extracted from one participant showing that spinal root activation did not occur. (**A)** When increasing the intensity of stimulator output there was no reduction in latency. (**B)** A lumbar stimulated doublet with 50 ms interval showing similar amplitudes between the stimulations. **(C)** Box and whisker plots showing unconditioned LEP responses normalized to M-max at different contraction intensities. The plot displays quartiles and whiskers (minimum and maximum), the median (line in the box) and mean (+ in the box). Increases in LEP amplitude with increases in torque shows that the stimulation was evoked trans-synaptically. *$p < 0.05$

Dorsal root activation was assessed via paired LS with a 50 ms time delay, where the second LEP amplitude was compared to the first. Evidence of dorsal root activation would be a decrease in the second LEP compared to the first due to post-activation depression at the motor-neuron pool from the first stimulus to the second (Hofstoetter et al. 2008; Hofstoetter et al. 2018) (Figure 13-B). If the participant failed any of the tests (i.e., dorsal or ventral stimulation protocols), the electrodes were relocated 1 cm higher, until the participant passed all tests, or the anode was place between the third and fourth thoracic vertebrae. In all Studies, to ensure the placement was the same in all sessions, the distance from the 7th cervical vertebra to the anode (21.7 ± 4.1 cm) and from the bottom of the anode to the top of the cathode (3.7 ± 1.1 cm) (i.e., inter-electrode distance) were taken. All participants reported that they found LS to be tolerable. Once the placement was confirmed, stimulation intensity was set at that which produced a LEP of 25% (low-intensity) or 50% (high-intensity) of the M-max in a resting state, and this stimulation intensity was used throughout the session (Table 1).

4.6 Transcranial magnetic stimulation

Single TMS pulses were delivered using a BiStim² magnetic stimulator operating in single-pulse mode (Magstim Co., Ltd., Whitland, UK) connected to a concave double-cone coil, positioned over the left cortical hemisphere for RF with a posterior-to-anterior current orientation. The hotspot was defined in every session, at rest, as the position eliciting the largest MEP recorded in the RF EMG using the same intensity (i.e., 50–70% stimulator output) producing a visible MEP. The coil position was marked on the scalp, once the hotspot was found, to maintain the same position throughout the protocol.

In Study I and III aMT was determined by increasing stimulator intensity in 5% steps, starting at 30% of the stimulator output until clear MEPs were visible. Thereafter, stimulator intensity was decreased in steps of 1% until 3 out of 5 stimulations elicited MEPs (>100 µV) during 10% of MVC (Rothwell et al. 1999; Temesi et al. 2014; Ziemann et al. 1996). Sets of 10 single TMS stimulations were delivered in a random order for each of the assigned conditions (i.e., 120%, 140% and 160% aMT). In Study II, TMS stimulator output intensity was adjusted to evoke a TMS-SP of ~200 ms, assigned as the stimulator artefact to the resumption of the voluntary EMG signal, during brief voluntary contractions at each torque (25%, 50% and 75% of MVC) displayed on the screen as visual feedback for the participant.

4.7 Knee extension one-repetition maximum (Study III)

In Study III, all participants performed a bilateral concentric knee extension (David 200, David Health Solutions Ltd, Helsinki, Finland) one-repetition maximum (1-RM) test during the five test periods (Figure 10-B). First, each participant went through anthropometric as well as body composition measurements. Body composition was assessed by bioelectrical impedance (Inbody 770, Inbody Co. Ltd, Seoul, Korea), which provided estimates of skeletal muscle and fat mass. Then, a 5 min cycling (1 kg load at 70 rpm) warm-up was performed followed by a series of submaximal warm-up sets (six repetitions at an estimated 10-RM load, three repetitions at an estimated 6-RM load, one rep at an estimated 3-RM load). Thereafter, single repetitions were performed until the participant could no longer lift the load from the beginning knee angle of ~85° to the required knee angle (≥ 170° knee angle), by visual inspection. The last successfully lifted load was recorded as the participant's 1-RM and used to prescribe the load for the first and fourth week of training. Four-to-eight attempts where needed to calculate 1-RM with 1.25 kg precision. Verbal encouragement was provided to motivate participants to produce a maximal effort. Three minutes rest were provided between attempts. The reliability of this method was excellent (CV = 8.4%; ICC = 0.991).

4.8 Resistance training intervention (Study III)

In Study III, over the course of the seven-week resistance training intervention, participants engaged in a total of thirteen supervised sessions of conventional resistance training, in which participants were allowed to miss only one training session. Mid-training testing was conducted after seven training sessions. Training sessions were conducted twice-a-week, with at least a 48-hour break between sessions. The resistance training program was created following the guidelines provided by Fragala et al. (2019), and this training program closely resembles the most potent program for older adults identified in a meta-analysis (Borde et al. 2015). The training program may be considered as whole-body, targeting both upper- and lower-limbs, although it is acknowledged that there were no dedicated abdominal or lower back exercises. Nevertheless, one or two exercises per muscle group were performed with a total volume of 8 sets per muscle group for the lower-limbs and back/biceps and 3 sets for chest/triceps (Fragala et al. 2019). All training sessions started with a warm-up, which consisted of 5 min of cycling and dynamic mobility exercises. Each training session consisted of five different exercises for the upper- and lower-limbs: leg press, knee extension, bicep curl, smith-machine bench press and chest-supported seated row in that order during normal training sessions. These training sessions consisted of five (knee extension and bicep curl) and three sets (leg press, smith-machine bench press and chest-supported seated row) of 8–10 repetitions at 75–80% of 1-RM. The participants were asked to perform a 2 s controlled eccentric phase, with no isometric phase and fast concentric phase. During the last set of the last session of the week, participants performed the maximum number of repetitions for each exercise to adjust either the volume or intensity (according to the estimated %RM) for the following week, so they could perform at least 8 repetitions.

During the initial training session, knee extension 1-RM testing was conducted. Subsequently, a 3–5 RM test was performed for the remaining exercises to determine the correct training load. During these 1-RM testing sessions (Pre, Mid, Post), the order was: knee extension, leg press, smith-machine bench press, bicep curl.

A four-week detraining period followed the resistance training intervention. Participants were allowed to maintain their normal aerobic physical activity (i.e., cycling, walking, running) during the whole intervention, but resistance training was terminated during the detraining period.

4.9 Data and statistical analysis

4.9.1 Data analysis

In all Studies, offline analyses were performed with Spike software (version 6.10, Cambridge Electronic Design, Cambridge, UK) to manually obtain M-max amplitude, MVC, TMS-SP and unconditioned LEP onset latencies. The other outcome measures were analysed by a customized MATLAB script (version R2020b, The MathWorks, Inc., Natick, USA). Peak-to-peak amplitude of LEPs and MEPs were analysed automatically between latencies-of-interest following peripheral nerve stimulation, LS or TMS (Taylor et al. 1999), respectively. Torque was averaged over the 100 ms before the stimulator artefact. TMS-SP duration was determined, through visual inspection, as the time from the stimulator artefact to the return of voluntary EMG (Damron et al. 2008). MEP (Study I and III) and LEP (Studies I-II) amplitudes were represented relative to M-max (Single N). In Study I, the MEP and LEP amplitudes were also represented relative to M-max and then voluntary root mean square (RMS) (Double N). Double N is typically performed to avoid the possibility that the background EMG level might modify the MEP or LEP amplitude (Sidhu et al. 2009; Škarabot et al. 2019a)

4.9.2 Statistical analysis

In all Studies, SPSS software (version 26.0, SPSS Inc., Chicago, USA) was used for all statistical methods. Means and standard deviation (SD) were calculated and reported throughout. Normality of the data was tested with the Shapiro–Wilk test and confirmed by z-score with an acceptance of +2 to -2 (e.g., skewness score/skewness score$_{SE}$ and kurtosis score/kurtosis score$_{SE}$) and Q-plots for visualization. Data that did not fulfil those requirements were Log_{10} transformed, which then fulfilled the requirements for normality.

In Study I, paired t-tests were used to examine differences between mean trials of Single N and Double N for MEP and LEP amplitude and TMS-SP and LS-SP. A two-way repeated measure ANOVA (3 aMT × 2 Contraction) was used to determine the effect of different stimulator outputs (%aMT) and contraction intensities (%MVC). Relative reliability, as the degree to which individuals maintain their position in a sample with repeated measurements, of TMS and LS variables were assessed using ICC. Absolute reliability, as the degree to which repeated measurements vary within individuals, was assessed using typical error (TE), coefficient of variance (CV) and standard error of the measurement (SEM) calculated as: averaged SD of test 1 and test 2 x √(1-ICC), (Portney, 2020) expressed in ratio (Single N or Double N) or time (SP) for MEPs and LEPs (Atkinson and Nevill, 1998; Portney, 2020). The minimal detectable change (MDC) was calculated as SEM x 1.96 x √2. Reliability, based on ICCs and their 95% CIs, was categorized as poor (ICC < 0.5), moderate (ICC: > 0.5 – < 0.75), good (ICC: > 0.75 – < 0.9) and excellent (ICC: > 0.9) (Koo and Li, 2016). Bland-Altman plots of

LEPs and MEPs in all conditions were used to assess the agreement and bias between the two sessions.

In Study II, paired t-tests were used to assess possible effects of fatigue between M-maxpre and M-maxpost, MVCpre and MVCpost, and to evaluate unconditioned LEP amplitude at different torque levels in the control measurements. One-way analysis of variance (ANOVA) was used to assess potential differences between the three contraction intensities in control measures: Unconditioned LEP latencies, MEP amplitude and TMS-SP. To determine whether normalized [Conditioned/Unconditioned LEP*100] LEPs responded differently at the tested time delays between the three different torque levels, two-way repeated measures ANOVA was employed (4 Time × 3 Contraction). When sphericity assumptions were violated, Greenhouse-Geisser corrections were used. Post hoc Bonferroni adjustments were used when significant main effects were found. When comparing Unconditioned and Conditioned LEP at each time delay, the Benjamin-Hochberg test corrected for multiple paired t-test comparisons with a 10% false discovery rate was used.

In Study III, a two-way repeated measures ANOVA (5 Time × 2 Group) was employed to assess most outcome variables (MVC, 1-RM, skeletal muscle mass, M-max, aMT, and silent periods of LEPs at 25% of the M-max and MEPs at 120%, 140%, 160% aMT) during contractions at 20% and 60% of MVC. When assumptions of sphericity were violated, Greenhouse-Geisser corrections were used. Post hoc Bonferroni adjustments were used when significant main effects were found. To investigate the influence of resistance training on the TMS- and LS-evoked MEP/LEP amplitude, and to accommodate for missing data points and baseline variability, a Linear Mixed Model (LMM) was employed (Wilkinson et al., 2023). This model served as a robust framework for analysing the data considering both fixed and random effects simultaneously. Cortico-spinal (MEPs at 120%, 140%, 160% aMT) and spinal (LEPs at 25% of the M-max) excitability at 20% and 60% of MVC were assessed using the LMM. The model included time (Con, Pre, Mid, Post, and De) and age group (young and older) as main effects and an interaction between age group (young and older) and time with participants as the random effect within the model. Bonferroni adjustments were used when significant main effects were found.

Data are presented in the tables and figures as mean and SD, and in the Results section by mean difference (MD). Effect sizes are represented as partial eta-squared values (η_p^2 = small: 0.01, medium: 0.06, large: 0.14) for the factors of the ANOVA and post hoc or paired t-test effect sizes are reported as Hedge's g (g = small: < 0.3, medium: 0.3–0.8, large: > 0.8). Alpha was set at 0.05.

5 RESULTS

5.1 Validity and reliability of cortico-spinal measurements (Study I and II)

5.1.1 Validity of spinal stimulation hotspot (Study I and II)

In Study I, there was no statistically significant differences between session 1 and 2 in LEP latencies at low- ($p = 0.685$, 95% CI [-3.5, 5.2], Hedges' $g < 0.01$) or high-intensity ($p = 0.647$, 95% CI [-3.5, 5.5], Hedges' $g = 0.06$). In Study II, there was a statistically significant difference between unconditioned LEP amplitude during 25% vs 50% of MVC ($p < 0.001$, 95% CI [-1.74, 15.25], Hedges' $g = -0.26$) and 25% vs 75% ($p = 0.001$, 95% CI [-0.21, -0.06], Hedges' $g = -0.27$) of MVC, although no statistical difference was found between 50% of MVC and 75% of MVC ($p = 0.956$, 95% CI [-0.05, 0.05], Hedges' $g = -0.01$) (Figure 13-C). These findings indicate that LS activated the cortico-spinal tract.

5.1.2 Reliability of MEP (Study I)

MEP amplitudes, elicited across different contraction intensities, remained consistent between test 1 and 2. Regardless the normalization method or the muscle contraction intensity, there were not statistically significant differences ($p > 0.05$). The data represented in Table 2 demonstrates the consistency in MEP responses independent of whether Single N or Double N was applied and the contraction intensity used.

Table 2 Mean and SD, 95% confidence intervals, effect sizes and results of paired t-test analyses for Single N and Double N MEP amplitudes comparisons between test sessions 1 and 2.

	Test 1	SD	Test 2	SD	p-value	95% CI	Hedges' *g*
20% of MVC							
120% aMT							
Single N (MEP/M-max)	0.366	0.223	0.389	0.48	0.421	[-0.08–0.03]	-0.06
Double N (MEP/M-max/RMS)	19.0	19.4	18.1	10.5	0.656	[-3.4–5.3]	0.06
140% aMT							
Single N (MEP/M-max)	0.472	0.195	0.479	0.224	0.812	[-0.06–0.05]	-0.03
Double N (MEP/M-max/RMS)	29.6	30.2	27.0	23.3	0.405	[-3.7–8.7]	0.09
160% aMT							
Single N (MEP/M-max)	0.509	0.224	0.519	0.240	0.742	[-0.07–0.05]	-0.04
Double N (MEP/M-max/RMS)	28.0	25.2	26.9	21.0	0.653	[-3.9–6.2]	0.05
60% of MVC							
120% aMT							
Single N (MEP/M-max)	0.540	0.317	0.558	0.351	0.246	[-0.01–0.03]	-0.05
Double N (MEP/M-max/RMS)	9.4	9.3	8.8	7.3	0.520	[-1.4–2.7]	0.08
140% aMT							
Single N (MEP/M-max)	0.552	0.321	0.582	0.366	0.399	[-0.10–0.04]	-0.09
Double N (MEP/M-max/RMS)	9.3	8.3	10.0	8.8	0.442	[-2.3–1.1]	-0.07
160% aMT							
Single N (MEP/M-max)	0.587	0.368	0.597	0.372	0.723	[-0.07–0.05]	-0.03
Double N (MEP/M-max/RMS)	10.0	9.1	9.8	8.2	0.810	[-1.7–2.1]	0.03

SD = standard deviation, CI = confidence interval, MVC = maximal voluntary contraction, Single N = single normalization, Double N = double normalization, M-max = maximal compound action potential, RMS= root mean square, aMT = active motor threshold, MEP= motor-evoked potential.

5.1.2.1 Low-level contraction intensity

Good reliability was found in Single N and Double N for all the TMS conditions (0.800 < ICC < 0.900) at 20% of MVC (Table 3). CVs for Single N was between 20–26% during 20% of MVC, whereas Double N was 29–35%. SEM for Single N was between 0.09–0.13 and MDC was between 0.24–0.36. Double N SEM was between 6–9 and MDC was between 6–25. Bland-Altman plots showed good agreement between test 1 and 2, with a low ratio (MEP/M-max) for the mean bias (-0.010) and data within the 95% limits of agreement (Figure 14-A and 14-B).

Table 3 Between-session test–retest reliability for Single *N* and Double *N* MEP amplitudes with ICC, TE, SEM, and MDC at low-level contraction intensities.

	TE [95%CI]		CV% [95% CI]		ICC [95% CI]		SEM	MDC
120% aMT								
Single N (MEP/M-max)	0.09	[0.07–0.13]	26.1	[19.5–39.3]	0.861	[0.69–0.94]	0.13	0.36
Double N (MEP/M-max/RMS)	7.0	[5.4–10.0]	34.8	[25.9–53.3]	0.816	[0.60–0.92]	6.40	17.74
140% aMT								
Single N (MEP/M-max)	0.09	[0.07–0.13]	20.6	[15.5–30.7]	0.831	[0.63–0.93]	0.09	0.24
Double N (MEP/M-max/RMS)	9.9	[7.6–14.2]	29.2	[21.8–44.3]	0.891	[0.75–0.95]	8.83	24.47
160% aMT								
Single N (MEP/M-max)	0.10	[0.08–0.14]	24.0	[17.9–35.9]	0.821	[0.61–0.92]	0.10	0.27
Double N (MEP/M-max/RMS)	8.1	[6.2–11.5]	29.9	[22.3–45.3]	0.851	[0.67–0.94]	8.93	24.74

TE= typical error, CI= confidence interval., CV=coefficient of variance, ICC= intra-class correlation, SEM: standard error of the measurement, MDC= minimal detectable change, MVC= maximal voluntary contraction, M-max = maximal compound action potential, RMS= root mean square, TMS = transcranial magnetic stimulation, aMT = active motor threshold, Single N = single normalization, Double N = double normalization, MEP= motor-evoked potential

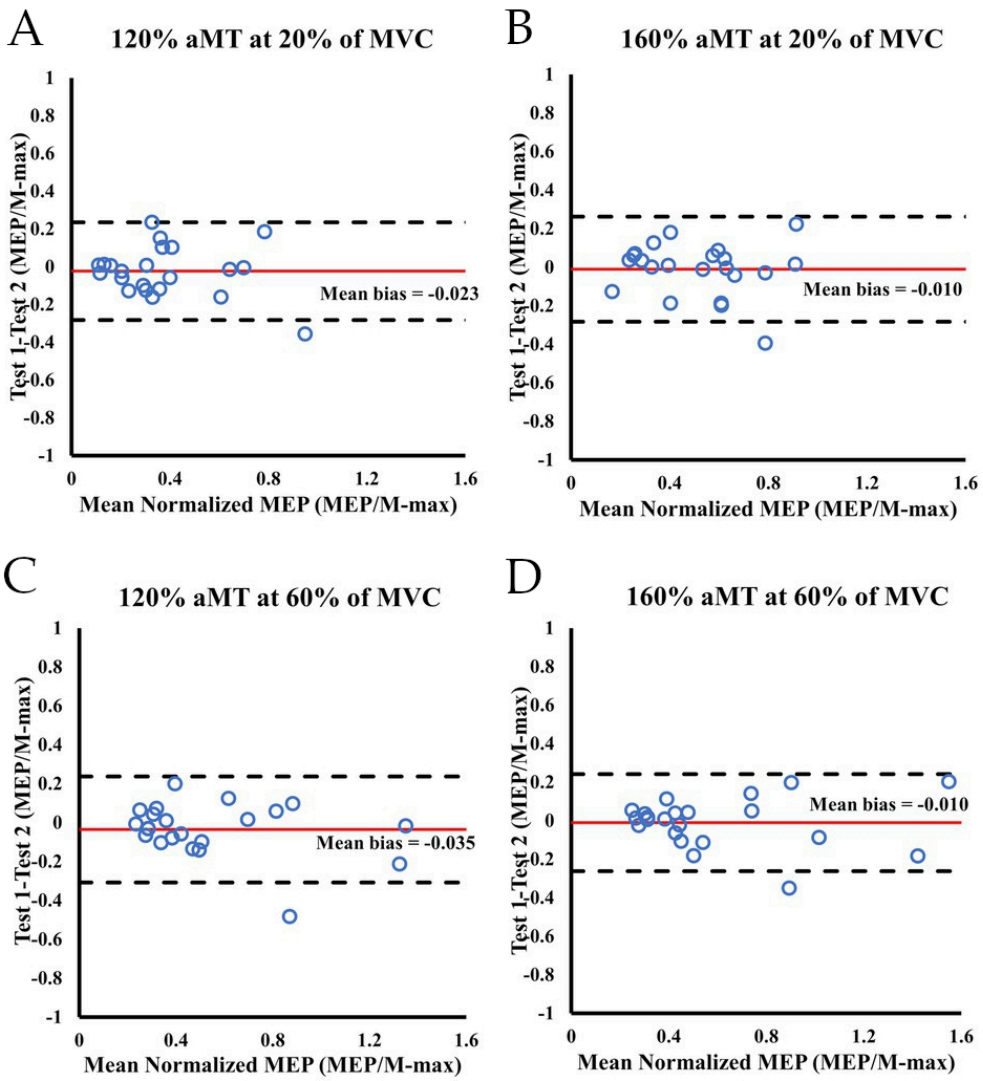

Figure 14 Bland Altman plots showing the level of agreement for MEP amplitude **(A–D)** during 20 and 60% of MVC between test sessions 1 and 2. Each panel shows the differences as a function of the average of the two testing sessions indicating the mean bias (solid line) and the 95% limits of agreement (dashed lines).

5.1.2.2 High-level contraction intensity

Excellent reliability was found in Single N for all the TMS conditions (ICC > 0.900) and at 140% aMT for Double N (ICC = 0.926) at 60% of MVC. CVs for Single N was between 14–18% during 60% of MVC, whereas Double N was 22–38%. SEM for Single N was between 0.09–0.11 and MDC was between 0.25–0.29. Double N SEM was 3 and MDC was between 6–8. Bland-Altman plots showed good agreement between test 1 and 2, with a low ratio (MEP/M-max) for the mean bias (-0.010) and data within the 95% limits of agreement (Figure 14-C and 14-D).

Table 4 Between-session test–retest reliability for Single *N* and Double *N* MEP amplitudes with ICC, TE, SEM, and MDC at high-level contraction intensities

	TE [95%CI]		CV% [95% CI]		ICC [95% CI]		SEM	MDC
120% aMT								
Single N (MEP/M-max)	0.10	[0.08–0.14]	18.4	[13.9–27.4]	0.901	[0.77–0.96]	0.11	0.29
Double N (MEP/M-max/RMS)	3.3	[2.5–4.7]	28.0	[20.9–42.2]	0.896	[0.76–0.96]	2.67	7.40
140% aMT								
Single N (MEP/M-max)	0.11	[0.09–0.16]	15.5	[11.7–22.9]	0.922	[0.82–0.97]	0.10	0.27
Double N (MEP/M-max/RMS)	2.7	[2.1–3.9]	22.5	[16.9–33.7]	0.926	[0.83–0.97]	2.33	6.45
160% aMT								
Single N (MEP/M-max)	0.09	[0.70–0.13]	14.0	[10.6–20.6]	0.941	[0.86–0.98]	0.09	0.25
Double N (MEP/M-max/RMS)	3.0	[2.3–4.3]	27.1	[20.3–40.9]	0.898	[0.77–0.96]	2.76	7.65

TE= typical error, CI= confidence interval., CV=coefficient of variance, ICC= intra-class correlation, SEM: standard error of the measurement, MDC= minimal detectable change, aMT = active motor threshold, Single N = single normalization, Double N = double normalization, MEP= motor-evoked potential, M-max = maximal compound action potential, RMS= root mean square

5.1.3 Reliability of LEP (Study I)

LEP amplitudes, elicited across different contraction intensities, remained consistent between test 1 and 2. Regardless the normalization method or the muscle contraction intensity, there were not statistically significant differences ($p > 0.05$). The data represented in Table 5 demonstrates the consistency in LEP responses independent of whether Single N or Double N normalization was applied, and the contraction intensity used.

Table 5 Mean and SD, 95% confidence intervals, effect sizes and results of paired *t*-test analyses for Single *N* and Double *N* LEP amplitudes between test sessions 1 and 2.

	Test 1	SD	Test 2	SD	p-value	95% CI	Hedges' *g*
20% of MVC							
Low-intensity							
Single N (LEP/M-max)	0.352	0.106	0.354	0.135	0.950	[-0.06–0.05]	-0.02
Double N (LEP/M-max/RMS)	19.1	13.7	17.8	13.1	0.451	[-2.1-–4.6]	0.09
High-intensity							
Single N (LEP/M-max)	0.597	0.219	0.566	0.226	0.483	[-0.06–0.12]	0.14
Double N (LEP/M-max/RMS)	29.0	22.5	29.0	27.2	0.986	[-4.2–4.3]	0.00
60% of MVC							
Low-intensity							
Single N (LEP/M-max)	0.510	0.184	0.454	0.189	0.115	[-0.01–0.10]	0.29
Double N (LEP/M-max/RMS)	7.9	6.2	7.0	4.8	0.110	[-0.3–2.6]	0.15
High-intensity							
Single N (LEP/M-max)	0.717	0.26	0.655	0.311	0.193	[-0.03–0.14]	0.21
Double N (LEP/M-max/RMS)	11.4	9.2	10.1	6.7	0.300	[-1.8–5.5]	0.16

SD = standard deviation, CI = confidence interval, LS = lumbar stimulation, LEP= Lumbar evoked potential, Single N = single normalization, Double N = double normalization, M-max = maximal compound action potential, RMS= root mean square.

5.1.3.1 Low-level contraction (Study I)

All reliability values for LEP amplitude at 20% of MVC can be found in Table 6. Good reliability was found for Double N for high-intensity elicited LEPs during 20% of MVC (ICC = 0.847), while moderate reliability was found for the rest of the conditions (Table 6). CVs for Single N was 23% and 38% for Double N for lower intensities, whereas higher intensities showed a CV of 33% for Single N and 30% for Double N. SEM for Single N was between 0.07 and 0.15 and MDC was between 0.20 -0.43 for low and high-intensity, respectively. SEM for Double N was between 7 and 10 and MDC was between 19 and 27, for low and high-intensity, respectively. Low-intensity stimulation during 20% of MVC showed a mean bias of -0.002 and 95% limits of agreement [-0.24, 0.24] (Figure 15)

Table 6 Between-session test–retest reliability for Single *N* and Double *N* LEP amplitudes with ICC, TE, SEM, and MDC at low-level contraction intensity.

	TE [95%CI]		CV% [95% CI]		ICC [95% CI]		SEM	MDC
Low-intensity								
Single N (LEP/M-max)	0.09	[0.07–0.12]	23.0	[17.3–34.5]	0.632	[0.29–0.83]	0.07	0.20
Double N (LEP/M-max/RMS)	5.2	[4.1–7.7]	38.5	[28.5–59.3]	0.737	[0.46–0.88]	6.86	19.02
High-intensity								
Single N (LEP/M-max)	0.13	[0.10–0.20]	33.4	[24.3–53.2]	0.520	[0.09–0.79]	0.15	0.43
Double N (LEP/M-max/RMS)	6.2	[4.7–9.3]	30.0	[22.0–47.5]	0.847	[0.64–0.94]	9.71	26.90

TE= typical error, CI= confidence interval., CV=coefficient of variance, ICC= intra-class co relation, SEM: standard error of the measurement, MDC= minimal detectable change, MVC= maximal voluntary contraction, LS = lumbar stimulation, LEP = lumbar evoked potential, , Single N = single normalization, Double N = double normalization, M-max = maximal compound action potential, RMS= root mean square

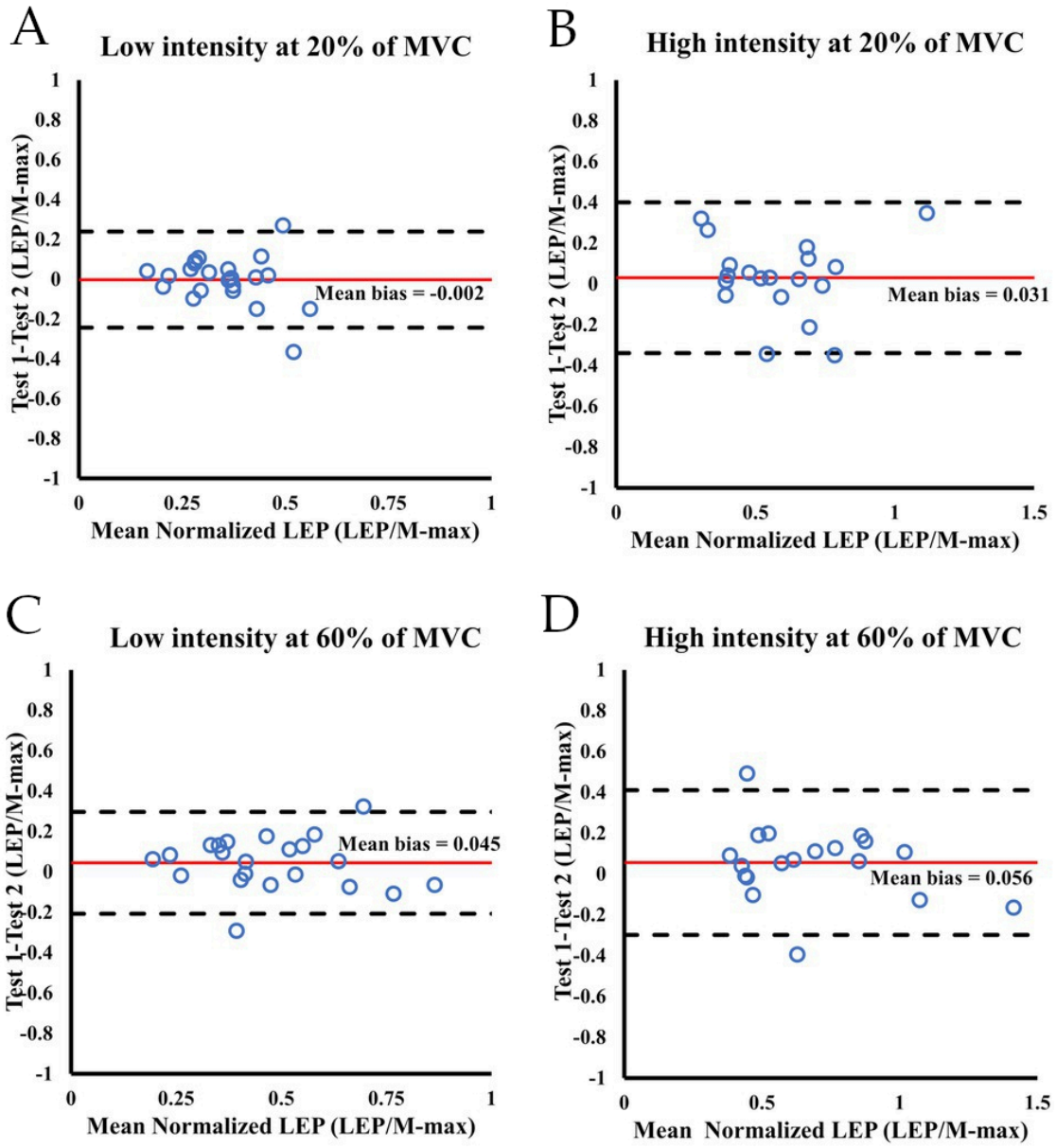

Figure 15 Bland Altman plots showing the level of agreement for LEP **(A–D)** amplitude during 20 and 60% of MVC between test sessions 1 and 2. Each panel shows the differences as a function of the average of the two testing sessions indicating the mean bias (solid line) and the 95% limits of agreement (dashed lines).

5.1.3.2 High-level contraction intensity

All reliability values for LEP amplitude at 60% of MVC can be found in Table 7. Good reliability was found in Double N for low-intensity elicited LEPs during 60% of MVC (ICC = 0.828), while moderate reliability was found for the rest of the conditions (Table 7). CVs for Single N was 23% and 32% for Double N for lower intensities, whereas higher intensities showed a CV of 29% for Single N and 40 for Double N. SEM for Single N was between 0.09–0.17 and MDC was between 0.26–0.47 for low and high-intensity, respectively. SEM for Double N was between 2–4 and MDC was between 6–11, for low and high-intensity, respectively. Bland-Altman plots showed a good agreement between test 1 and

test 2 for all LS conditions (Figure 15-C and Figure15-D). Low-intensity stimulation during 60% of MVC showed a mean bias of -0.035 and data within 95% limits of agreement (Figure 15-C and Figure15-D).

Table 7 Between-session test–retest reliability for Single *N* and Double *N* LEP amplitudes with ICC, TE, SEM, and MDC at low contraction intensities.

	TE [95%CI]		CV% [95% CI]		ICC [95% CI]		SEM	MDC
Low-intensity								
Single N (MEP/M-max)	0.09	[0.07–0.13]	22.8	[17.1–34.1]	0.749	[0.48–0.89]	0.09	0.26
Double N (MEP/M-max/RMS)	2.3	[1.8–3.3]	31.9	[23.8–48.5]	0.828	[0.62–0.93]	2.27	6.30
High-intensity								
Single N (MEP/M-max)	0.13	[0.10–0.19]	28.8	[21.1–45.4]	0.643	[0.24–0.82]	0.17	0.47
Double N (MEP/M-max/RMS)	5.4	[4.1–7.9]	39.5	[28.6–63.7]	0.742	[0.43–0.90]	4.04	11.20

TE= typical error, CI= confidence interval., CV=coefficient of variance, ICC= intra-class co relation, SEM: standard error of the measurement, MDC= minimal detectable change, MVC= maximal voluntary contraction, LS = lumbar stimulation, LEP = lumbar evoked potential, , Single N = single normalization, Double N = double normalization, M-max = maximal compound action potential, RMS= root mean square

5.1.4 Reliability of TMS-SP and LS-SP (Study I)

TMS-SP showed a statistically significant difference at 120% aMT during 20% of MVC ($p = 0.031$) between test 1 and test 2, although the effect size was small (Hedges' $g = -0.28$). No other condition showed any significant differences (Table 8).

Table 8 Mean and SD, 95% confidence intervals, effect sizes and results of paired *t*-test analyses for TMS-SP and LS-SP length between test sessions 1 and 2.

	Test 1	SD	Test 2	SD	p-value	95% CI	Hedges' *g*
20% of MVC							
TMS-SP (ms)							
120% aMT	107	19	112	16	0.031	[-9–-1]	-0.28
140% aMT	127	27	131	27	0.106	[-11– 1]	-0.15
160% aMT	143	31	144	28	0.468	[-7–3]	-0.03
LS-SP (ms)							
Low-intensity	79	12	77	12	0.255	[-2–6]	0.16
High-intensity	87	15	86	12	0.618	[-3–5]	0.07
60% of MVC							
TMS-SP (ms)							
120% aMT	106	22	107	17	0.463	[-7–3]	-0.05
140% aMT	122	23	124	19	0.245	[-8–2]	-0.09
160% aMT	140	35	145	34	0.081	[-10–1]	-0.14
LS-SP (ms)							
Low-intensity	69	14	67	16	0.348	[-2–7]	0.13
High-intensity	68	10	67	9	0.528	[-2–4]	0.10

SD = standard deviation, CI = confidence interval, TMS-SP= Transcranial magnetic stimulation-evoked silent period, aMT = active motor threshold, LS-SP = lumbar stimulation-evoked silent period, ms = millisecond.

During a low-level contraction intensity, reliability of TMS-SP and LS-SP at 20% MVC can be found in Table 9. Excellent reliability was found for TMS-SP at 160% aMT during 20% (ICC = 0.920) and good reliability for the other TMS conditions. While good reliability was observed for high-intensity LS-SP, low-intensity LS-SP showed moderate reliability. CV for TMS-SP was between 5–8% and CV was 7% for LS-SP. SEM for TMS-SP was between 7–11 and MDCs were 21–30. SEM for LS-SP was 6 and MDC was between 15–18. Bland-Altman plots showed good agreement between test 1 and test 2 regardless of the stimulation method, intensity (TMS-SP: Figure 16 and LS-SP: Figure 17). TMS-SP 160% aMT and LS-SP at high-intensity during 20% of MVC showed a mean bias of -1.76 ms. In addition, LS-SP high-intensity during 20% of MVC showed a mean bias of -0.86 ms. Data was within 95% limits of agreement.

Table 9 Between-session test–retest reliability for TMS-SP and LS-SP length with ICC, TE, SEM, and MDC at low-level contraction intensity.

	TE [95%CI]		CV% [95% CI]		ICC [95% CI]		SEM	MDC
TMS-SP (ms)								
120% aMT	7.2	[5.5–10.3]	7.2	[5.5–10.4]	0.820	[0.61–0.92]	7.42	20.58
140% aMT	9.3	[7.2–13.3]	7.9	[6.0–11.5]	0.840	[0.64–0.93]	10.80	29.94
160% aMT	7.7	[6.0–11.1]	5.5	[4.2–8.0]	0.920	[0.81–0.97]	8.34	23.13
LS-SP (ms)								
Low-intensity	5.9	[4.6–8.5]	7.5	[5.7–10.9]	0.713	[0.42–0.87]	6.43	17.82
High-intensity	5.4	[4.1–8.1]	6.7	[5.0–10.0]	0.830	[0.60–0.93]	5.57	15.43

CI = confidence interval., CV = coefficient of variance, ICC = intra-class correlation, SEM: standard error of the measurement, MDC = minimal detectable change, MVC = maximal voluntary contraction, TMS-SP = Transcranial magnetic stimulation-evoked silent period, aMT = active motor threshold, LS-SP = lumbar stimulation-evoked silent period, ms = millisecond.

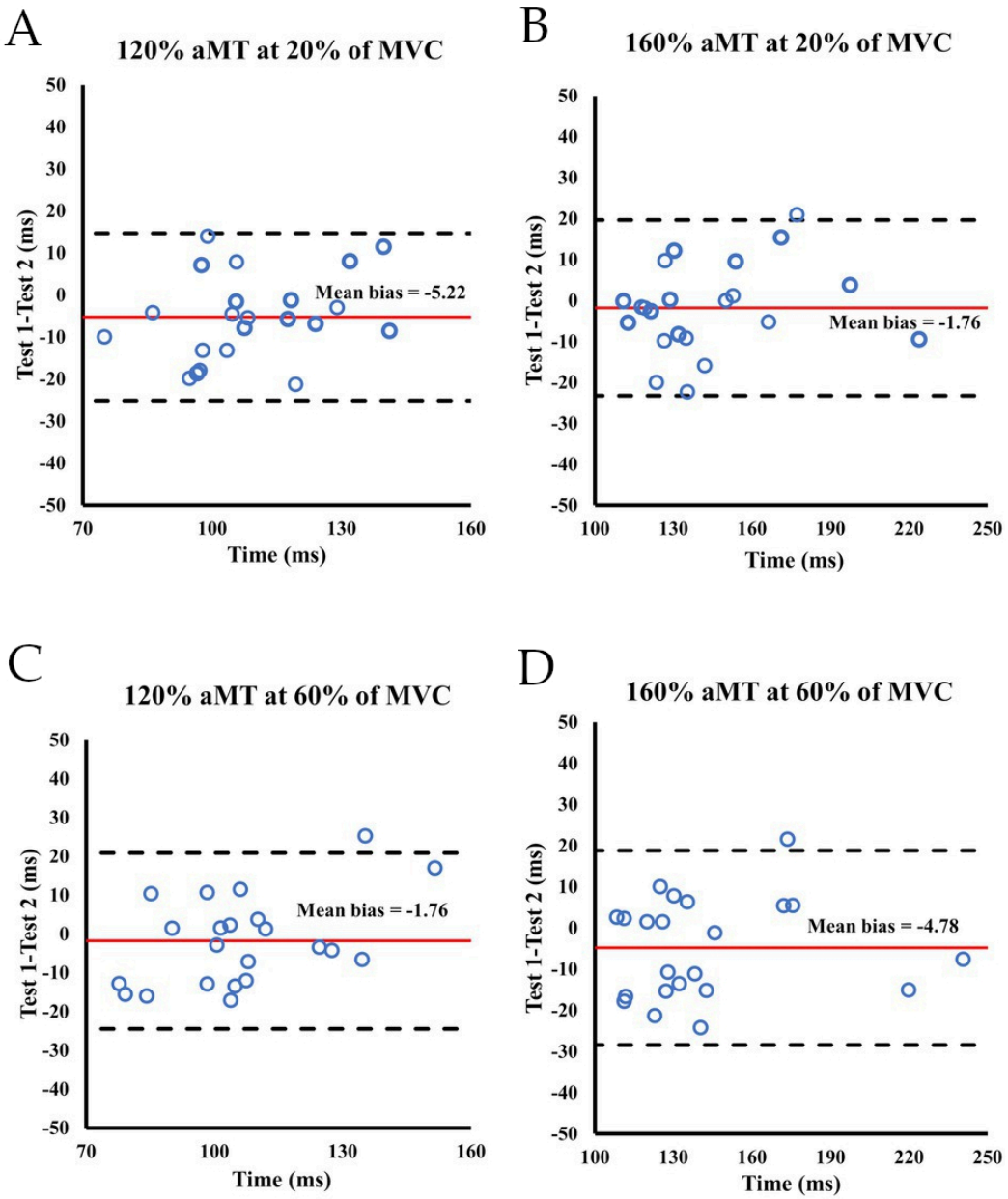

Figure 16 Bland Altman plots showing the level of agreement for TMS-SP **(A–D)** during 20 and 60% of MVC between test sessions 1 and test 2. Each panel shows the differences as a function of the average of the two testing sessions indicating the mean bias (solid line) and the 95% limits of agreement (dashed lines).

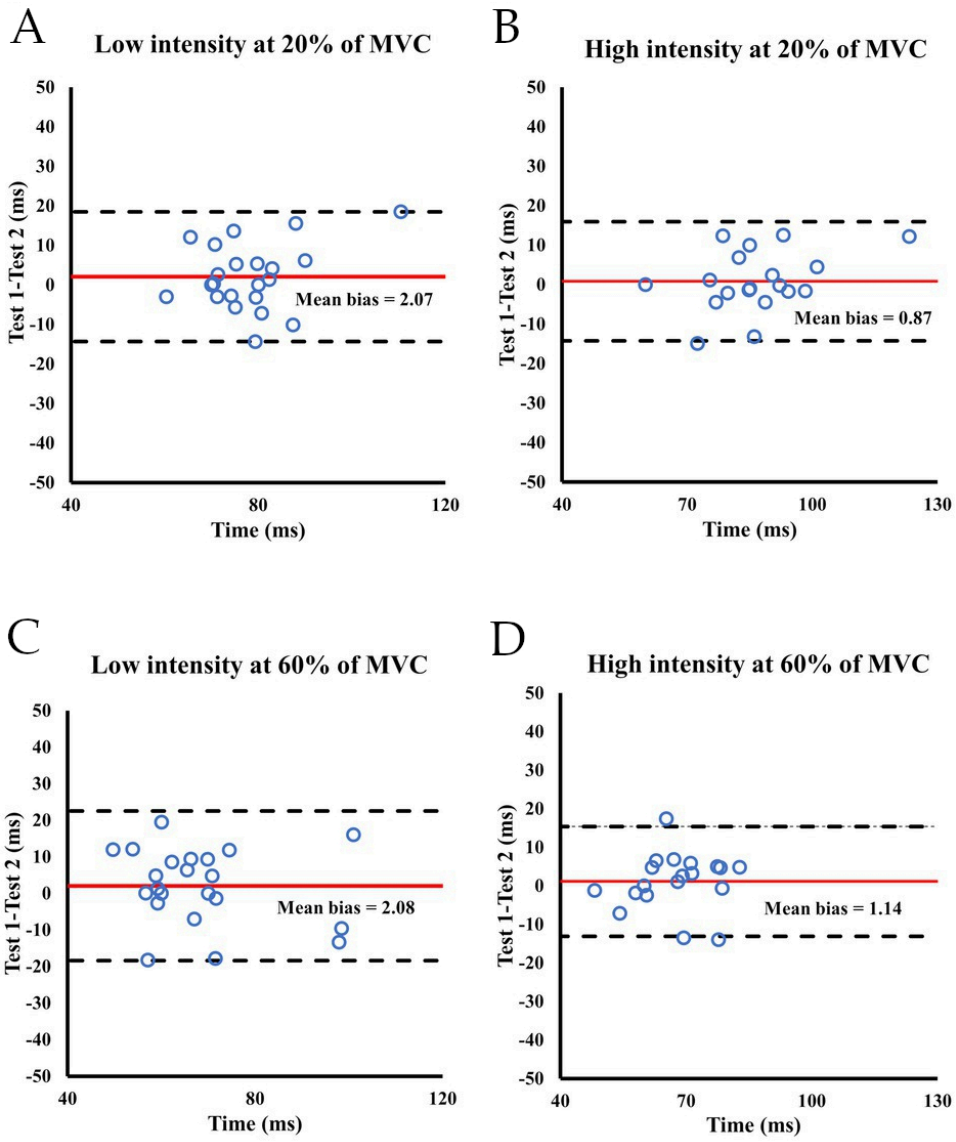

Figure 17 Bland Altman plots showing the level of agreement for LS-SP **(A–D)** during 20 and 60% of MVC between test sessions 1 and test 2. Each panel shows the differences as a function of the average of the two testing sessions indicating the mean bias (solid line) and the 95% limits of agreement (dashed lines).

Reliability of TMS-SP and LS-SP at 20% and 60% of MVC can be found in Table 10. Excellent reliability was found for TMS-SP elicited by TMS at 160% aMT during 60% of MVC (ICC = 0.920). Good reliability was found for high-intensity LS-SP and the other TMS conditions. CV for TMS-SP was between 6–8% and CV was between 8–12% for LS. SEM for TMS-SP was between 8–10 ms and MDC was between 23–27 ms. SEMs for LS-SP were between 5–8 ms and MDCs were 13–22 ms. Bland-Altman plots showed good agreement between test 1 and test 2 regardless of the stimulation method, intensity, or contraction intensity (TMS-SP: Figure 16 and LS-SP: Figure 17). TMS-SP at 120% and high-intensity LS-SP during 60% of MVC showed a mean bias of -1.76 ms and -1.14 ms. Data was within 95% limits of agreement.

Table 10 Between-session test–retest reliability for TMS-SP and LS-SP length with ICC, TE, SEM, and MDC at high-level contraction intensity.

	TE [95%CI]		CV% [95% CI]		ICC [95% CI]		SEM	MDC
TMS-SP								
120% aMT	8.2	[6.3–11.7]	8.2	[6.2–11.9]	0.820	[0.60–0.92]	8.27	22.93
140% aMT	7.9	[6.1–11.3]	7.0	[5.3–10.1]	0.835	[0.64–0.93]	8.53	23.64
160% aMT	8.5	[6.6–12.2]	6.5	[4.9–9.4]	0.920	[0.81–0.97]	9.76	27.05
LS-SP								
Low-intensity	7.3	[5.7–10.5]	11.8	[9.0–17.3]	0.710	[0.41–0.87]	8.08	22.39
High-intensity	5.1	[3.9–7.6]	7.9	[5.9–11.9]	0.750	[0.44–0.90]	4.75	13.17

CI = confidence interval., CV = coefficient of variance, ICC = intra-class correlation, SEM: standard error of the measurement, MDC = minimal detectable change, MVC = maximal voluntary contraction, TMS-SP = Transcranial magnetic stimulation-evoked silent period, aMT = active motor threshold, LS-SP = lumbar stimulation-evoked silent period, ms = millisecond.

5.2 Corticospinal excitability (Study I and III)

5.2.1 Effect of contraction intensity (Study I)

There was an effect for TMS intensity ($F_{(1.3,55.8)} = 4.93$, $p = 0.021$, $\eta_p^2 = 0.105$), but there were no aMT*Contraction ($p = 0.237$) nor contraction effects ($p = 0.186$). Post hoc comparisons showed that MEP amplitude at 120% aMT were smaller than 160% aMT at 20% of MVC (Figure 18).

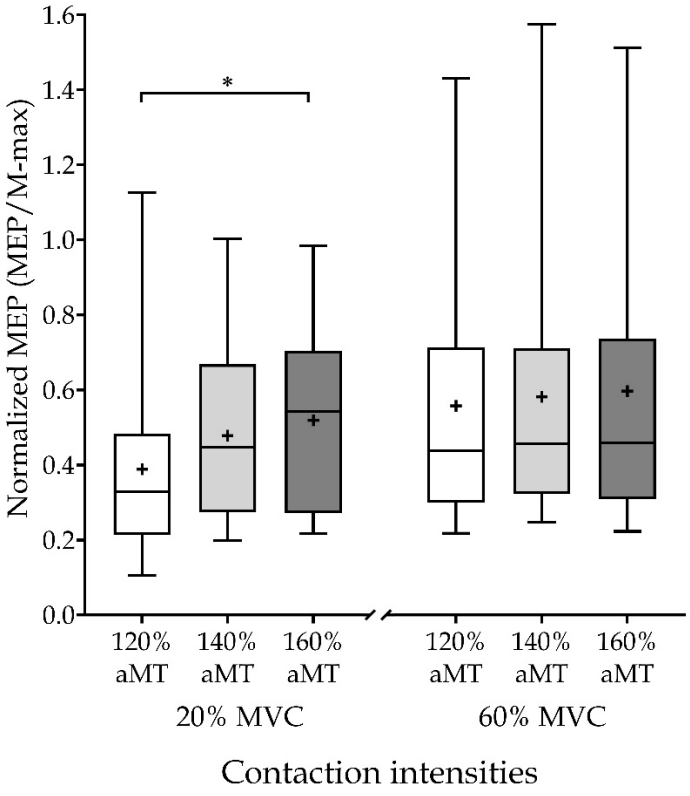

Figure 18 Box and whiskers plots showing the comparisons of aMT at different contraction intensities. Each figure shows quartiles and whiskers (minimum and maximum), the median (line in the box), mean (+ in the box) for each condition (120% aMT: blank box; 140%aMT: filled light grey box; 160% aMT: filled dark grey box filled) and session. *$p < 0.05$

5.2.2 Effect of age (Study III)

Main effects for Group were observed for aMT ($F_{(1,19)} = 11.75$, $p = 0.038$), MEP amplitude during 60% of MVC with 120% aMT ($F_{(1,19)} = 4.65$ $p = 0.044$) and 140% aMT ($F_{(1,19)} = 4.62$ $p = 0.045$). During the first measurement session (i.e., control), young adults had a lower aMT (Table 1). Further, during control, MEP amplitude at 120% and 140% aMT was greater in the older group during 60% of MVC (Figure 19-D and 19-E).

5.2.3 Effects of training (Study III)

There was no main effect for Time or Time*Group for aMT. However, significant main effects for Time and Time*Group interaction were observed for MEP amplitude during 20% of MVC at 120% aMT (Time: $F_{(4,1021)} = 3.09$, $p = 0.015$; Time*Group: $F_{(4,1021)} = 4.10$, $p = 0.003$), 140% aMT (Time: $F_{(4,1021)} = 4.89$, $p = 0.001$; Time*Group: $F_{(4,1021)} = 14.44$, $p < 0.001$), and 160% aMT (Time: $F_{(4,1021)} = 8.12$, $p < 0.001$; Time*Group: $F_{(4,1021)} = 4.10$, $p = 0.003$). In the young adults, significant increases occurred Pre to Post at 140% aMT ($p = 0.023$) and Pre to Mid at 160%

aMT ($p = 0.005$). In older adults, significant decreases were observed Pre to Post at 140% aMT ($p < 0.001$) and 160% aMT ($p < 0.001$, Figure 19-B and 19-C).

Significant main effects for Time and Time*Group interaction were observed for MEP amplitude during 60% of MVC at 120% aMT (Time: $F_{(4,1021)} = 4.24$, $p = 0.002$; Time*Group: $F_{(4,1021)} = 10.53$, $p < 0.001$), 140% aMT (Time: $F_{(4,1021)} = 7.97$, $p < 0.001$; Time*Group: $F_{(4,1021)} = 13.69$, $p < 0.001$), and 160% aMT (Time: $F_{(4,1021)} = 13.50$, $p = 0.002$; Time*Group: $F_{(4,1021)} = 14.08$, $p < 0.001$). Post hoc comparisons showed that only older adults decreased Pre to Post with all stimulation intensities ($P < 0.001$, Figure 19-D–19-F).

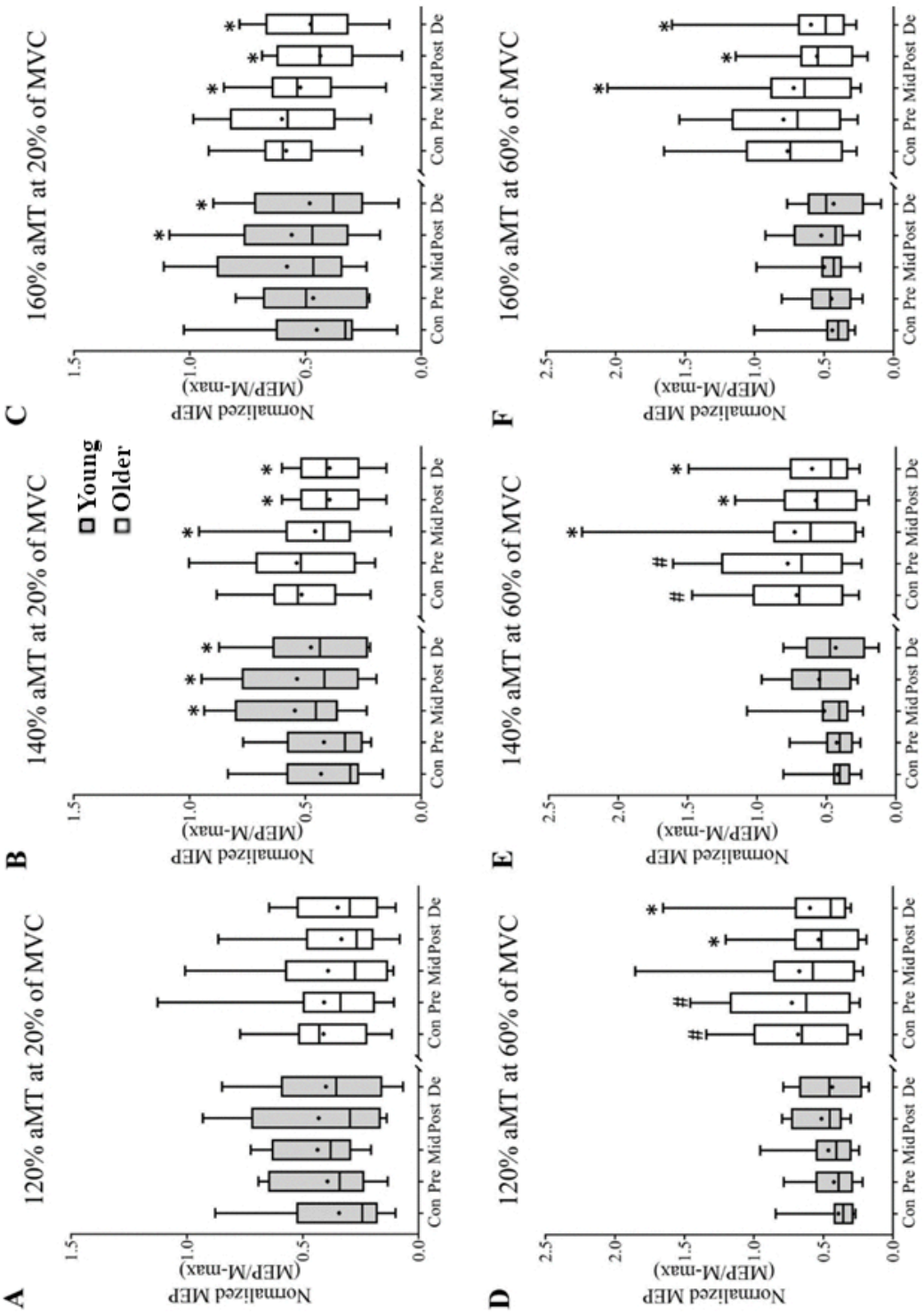

Figure 19 Box and whisker plots showing the comparisons of Group and Time effect in young and older adults for different aMT intensities at 20% of MVC (120% aMT: **(A)**; 140% aMT: **(B)**; 160% aMT: **(C)** and 60% of MVC (120% aMT: **(D)**; 140% aMT: **(E)**; 160% aMT: **(F)**. Each figure shows quartiles and whiskers (minimum and maximum), the median (line in the box), mean (+ in the box) for each group (young: filled box and older: blank box) and session. *$p < 0.05$ post hoc within-group analysis compared to pre-training. #$p < 0.05$ post hoc between-group analysis compared to the older group. Figure extracted from publication III.

5.3 Spinal excitability (Study II and III)

5.3.1 Effect of contraction intensity (Study II)

A significant main effect for Time ($F_{(2,5,\ 102.4)} = 6.542$, p = 0.001, $\eta_p^2 = 0.135$) and Time*Contraction interaction ($F_{(4.9,\ 102.4)} = 2.953$, p = 0.016, $\eta_p^2 = 0.123$) for the normalized LEP was observed. Post hoc analyses revealed significant differences in LEP amplitude between 60 ms (0.73 ± 0.27) and 150 ms (0.95 ± 0.34 (p = 0.007, 95% CI [-0.398, -0.046], Hedges' *g* = -0.27) and 90 ms (0.75 ± 0.35) and 150 ms (p = 0.004, 95% CI [-0.352, -0.050], Hedges' *g* = -0.25) during 25% of MVC.

When Unconditioned LEP was compared to the conditioned LEP at each time delay at the three contraction intensities, during 25% of MVC, conditioned LEP amplitude was statistically lower than unconditioned LEP at 60 ms ($t_{(14)}$ = -3.128, p = 0.007, 95% CI [-0.464, -0.087], Hedges' *g* = -0.62), but not at 90 ms ($t_{(14)}$ = -2.397, p = 0.075, 95% CI [-0.505, -0.028], Hedges' *g* = -0.58), 120 ms ($t_{(14)}$ = -1.285, p = 0.220, 95% CI [-0.292, 0.073], Hedges' *g* = -0.18), nor 150 ms ($t_{(14)}$ = 0.722, p = 0.482, 95% CI [-0.248, 0.123], Hedges' *g* = -0.13).

During 50% of MVC, statistical differences were found at 60 ($t_{(14)}$ = -3.052, p = 0.009, 95% CI [-0.634, -0.111], Hedges' g = -0.76), 90 ($t_{(14)}$ = -2.843, p = 0.013, 95% CI [-0.446, -0.062], Hedges' *g* = -0.44) and 150 ms ($t_{(14)}$ = -3.099, p = 0.008, 95% CI [-0.502, -0.091], Hedges' *g* = -0.52), where the conditioned LEP was lower than the unconditioned LEP. There were no statistically significant differences in conditioned versus unconditioned LEP amplitude at 120 ms ($t_{(14)}$ = -2.073, p = 0.057, 95% CI [-0.451, 0.008], Hedges' *g* = -0.36).

During 75% of MVC, the conditioned LEP amplitude was significantly lower than unconditioned LEP (Figure 20) at 60 ms ($t_{(14)}$ = -3.348, p = 0.005, 95% CI [-0.602, -0.132], Hedges' *g* = -0.78)and 150 ms ($t_{(14)}$ = -3.377, p = 0.005, 95% CI [-0.610, -0.136], Hedges' *g* = -0.70). But no statistically significant differences were observed at 90 ms ($t_{(14)}$ = -2.511, p = 0.067, 95% CI [-0.429, -0.034], Hedges' *g* = -0.51) nor 120 ms ($t_{(14)}$ = -2.626, p = 0.083 (corrected), 95% CI [-0.394, -0.040], Hedges' *g* = -0.52).

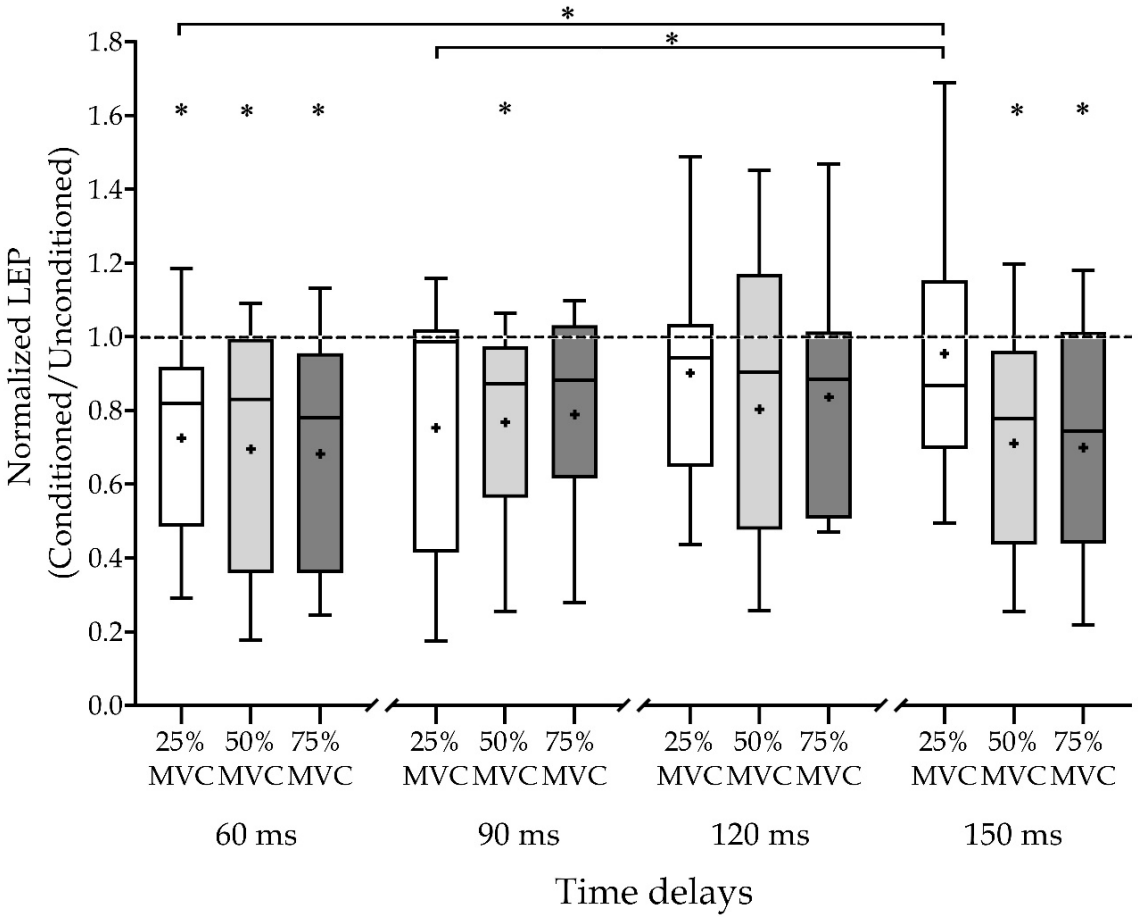

Figure 20 Box and whiskers plots showing the LEP normalized to the unconditioned LEP. The dashed line represents the unconditioned LEP amplitude. Each figure shows quartiles and whiskers (minimum and maximum), the median (line in the box), mean (+ in the box) for each condition (25% MVC: blank box; 50% MVC: filled light grey box; 75% MVC: filled dark grey box filled) at different time delays. Any mean value below the dash line represents inhibition and any mean value above the dash line represent facilitation. *$p < 0.05$.

5.3.2 Effect of age (Study III)

Results showed that there was no Group effect for LEP amplitude during the first measurement at 20% ($F_{(1,19)} = 0.03$, $p = 0.857$) or 60% ($F_{(1,19)} = 1.07$, $p = 0.313$) of MVC. For a comprehensive overview of LEP amplitudes for the young and older group at different time points during 20% and 60% of MVC, see Table 11.

5.3.3 Effects of training (Study III)

Significant main effects for Time ($F_{(4, 1021)} = 3.09$, $p = 0.015$) and Time*Group interaction ($F_{(4, 1021)} = 4.10$, $p = 0.003$) were observed for LEP amplitude during 20% of MVC. Young adults significantly increased Pre to Post ($p < 0.001$) and subsequently decreased Post to De ($p < 0.001$). Also, in the young adults, there was a significant decrease from Con to Pre ($p = 0.022$). In older adults, a significant decrease occurred Pre to Post ($p < 0.001$) (Table 11).

Significant main effects for Time ($F_{(4, 1021)} = 8.45$, $p < 0.001$) and Time*Group interaction ($F_{(4, 1021)} = 6.66$, $p < 0.001$) were observed for LEP amplitude during 60% of MVC. Post hoc analyses showed that young significantly decreased from Con to Pre ($p < 0.001$), and further decreased Pre to Mid ($p = 0.023$), and then increased Mid to Post ($p < 0.001$) (Table 11).

Table 11 Mean (± SD) and statistical results from Linear Mix Models fixed effects of normalized LEP amplitude (LEP/M-max) for young and older groups at different contraction intensities and post hoc comparison.

	Control	Pre-training	Mid-training	Post-training	Detraining	Time p-value	Time*Group p-value	Group p-value
20% MVC								
Young adults	0.36 ± 0.13*	0.30 ± 0.10	0.31 ± 0.23	0.48 ± 0.23*	0.36 ± 0.22 †			
95%CI	[0.30, 0.43]	[0.21, 0.39]	[0.26, 0.51]	[0.37, 0.59]	[0.30, 0.48]	p = 0.003	p < 0.001	p = 0.857
Older adults	0.35 ± 0.11	0.40 ± 0.21	0.37 ± 0.22	0.30 ± 0.10*	0.35 ± 0.13			
95%CI	[0.30, 0.43]	[0.30, 0.49]	[0.25, 0.51]	[0.18, 0.42]	[0.25, 0.45]			
60% MVC								
Young adults	0.51 ± 0.22*	0.41 ± 0.21	0.34 ± 0.21* †	0.47 ± 0.20	0.41 ± 0.20			
[95%CI]	[0.39, 0.62]	[0.29, 0.54]	[0.25, 0.54]	[0.33, 0.60]	[0.31, 0.55]	p < 0.001	p < 0.001	p = 0.313
Older adults	0.50 ± 0.21	0.51 ± 0.23	0.48 ± 0.30	0.48 ± 0.25	0.51 ± 0.24			
[95%CI]	[0.39, 0.63]	[0.37, 0.63]	[0.34, 0.64]	[0.34, 0.62]	[0.39, 0.64]			

MVC = maximal voluntary contraction, *LEP* = lumbar-evoked potential, *M-max* = maximal compound action potential, *CI* = confidence intervals

*$p < 0.05$ post hoc within-group analysis compared to pre-training

$p < 0.05$ post hoc within-group analysis compared to post-training

5.4 Cortico-spinal inhibition (Study III)

Main effects for Group were observed for TMS-SP during 20% of MVC with 120% aMT ($F_{(1,19)} = 13.96$, $p = 0.001$, $\eta_p^2 = 0.42$), LS-SP duration during 20% of MVC ($F_{(1,19)} = 5.60$, $p = 0.029$, $\eta_p^2 = 0.229$), TMS-SP during 60% of MVC with 120% aMT ($F_{(1,19)} = 23.39$, $p < 0.001$, $\eta_p^2 = 0.650$), and LS-SP duration during 60% of MVC ($F_{(1,19)} = 23.39$, $p < 0.001$, $\eta_p^2 = 0.552$). However, there was no effect of Time or Time*Group interaction for TMS-SP and LS-SP.

During the first measurement session (i.e., control), TMS-SP duration was longer in older adults during both 20% of MVC (99 ± 15 ms versus 117 ± 18 ms, $p = 0.027$, 95% CI [−33 , −2], Hedges' g = -1.05) and 60% of MVC (94 ± 12 ms versus 121 ± 20 ms, $p = 0.001$, 95% CI [−43 , −12], Hedges' g = -1.59) when stimulated at 120% aMT. Low-intensity LS-SP was also longer for the older adults, when compared to the young adults, during 60% of MVC (62 ± 7 ms versus 82 ± 20 ms, $p = 0.006$, 95% CI [−34, −7], Hedges' g = -1.31).

5.5 Maximum strength and skeletal muscle mass from training and detraining (Study III)

Main effects for Group were observed for 1-RM ($F_{(1,19)} = 15.94$, $p = 0.001$, $\eta_p^2 = 0.46$), and MVC ($F_{(1,19)} = 9.60$, $p = 0.006$, $\eta_p^2 = 0.34$). During the first measurement session (i.e., control), young adults showed greater 1-RM (~ 50 kg, $p = 0.002$, 95% CI [− 79.93, − 21.12], Hedges' g = -1.51) and MVC (~ 63 N·m, $p = 0.006$, 95% CI [− 105.54, − 20.84], Hedges' g = -1.31) than older adults.

For 1-RM, main effects for Time ($F_{(2.3,42.9)} = 28.29$, $p < 0.001$, $\eta_p^2 = 0.60$) and Time*Group interactions ($F_{(2.3,42.9)} = 11.06$, $p<0.001$, $\eta_p^2 = 0.38$) were observed. Post hoc comparisons showed that young adults increased from Pre to Post (+19 kg, $p < 0.001$, 95% CI [12, 26], Hedges' g = 0.40) and then decreased from Post to De (-5 kg, $p = 0.011$, 95% CI [− 10, − 1], Hedges' g = -0.10). Older adults did not increase statistically from Pre to Post but did Mid to Post (+3 kg, $p = 0.027$, 95% CI [0, 6], Hedges' g = 0.16) and they also decreased Post to De (-5 kg, $p = 0.012$, 95% CI [-10, -1], Hedges' g = -0.25)).

MVC demonstrated a significant main effect for Time ($F_{(4,76)} = 10.13$, $p < 0.001$, $\eta_p^2 = 0.35$). Post hoc analysis showed that young adults increased significantly Mid to Post (+10 N·m, $p = 0.024$, 95% CI [1, 19], Hedges' g = 0.17) and older adults increased significantly Pre to Post (+13 N·m, $p = 0.014$, 95% CI [2, 24], Hedges' g = 0.31).

Skeletal muscle mass demonstrated a significant main effect for Time ($F_{(2.5,47.8)} = 3.16$, $p < 0.001$, $\eta_p^2 = 0.323$). Here, only young adults increased Pre to Post (+0.9 kg, $p = 0.009$, 95% CI [0.18, 1.70], Hedges' g = 0.08) and then decreased Post to De (-0.8 kg, $p < 0.001$, 95% CI [-1.24, -0.41], Hedges' g = -0.09).

6 DISCUSSION

This dissertation's main findings showed that even though LEP reliability was moderate-to good, MEP reliability was good-to excellent, and both MEP and LEP reliability was better with higher submaximal contraction intensities (Study I). Furthermore, contraction intensity modulated spinal excitability differently within the TMS-SP, but it did not influence MEP amplitude (Study I and II). These findings predominantly, but not fully, support the hypotheses. Additionally, young adults were stronger, had lower cortico-spinal excitability and lower motor threshold than older adults. Resistance training improved strength in both young and older adults, but only skeletal muscle mass improved in the younger group. Cortico-spinal excitability increased in the younger group while it decreased in the older group, reaching the level of the younger group. Again, the hypothesis for Study III was predominantly supported, but with the exception of direct evidence for reduced inhibition.

6.1 Measurements of cortico-spinal excitability and inhibition

6.1.1 Reliability of MEP

In Study I, MEPs elicited by TMS showed very good-to-excellent reliability (0.821–0.941), depending on the normalization procedure and the contraction intensity. Reliability was good during 20% of MVC and excellent during 60% of MVC for Single N. Interestingly, Brownstein et al. (2018) reported ICC of 0.770–0.870 in RF during 10% of MVC, Temesi et al. (2017) reported ICC > 0.800 for MEPs elicited in RF during 20% of MVC, which are similar to the ones reported in Study I at 20% of MVC (0.821–0.861), but poor reliability (ICC = 0.590) was observed by Malcolm et al. (2021) who used maximal voluntary contractions. Malcom et al. (2021) proposed some factors for their poor reliability at high contractions intensities, such as high variability of individual EMG between measurement sessions, motor-unit synchronization and signal cancelation, as

well as intrinsic fluctuation in cortical and spinal excitability. It has been shown that MEPs increased in size with increasing contraction intensity seemingly up to 50–75% of MVC (Goodall et al. 2009; Martin et al. 2006; Oya et al. 2008; Škarabot et al. 2019b) depending on the targeted muscle. Particularly during higher contraction intensities (> 75% of MVC), the firing rate of motor-neurons increases, along with an increase in refractory period that could reduce the magnitude of the MEP (Goodall et al. 2009; Todd et al. 2003). In Study I, 60% of MVC was used as the higher contraction intensity, where MEP amplitude seemed to plateau (Figure 18), possibly reaching the maximum MEP amplitude. In addition, MEP amplitude ICCs for 60% of MVC were higher (0.896–0.941) than the ones observed at 20% of MVC in Study I, and previously reported maximum voluntary contraction (Malcolm et al. 2021). Therefore, MEP reliability could benefit from testing at this contraction intensity and the lower level of motor-neuron activation compared to the maximal voluntary contraction used in Malcom et al. (2021) by reducing variability, leading to higher ICC values.

Furthermore, Study I showed similar CV (21–26%) at 20% of MVC than those reported during 10% (CV = 18–20%) (Brownstein et al. 2018; Leung et al. 2018) and 20% (CV = 21%) (O'Leary et al. 2015) of MVC. Moreover, the values for systematic bias reported in Study I suggest that TMS-elicited responses during 20% and 60% of MVC are a reliable tool to measure the cortico-spinal tract, for example, in studies expecting changes in the magnitude of 0.24–0.36 for Single N and 6–25 for Double N (Leung et al. 2018).

6.1.2 Reliability of LEP

In Study I–III, LS hotspot was located by observing the latencies of the LEP response when the intensity of the stimulator output was increased to the maximum required intensity of each study (Figure 13-A). This test was first reported by Petersen et al. (2002), where an increase in stimulation output showed limited decreases in the latency of the CMEP in the biceps brachii, until the stimulator reached an intensity of 62–70% when they observed a decrease of ~1.5 ms in the latency. The authors discussed that such a decrease in latency could represent a change in the stimulation site, from the cortico-spinal tract axons to the ventral roots. In addition, Taylor et al. (2002) observed that CMEP area increased ~18% from a relaxed conditioned with non-significant changes in latency, when both were normalized to M-max and the stimulator output was the same, in the biceps brachii during contraction at 20% MVC. This suggests that voluntary activation decreases the threshold of higher motor-neurons, increasing the probability of recruitment at the spinal level (Ugawa et al. 1995; Petersen et al. 2002; Taylor et al. 2002; Di Lazzaro et al. 1998). In the present dissertation, LEP amplitude increased ~9% from 25–50% MVC and a plateau of response from 50–75% of MVC was observed (Figure 13-C). These results are similar to those reported by Škarabot et al. (2019b), who showed an increase of LEP amplitude ~8% (n.s.) in the RF from contractions of 25–50% of MVC followed by a plateau from 50–75% of MVC. Even though 8 participants were removed during the data analysis from Studies I–III during the offline data analysis because of ventral

roots activation, the combined results from the remaining participants suggests that LS in the present dissertation did not stimulate ventral roots.

In addition, Hofstoetter et al. (2008) created a protocol to test possible activation of the dorsal roots, by positioning the electrical stimulation between L_2–S_1 and paired electrical stimuli with 50 ms inter-stimulation interval. The authors demonstrated that the decrease in the amplitude of the second responses is attributable to a prolonged refractory period induced by the posterior root reflex. In the present dissertation, 4 participants presented a decrease in the second response and were not considered to further participate in Study II. In the remaining participants, LS did not produce such a decrease in the second response (Figure 13-B). Thus, it can be assumed that the LS was valid to stimulate the cortico-spinal tract here, as suggested by Škarabot et al. (2019b).

Study I is the first study reporting LEP reliability during different submaximal contraction intensities. LS can elicit a LEP in the target muscle, which represents the excitability of the motor-neurons (Brownstein et al. 2021; Škarabot et al. 2019b) activated by the intensity of the stimulator output in use (Yacyshyn et al. 2016). The results suggest moderate-to-good reliability of LEPs from 10 stimulations of different contraction intensities and stimulator output intensities, independent of the normalization procedure. Despite the moderate reliability shown for some conditions (e.g., low-intensity stimulation during 20% of MVC with Single N), these LEP values are within the range reported by previous reliability studies investigating MEPs in the lower-limbs (ICC = 0.600–0.900) (Brownstein et al. 2018; Leung et al. 2018; Malcolm et al. 2021). Furthermore, the CV data reported in the present study are lower than ones reported previously (e.g., 59% CV in Ansdell et al. 2020) for LEPs normalized to M-max. It is, however, important to mention that MDC was more than 100% in some conditions with Double N. Thus, LS could be used as a tool to understand spinal excitability in the lower-limbs in various experiments typical in clinical neurophysiology or exercise science fields (e.g., pharmacological treatment, training intervention, fatigue intervention, balance perturbation). However, researchers should consider and utilize the most appropriate normalization procedure or there may be a high degree of variability and possible statistical error.

6.1.3 Reliability of TMS-Silent Period

The duration of the silent period can provide information about the inhibition at the cortical or spinal level (Inghilleri et al. 1993). ICCs for TMS-SP at different stimulation intensities and contraction intensities were good and excellent, respectively. Results in Study I were in concordance with others reported by other groups (Leung et al. 2018; O'Leary et al. 2015; Pagan et al. 2023). Furthermore, the CV of the TMS-SP was within the ranges mentioned above. Moreover, ICC of LS-SP at low-and high-intensity during 20% and 60% MVC were moderate and good, which were slightly higher than the ICCs (0.610–0.700) for TMS-SP reported by Di Virgilio et al. (2022), but similar of the ICC value (0.866) reported by Leung et al. (2018) for TMS-SP. Furthermore, CV were similar to

those reported previously (CV = 7–15%) (Di Virgilio et al. 2022; Leung et al. 2018; O'Leary et al. 2015). One of the major differences observed is the number of stimuli, while 10 stimuli were used in Leung et al. (2018) and Study I, only 3 stimuli were used in Di Virgilio et al., (2022), which can have an impact on reliability (Brownstein et al. 2018). Thus, results in Study I suggest that TMS-SP and LS-SP could be used to understand inhibitory processes at cortical and spinal segments by utilizing them concurrently.

6.2 Effect of contraction intensity on cortico-spinal excitability and inhibition

Study I showed an increase in MEP amplitude, with increases in stimulator output, but did not show differences between contraction intensities. This increase in MEP amplitude was first reported by Bawa and Lemon (1993) who recorded single motor-units and EMG of different hand muscles from 100 stimuli between 25–40% of the stimulator output, while maintaining a steady discharge of an easily discriminable rate. The authors found that an increase in intensity activated higher threshold motor-units following the orderly recruitment 'size principle'. However, a plateau in the response has been observed at certain stimulator output intensities (140% rMT that corresponded to 170% aMT) (Groppa et al. 2012), depending on the target muscle (Temesi et al. 2014). A study conducted by Temesi et al. (2014) assessed the effect of different stimulator output intensities (20–80% stimulator output) and different contraction intensities (10%, 20% and 50% of MVC) in different muscles (m.vastus lateralis, vastus medialis and RF) on MEP responses. The authors showed that the plateau in the M-max normalized MEP was reached with a lower stimulator output (~-15%) when contraction increased from 10–20% to 50% of MVC. However, there were no statistical differences in Study I between 20% and 60% of MVC. In Study I, the dataset encompassed a broad spectrum of ages, which contrasted the differential responses observed in MEP amplitude at 60% of MVC during pre-training in Study III (Figure 18 and 19). This suggests that the MEP amplitude heterogeneity during aging could have contributed to an increased dispersion of the data at 60% of MVC. Nevertheless, a MEP amplitude plateau was observed at 60% of MVC (Figure 18), which could be related to spinal mechanisms that reduce the ability to discharge high threshold motor-units in response to an excitatory input (i.e., firing rate, after-hyperpolarization, recurrent inhibition, etc.) (Goodall et al. 2009; Nuzzo et al. 2021; Sidhu et al. 2009; Taylor et al. 1997; Todd et al. 2003).

Study II is the first study to directly test spinal excitability at different time delays during TMS- SP, and during different contraction intensities, in the lower-limbs (specifically RF). The results in Study II showed reduced spinal excitability during the first 60 ms in RF during all contraction intensities, extending to 90 ms

at 50% of MVC and further reductions were observed at 150 ms during 50% and 75% of MVC.

These results conflict with a previous study that used CMEPs during a 25% of MVC contraction in the upper-limb (Yacyshyn et al. 2016); in which the conditioned CMEP showed differences from the unconditioned response also at 120 ms and 150 ms after TMS. However, the results in Study II agree with earlier studies conducted using H-reflex methodology in both upper- and lower-limbs (Fuhr et al. 1991; Ziemann et al. 1996) despite H-reflex data being possibly influenced by changes in pre-synaptic inhibition, which is absent in the present methods. The results suggest that reduced spinal excitability is present but largely limited to ≤ 90 ms after TMS in lower-limb muscles, at low contraction intensities (i.e. < 25% of MVC). Differences between upper- and lower-limbs have previously been presented by Giesebrecht et al. (2010). They reported contrasting responses to spinal stimulation in biceps brachii and tibialis anterior after 10 s and 1 min MVC, discussing different physiological mechanisms in upper- and lower-limbs muscles, such as interneuron activity and efficiency, cortical-motor-neuron efficacy or concentration of Ca^{2+} in pre-synaptic terminals, which could be activity and muscle dependent. Thus, caution should be taken when extrapolating different neurophysiological phenomena to different limbs and muscles.

Compiling the existing literature provides indirect support for Study II's finding in that contraction intensity influenced the duration of reduced spinal excitability during TMS-SP. First, Finn et al. (2018) did not observe reduced spinal excitability at 100 ms (TMS induced a 200 ms SP), given that the conditioned TMEP was similar to the amplitude of the unconditioned TMEP when standardized to 50% of the M-max (as in the current study). Conversely Brownstein et al. (2021) did observe reduced spinal excitability since both conditioned TMEP and conditioned LEP amplitude at 100 ms (TMS included 200 ms SP) were lower than their respective unconditioned amplitudes, again when spinal stimulation was standardized at 50% of the M-max. As Finn et al. (2018) employed contraction intensities of 25% of MVC, whereas Brownstein et al. (2021) employed 50% of MVC, this suggests that contraction intensity influences the duration of reduced spinal excitability. In directly assessing this hypothesis, spinal excitability was reduced at 60 ms but no longer at 90 ms after TMS contracting to 25% of MVC, matching the findings of Finn et al. (2018). However, reductions in conditioned LEP were observed at 90 ms during 50% of MVC and at 150 ms during 50% and 75% of MVC, providing support for and extending the findings of Brownstein et al. (2021). Thus, I suggest that increased contraction intensity modulates spinal excitability distinctly in that reduced stimulation-induced responses are apparent at longer time delays when contracting at a higher intensity.

The suggested mechanisms for the decrease in spinal excitability during TMS-SP are: afterhyperpolarization, recurrent inhibition via Renshaw cells, Ia interneuron unloading through reciprocal inhibition, and/or Golgi tendon organ inhibition (Fuhr et al. 1991; Mills 1988; Yacyshyn et al. 2016; Ziemann et al. 1993).

Although afterhyperpolarization, recurrent inhibition and Golgi tendon organ inhibition are dependent on the preceding motor-neuron activity (Hultborn and Pierrot-Deseilligny 1979; Ziemann et al. 1993) and the size of the conditioned test stimuli (Hultborn and Pierrot-Deseilligny 1979), afterhyperpolarization may not account for more than ~56 ms, since discharge rate at 50% of MVC is ~18 pps in the vastus lateralis muscle (Kamen and Knight 2004; Watanabe et al. 2016). There is evidence that afterhyperpolarization could impact excitability up to approx. 100 ms, depending on motor-neuron firing rate (Piotrkiewicz et al. 2007), as observed in upper-limb muscles. Thus, the exact duration of the influence of afterhyperpolarization is still unresolved in different muscles. However, converging evidence and the results of Study II suggests that afterhyperpolarization may not be the case in explaining the difference between conditioned LEP amplitude during 25% versus 50% of MVC at 90 ms.

Among the TMS-SP studies, Ziemann et al. (1993) found that the conditioned/unconditioned H-reflex amplitude progressively decreased with increasing contraction intensity in the soleus muscle. The authors argued that Renshaw cells might have a stronger influence on TMS-SP inhibition, rather than Golgi tendon organs or muscle spindles, since the decrease in spinal excitability was ~50 ms, and those monosynaptic feedback mechanisms start to exert an influence after ~40 ms in soleus muscle. Although recurrent inhibition may only account for ~40 ms (Pierrot-Deseilligny and Burke 2005, pp. 138) it could influence discharging rate (Granit et al. 1960). Since stimulator output was not statistically different in 25% and 50% of MVC conditions, a plausible mechanism to explain the prolonged decrease from 60 ms to 90 ms in spinal excitability at higher contraction intensities could be recurrent inhibition via Renshaw cells.

In Study II, the interstimulus intervals of 60 ms and 90 ms could also be affected by modified muscle spindle or Golgi tendon organ activity to the cortico-spinal tract. The spindles provide muscle length feedback and Golgi tendon organs provide tensile feedback (Enoka 2008, pp. 249–288; Nichols 2018). When there is an increase in contraction intensity, Golgi tendon organs increase their discharge rate, increasing Ib inhibition (Houk et al. 1970). Further, the TMS-induced muscle twitch has been suggested to also engage Golgi tendon organs increasing Ib inhibition (Yacyshyn et al., 2016). It is conceivable that the combination of higher intensity contractions and muscle twitch-induced Ib inhibition could be enhanced in the 50% of MVC trials of the present study. Therefore, Golgi tendon organs may be one candidate for the continued decrease (i.e., > 90 ms) of spinal excitability with increasing contraction intensity.

One interesting finding in Study II was the observed return of conditioned/unconditioned LEP to baseline during 25% and 75% of MVC at 90 ms and at 120 ms for all conditions, but then a second reduction in spinal excitability at 150 ms during 50% and 75% of MVC (Figure 20 and 21). An involuntary EMG activity burst (80–150 ms) has been previously observed in upper- (Butler et al. 2012; Calancie et al. 1987; Holmgren et al. 1990) and lower-limbs (Dimitrijević et al. 1992), categorized as "low level EMG" (Butler et al. 2012) or "breakthrough EMG" (Hupfeld et al. 2020), and its origin is not known. But

this involuntary EMG activity has been postulated to arise from cortical pathways (Holmgren et al. 1990; Dimitrijević et al. 1992), spinal reflex (Dimitrijević et al. 1992; Butler et al. 2012) and/or agonist and antagonist muscle activity through polysynaptic excitatory and inhibitory potentials to the motor-neuron (Calancie et al. 1987). This involuntary activity was also observed in 11 of the 15 participants (Figure 21), with onset latencies between 83–130 ms and lengths of 28–91 ms. Additionally, the size of the response was greater at 75% vs 25% of MVC. Muscle spindles have been considered as a mechanism for the involuntary EMG activity. After the TMS-evoked twitch, there is a period of relaxation, where sarcomeres lengthen and the muscle spindles could induce a monosynaptic reflex (Hupfeld et al. 2020; Škarabot et al. 2019c). Since increases in voluntary contraction increased the relaxation ratio and reduced the time to peak relaxation in knee extensor (Vernillo et al. 2022), muscle spindles could be responsible for the involuntary EMG activity. However, latencies of the patellar tendon reflex in RF were 16–22 ms (Frijns et al. 1997), and time to peak relaxation in knee extensors were ~140 ms and ~160 ms during contractions of 75% and 50% of MVC, respectively (Vernillo et al. 2022). Thus, muscle spindles could provide feedback but not as early as the involuntary EMG activity observed in the present study. Consequently, one possible explanation for the return to baseline in spinal excitability at 90 ms during 75% of MVC and 120 ms during contractions >50% of MVC could be afferent feedback provided by synergist and/or antagonist muscles from the same limb and contralateral limb (i.e., heteronymous feedback) (Baudry et al. 2010; Calancie et al. 1987; Houk et al. 1970; Manning and Bawa 2011; Wilmink and Nichols 2003; Zehr et al. 2001). Wilmink and Nichols (2003) found both excitatory and inhibitory effects from the vastii muscles on RF following stretches in cat forelimbs. Furthermore, Zehr et al. (2001) showed a long-latency reflex in various muscles of the contralateral limb at 90 ms after peroneal nerve stimulation. Thus, at higher contraction intensities, heteronymous afferent signalling could be responsible for the return of spinal excitability at 90–120 ms, via an excitatory reflex that alters motor-neuron excitability at such time delays. Thus, it is speculated that heteronymous feedback specifically affected the 120 ms time delay (and to a certain extent also the 90 ms delay) but it no longer influenced the conditioned LEP amplitude at 150 ms, allowing reduced spinal excitability to be observed with the LS method at higher contraction intensities. Despite this being a physiological possibility, this proposal should be specifically investigated in future.

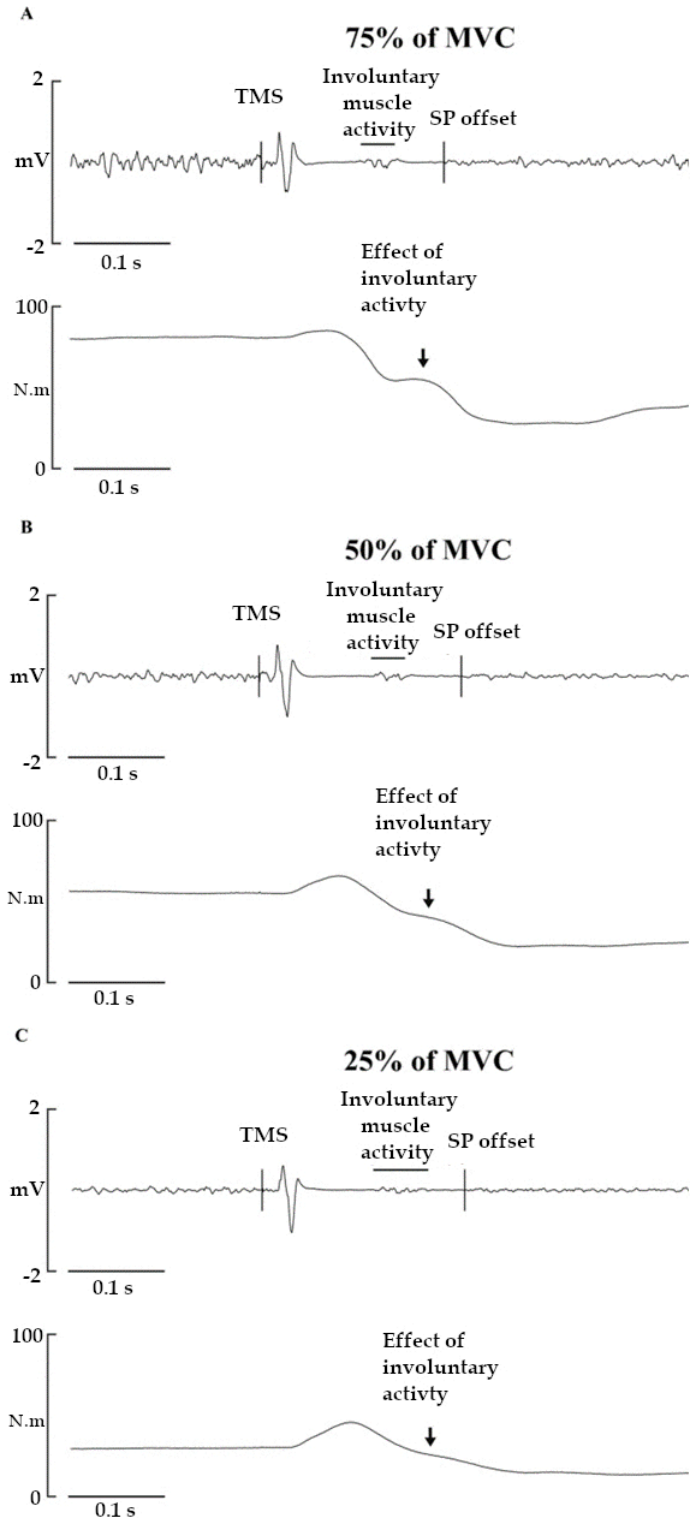

Figure 21 Involuntary EMG activity during the SP of a participant during different trials at **(A)** 75% of MVC, **(B)** 50% of MVC and **(C)** 25% of MVC. Upper traces represent the EMG signal and lower traces represent torque signal. The arrow points to the possible effect of the involuntary EMG in the torque trace. This phenomenon was observed in 11/15 participants. *TMS* transcranial magnetic stimulation, *SP* silent period. Figure extracted from publication II.

6.3 Effect of aging on cortico-spinal excitability and inhibition

Study III revealed that older adults required greater stimulation intensity to elicit a MEP (i.e., aMT), cortico-spinal excitability at higher contraction intensity was greater, and cortical and spinal inhibition was greater in older adults at baseline accompanying the between-group strength differences.

The observed differences in 1-RM and MVC between young and older adults would be expected due to the age-related reduction in maximal strength (Bemben et al. 1991). The decrease in force with aging has been related to neural and structural loss (i.e., decrease in muscle mass, brain thickness, motor-neurons, etc) (Clark and Taylor 2011). Although in Study III there was no differences between groups in skeletal muscle mass, the results showed higher aMT in older adults compared to younger adults, which is purportedly an indicator of cortico-spinal excitability (Pascual-Leone et al. 1995; Wassermann 2002). Should this reflect a decline in cortico-spinal excitability with age, as interpreted in previous studies (Bashir et al. 2014; Cirillo et al. 2011), this would directly conflict the MEP amplitude data of the present study. The aging process may lead to reduced activation of cortico-spinal neurons or disrupted synchronization among these neurons leading to a cancellation phase (Magistris et al. 1998; Pitcher et al. 2003). Notably, despite the impact of resistance training and subsequent detraining on MEP and LEP amplitudes, aMT remained unchanged across intervention and age groups suggesting a discrepancy between the measures as an indicator of excitability. Previous studies have discussed (Wassermann 2002; Hassanlouei et al. 2017) that caution is advised in interpreting aMT due to factors such as a reduction in motor cortex size (Marner et al. 2003; Salat et al. 2004) and increase in skull thickness (Lillie et al. 2016) with older age that potentially increase the coil-to-cortex distance, meaning a requirement for higher stimulation intensities to generate action potentials. It may be that the between-group differences in aMT of the present study is due to cortex size and/or skull thickness rather than cortico-spinal excitability *per se*, and this could have an impact in strength, possibly due to the decrease of pyramidal cells, that could impact the number of motor-neurons activated (Clark and Taylor 2011). While the present dissertation did not directly address these factors, the results underscore the need for further investigation to identify the precise mechanisms: 1) whether aging increases aMT due to reduced cortico-spinal excitability or decrease in cortex size and/or skull thickness and 2) whether the decrease in cortex size contributes to a decrease in maximal strength.

In addition, in Study III it was observed that M-max amplitude, which represents the activation of all motor-units in the motor neuron pool (Rodriguez-Falces and Place 2018), was lower in older adults compared to younger ones. Furthermore, the decrease in force in older adults is concomitant with slower neuromuscular properties and a reduced number of motor-units, as reviewed by Roos et al. (1997). The purported remodelling of motor-units, particularly the selective denervation of Type II fibres and their potential reinnervation by Type

I motor-unit axons, could contribute to this observed difference in M-max and force production (Roos et al. 1997). M-max can also be influenced by fat mass, which can reduce the effectiveness of electrical stimulation (Petrofsky 2008). Older adults typically have more fat mass than younger ones (Schilling et al. 2023), although Study III found no significant differences in fat mass between the two groups. For body composition assessment in this dissertation, bioimpedance was preferred over dual-energy X-ray absorptiometry, which is generally considered a more valid method to assess body composition (Branski et al. 2010).

At baseline, the results showed that MEP-SP at 120% aMT and LEP-SP were significantly longer for the older group independently of the contraction intensity used. TMS-SP is an indication of intracortical inhibition (Inghilleri et al., 1993) mediated by GABA inhibitors, particularly involving the activity of $GABA_B$ receptors (Siebner et al. 1998). Consequently, prolonged TMS-SP indicates greater $GABA_B$ activity and longer intracortical inhibition in the older group. These results contradict previous findings, where TMS-SP durations were reported as shorter (Christie and Kamen 2014; Sale and Semmler 2005) or not different (Fujiyama et al. 2012) compared to younger adults at baseline. However, it should be noted that MEPs were either of similar amplitude (Christie and Kamen 2014) or smaller (Sale and Semmler 2005) than the younger adults in those previous studies, which contrasts the higher MEP and LEP amplitudes for the older adults here. Given the correlation between TMS-SP and MEP amplitude (Orth and Rothwell 2004), it is plausible that normalization of TMS-SP to MEP amplitude in the young and older group might have led to an interpretation of similar or less inhibition in older adults at baseline.

6.4 Effects of short-term resistance training and detraining

In Study III, both young and older adults responded positively to a short-term resistance training intervention observed through increases in 1-RM and MVC, again as expected from previous studies (Christie and Kamen 2014; Häkkinen et al. 2000; Walker and Häkkinen 2014). The 1-RM increases in Study III of Δ14% and Δ9% in young and older adults, respectively, are similar to those reported by Walker and Häkkinen (2014) over ten weeks of training. Interestingly, increases in lean leg mass in that study occurred only in the younger group (Walker and Häkkinen 2014), and only the young group increased skeletal muscles mass in Study III. These converging results suggest that (solely) neural mechanisms, rather than morphological, may be responsible for increased maximal strength in previously untrained older adults when initiating resistance training. Previously untrained young adults, on the other hand, appear to improve maximal strength through a combination of neural and morphological mechanisms.

An interesting observation was the consistent decrease in MEP excitability in the older group, independent of the contraction intensity. These changes became apparent as early as three weeks into the training. The results in Study III differ from those reported by Christie and Kamen (2014) where two weeks of

training (six training sessions) did not induce significant changes in MEP amplitude in the tibialis anterior muscle. The authors noted decreases of 4–6% (n.s) in MEP amplitude in the older adults and the magnitude of those results was similar to the present results (-7–8%) after 3 weeks/6 sessions of resistance training, but MEP amplitude further decreased (to -12–21%) after 7 weeks/ 13 sessions of resistance training in the present intervention. Therefore, cortico-spinal adaptation in older adults seems to require more training duration than in young adults.

Furthermore, the interaction, and within-group changes of LEP amplitude parallel those of MEP amplitude; older adults showing a reduction in LEP amplitude at 20% of MVC. In addition, LEP amplitude increased in the young group from pre- to post-training at 20% of MVC and then decreased back to baseline after detraining. Although no clear or systematic changes in LEP amplitude were observed in either group during 60% of MVC trials, the results in Study III suggest that the adaptation in cortico-spinal excitability are predominantly at the spinal level. The observed LEP amplitude fluctuation at 60% of MVC may be due to the relatively high typical error/reliability values of this method shown in Study I. Nevertheless, one previous study investigating short-term resistance training effects (Ansdell et al., 2020) observed no changes in MEP nor LEP amplitude at a group level; where large inter-individual differences apparent with approximately half of the group increasing and half decreasing amplitude after 12 sessions of 4 sets of 6–8 back squat repetitions. In contrast, Jensen et al. (2005) demonstrated decreased cortico-spinal excitability in untrained healthy young adults after thirteen training sessions spread over 4 weeks. This effect was observed at several higher TMS stimulator output intensities (160–220% rMT), similar to the differences observed here at 140% and 160% aMT. Previous authors discussed that those changes could potentially be at subcortical levels through changes in spinal motor-neuron firing rate and/or intrinsic firing properties, although this was not specifically tested. In support, Vila-Chã et al. (2012) and Aagaard et al. (2002) observed spinal adaptations, through better modulation of inhibitory pathways, after 3 weeks and 14 weeks of resistance training in younger adults. Thus, in the present study, the older group adapted to the training by reducing their MEP amplitude down to the level of the young and these adaptations could be at the spinal level.

Conversely, small magnitude but statistically significant increases in MEP excitability occurred in the young group after resistance training, as has been previously reported (Goodwill et al. 2012; Kidgell et al. 2017; Weier et al. 2012). Goodwill et al. (2012) and Weier et al. (2012) found that a short-term training intervention, twelve sessions, produced an increase in MEP amplitude of RF when measured at 10% of MVC. Those results are in line with the present results at 20% of MVC. However, and importantly for the following interpretation, MEP excitability assessed at 60% of MVC did not show significant changes in the young. Resistance training and maximal strength has been proposed as a specific skill (Buckner et al. 2017), and 12 sessions of arm flexion-extension visuo-motor tracking skill training (Lundbye-Jensen et al. 2005) along with 12 sessions of 3 s

concentric and 4 s eccentric tempo-controlled bicep curl resistance training (Leung et al. 2017) has been shown to increase MEP amplitude after four weeks. Since the participants were required to hold the force level constant prior to stimulation (~2 s), it may be that lower force levels challenge the sensorimotor system to a greater extent than higher contraction levels, as previously evident in force steadiness tasks (Laidlaw et al. 2000). Therefore, it is proposed that the statistically significant but small magnitude changes in excitability in the young observed only during 20% of MVC trials reflect the sensorimotor integration needed for force steadiness, a so-called 'skill element' of resistance training.

Moreover, the present study showed decreased MEP amplitude following resistance training while the TMS-SP duration from cortical and spinal stimulation remained unchanged. Therefore, normalizing the TMS-SP and LS-SP to MEP or LEP amplitude, respectively, would modify the interpretation of excitatory and inhibitory processes influencing the observed outcomes. Thus, the observed decrease in MEP/LEP amplitude and the conserved TMS-SP/LS-SP may indicate greater contribution of cortical and/or spinal inhibition in older adults after training, which may improve movement efficiency and result in increased strength.

Furthermore, other cortical inhibitory process (i.e., inter-hemispheric inhibition (Talelli et al. 2008), SICI (Heise et al. 2013), and cortical reciprocal inhibition (Hortobágyi and Devita 2006)) have been reported to decrease in aging and could have a potential effect in motor control and performance (Levin et al. 2014). Although there is no current evidence of the effects of resistance training on cortical inhibition, a meta-analysis (Gómez-Feria et al. 2023) has reported that SICI does not change after resistance training in young adults, but there is an increase in MEP amplitude. Therefore, it could be speculated that the differences in MEP and LEP excitability at 60% of MVC could be due to the above mentioned inhibitory process and that resistance training could modulate activity, in older adults, decreasing MEP and LEP amplitude to the same level of the young adults. Further exploration, particularly focussing on inhibitory processes in older adults following resistance training, is warranted.

6.5 Strengths and limitations

This dissertation is the first to provide reliability statistics for two methods to assess cortico-spinal and spinal excitability during different submaximal contraction levels and stimulation intensities in Study I. Clearer between-group differences (at baseline) were observable at 60% of MVC compared to 20% of MVC, and this finding could direct future studies comparing differences between groups. Although previous studies have reported the reliability of motor-evoked potentials (MEPs) at low submaximal contraction levels, this is the first that provides reliability for submaximal contraction levels higher than 20% of MVC (Brownstein et al. 2018; Leung et al. 2018; Temesi et al. 2017). Moreover, this is the first study reporting reliability of LEPs at different submaximal contraction

levels. This study also provides reliability data of a normalization technique for MEPs and lumbar-evoked potentials (LEPs) that aims to take into account the possible effect of EMG background activity on the induced responses (Sidhu et al. 2013; Škarabot et al. 2018). Furthermore, in all Experiments, Transcranial Magnetic Stimulation (TMS) and Lumbar Stimulation (LS) was employed to assess MEP and LEP, respectively, with meticulous control over factors that could influence MEP and/or LEP amplitude. These factors included limb orientation (Mogk et al. 2014), attention levels (Ruge et al. 2014), surrounding noise (Rossi et al. 2009), and EMG activity (Bawa and Lemon 1993; Škarabot et al. 2019a). The limb being tested was secured to ensure stability even during 60% maximum voluntary contraction (MVC) efforts. Additionally, upper-limb positioning was standardized across all stimulation sessions. Tasks were conducted with the muscle relaxed, by fixing the eyes on a continuous trace displayed in the screen and counting down; and during contraction, by achieving the specified contraction intensity (20% or 60% of MVC). Noise and other distractions were minimized 30 seconds prior to and during stimulation to maintain participant alertness. EMG activity was constantly monitored, especially during stimulations at rest, to prevent any ongoing muscle activity.

These methods were used to understand how contraction intensity could affect cortical-spinal excitability (Study I and II). Additionally, those methods were used to provide evidence of cortical and spinal excitability and inhibition adaptations to a 7-week strength training intervention in young and older adults. Study III also provides information from a detraining period, which strengthens inferences that can be drawn regarding the causality of the intervention.

In terms of limitations, the number of stimuli in Study I might have been a possible factor for the LEPs moderate reliability. Although previous studies have used 10 stimuli to induce LEPs, there is evidence from MEP reliability studies that increasing the number of stimuli (> 15) could improve reliability of MEPs (Bastani and Jaberzadeh 2012; Brownstein et al. 2018; Cavaleri et al. 2017).

In Study II, TMS was not employed, in addition to spinal electrical stimulation, to compare cortico-spinal and spinal excitability at the same time delays (60, 90, 120 and 150 ms). This could have provided information regarding ongoing cortical inhibition along with spinal level inhibition (as employed by Fuhr et al. (1991) and Inghilleri et al. (1993)). However, the number of trials needed to employ both TMS and LS would have compromised the present study's ability to restrict neuromuscular fatigue during the testing session and tripled the number of transcranial stimulations. Second, it should be acknowledged that employing voluntary contractions in the present study's methodology does not allow controlling for the background EMG activity/torque (Škarabot et al. 2019a) when unconditioned and conditioned LEP were elicited, since the unconditioned LEP was elicited during a period of voluntary muscle activity as opposed to during the TMS-SP. Third, sample size estimation suggested that 18 participants were needed to obtain medium effect sizes for torque × time delay interaction. A significant interaction in normalized LEP was observed but post hoc comparisons have likely been underpowered to

detect pairwise differences as only 15 participants were available for the final analysis.

In Study III, the resistance training programme was performed dynamically and mainly bilaterally. Thereby, the unilateral isometric test was non-specific and could have influenced the ability to identify neural adaptations. Furthermore, bioimpedance was used to measure skeletal muscle mass, instead of ultrasound, being the later more useful to assess muscle thickness (Isaka et al. 2022), especially in older adults (Rustani et al. 2019) or dual-energy X-ray absorptiometry, is generally considered a more valid method to assess body composition (Branski et al. 2010). TMS paired-pulse paradigms (i.e., SICI, LICI, ICF), peripheral stimulation paradigms (H-reflex) and/or paired H-reflex-TMS (cortical recurrent inhibition) were not measured in this study because an increased number of contractions per session would have increased the risk of fatigue. These procedures, however, could have provided more specific information about how resistance training modulates cortical and spinal inhibitory process in young and older adults alongside cortico-spinal and spinal excitability.

7 MAIN FINDINGS AND CONCLUSION

The results of the present dissertation indicate that LS appears to be a valid method to assess spinal excitability and inhibition, as indicated by the three control tests employed and the close symmetry of the MEP and LEP responses to aging and resistance training/detraining. Furthermore, single-pulse MEPs, LEPS, TMS-SP and LS-SP are reliable tools to assess changes in cortico-spinal excitability (MEPs and LEPs) as well as cortical and spinal inhibition (TMS-SP and LS-SP). Although contraction intensity did not show an effect on MEP amplitude, spinal excitability might have a higher impact during the TMS-SP than previously thought when contraction intensities are higher or equal to 50% of MVC. Thus, it is important to assess both TMS-SP and LS-SP for a better understanding of inhibitory processes at cortical and/or spinal levels.

Consequently, assessment of cortico-spinal excitability and inhibition, using MEPs, LEPs, TMS-SP and LS-SP, during a short-term resistance-training intervention and detraining has provided new insight on neural adaptations in young and older adults. Improvements in maximum strength occurred in both groups, as well as early cortico-spinal adaptations, but these latter adaptations appeared to be age-dependant and specific to contraction intensity. Despite no changes in TMS-SP and LS-SP, the decrease in MEP amplitude at 20% and 60% of MVC indicates cortico-spinal adaptations occurred in the older adults. MEP decreases could have a spinal origin since LEP amplitude decreased in parallel in older adults, possibly due to an increase in inhibitory feedback to the spine, regulating spinal excitability to the control level of physically active young adults. On the other hand, physically active young adults increased MEP and LEP amplitude supporting the suggestion that the primary site of adaptation after resistance training in young and older adults is at the spinal level. In conclusion, it can be stated that resistance training has reversed some of the age-related neural maladaptations in older adults and increased maximum strength. This dissertation confirmed the usefulness of cortico-spinal excitability assessment methods and showed spinal adaptation after a resistance training intervention in both young and older adults.

YHTEENVETO (SUMMARY IN FINNISH)

Hermosto on tärkeä osa ihmisen liikkumisen kannalta. Se mahdollistaa viestinnän aivojen, selkäytimen ja raajojen välillä. Kortikospinaalirata on keskeisessä asemassa tässä järjestelmässä, välittäen käskyjä aivoista lihaksille liikkeen käynnistämiseksi. Tämän radan toiminnan tehokkuutta voidaan tutkia transkraniaalisella magneettistimulaatiolla (TMS) sekä selkärangan sähköstimulaatiolla. TMS tuottaa magneettikentän, joka indusoi sähköisen aktiivisuuden motoriselle aivokuorelle, joka voidaan mitata kohdelihaksen sähköisenä vasteena (MEP). Jotta näiden mittausten merkitys täysin ymmärrettäisiin, on tärkeää ottaa huomioon sekä aivojen että selkäytimen eri mekanismien osuus ko. lihasvasteen muodostumisessa.

Selkärangan liikehermosolujen toiminnan syvällisemmässä tutkimuksessa selkärankaa voidaan stimuloida myös magneetti- sekä sähköstimulaatiolla. Transkutaaninen sähköstimulaatio selkärangan tasolla voi suoraan laukaista lihasvasteen (esim. LEP). Erityisesti lannerangan stimulaatio (LS) on validi ja hyvin siedetty menetelmä, joka tarjoaa arvokasta tietoa selkäytimen liikehermosolujen toiminnasta. Lihassupistusten aikana suoritettu TMS-stimulaatio voi aiheuttaa "hiljaisen jakson (SP)" – eli tilapäisen lihaksen sähköisen vasteen keskeytymisen, jonka keston perusteella voidaan arvioida aivojen tuottaman liikekontrollin estotoimintojen (inhibitio) tilaa. Viimeaikaiset tutkimukset viittaavat siihen, että myös selkäytimen estomekanismit saattavat olla osallisena tässä ilmiössä.

Ikääntyminen aiheuttaa muutoksia hermostossa, johtaen sekä aivojen että selkäytimen toimintojen heikkenemiseen, mikä voi ilmetä vähentyneenä lihasvoimana ja heikentyneenä fyysisenä suorituskykynä. Voimaharjoittelun on osoitettu hidastavan tätä prosessia, sekä parantavan toimintakykyä iäkkäillä aikuisilla. On kuitenkin epäselvää, tapahtuuko voimaharjoittelun aikaansaamat hyödylliset muutokset aivojen vai selkäytimen tasolla vai molemmissa. Tämän väitöskirjan tarkoituksena oli selvittää, kuinka voimaharjoittelu vaikuttaa kortikospinaalijärjestelmän toimintaan sekä nuorilla että iäkkäillä aikuisilla, ja väheneekö selkäydintason herkkyys TMS:n aiheuttaman hiljaisen jakson aikana alaraajojen lihaksissa.

Tulokset osoittavat, että LS on validi menetelmä selkäydintason herkkyyden ja eston arvioimiseksi. Tämä väitöskirja on osoittanut, että MEP-, LEP- ja TMS-SP vasteet ovat luotettavia mittareita osoittamaan muutoksia kortikospinaalisen radan herkkyydessä. Mielenkiintoista on, että vaikka lihassupistuksen voimakkuus ei vaikuttanut MEP-amplitudiin, selkäydintason mekanismit saattavat vaikuttaa TMS-SP:hen merkittävämmin kuin aikaisemmin on ajateltu. Näin vaikuttaisi tapahtuvan erityisesti suuremmilla lihaksen supistusvoimakkuuksilla. Tutkimus osoitti, että voimaharjoittelulla voidaan kumota osittain ikääntymiseen liittyvää hermostollista lihastoiminnan heikkenemistä, lisäten maksimaalista voimantuottoa. Tämä väitöskirja korostaa kortikospinaalisen radan herkkyyden arvioinnin tärkeyttä sekä osoittaa voimaharjoittelun aiheuttaman hermostollisen sopeutumisen tapahtuvan selkäydintasolla sekä nuorilla, että ikääntyneillä aikuisilla.

REFERENCES

Aagaard, P., Simonsen, E. B., Andersen, J. L., Magnusson, P., & Dyhre-Poulsen, P. (2002). Neural adaptation to resistance training: Changes in evoked V-wave and H-reflex responses. *Journal of Applied Physiology (1985)*, *92*(6), 2309–2318. https://doi.org/10.1152/japplphysiol.01185.2001

Aagaard, P., Suetta, C., Caserotti, P., Magnusson, S. P., & Kjaer, M. (2010). Role of the nervous system in sarcopenia and muscle atrophy with aging: Strength training as a countermeasure. *Scandinavian Journal of Medicine & Science in Sports*, *20*(1), 49–64. https://doi.org/10.1111/j.1600-0838.2009.01084.x

Ahdab, R., Ayache, S. S., Brugières, P., Farhat, W. H., & Lefaucheur, J.-P. (2016). The Hand Motor Hotspot is not Always Located in the Hand Knob: A Neuronavigated Transcranial Magnetic Stimulation Study. *Brain Topography*, *29*(4), 590–597. https://doi.org/10.1007/s10548-016-0486-2

Akay, T. (2020). Sensory Feedback Control of Locomotor Pattern Generation in Cats and Mice. *Neuroscience*, *450*, 161–167. https://doi.org/10.1016/j.neuroscience.2020.05.008

Alvarez, F. J., Jonas, P. C., Sapir, T., Hartley, R., Berrocal, M. C., Geiman, E. J., Todd, A. J., & Goulding, M. (2005). Postnatal phenotype and localization of spinal cord V1 derived interneurons. *The Journal of Comparative Neurology*, *493*(2), 177–192. https://doi.org/10.1002/cne.20711

American College of Sports Medicine. (2009). American College of Sports Medicine position stand. Progression models in resistance training for healthy adults. *Medicine and Science in Sports and Exercise*, *41*(3), 687–708. https://doi.org/10.1249/MSS.0b013e3181915670

Amunts, K., & Zilles, K. (2015). Architectonic Mapping of the Human Brain beyond Brodmann. *Neuron*, *88*(6), 1086–1107. https://doi.org/10.1016/j.neuron.2015.12.001

Ansdell, P., Brownstein, C. G., Škarabot, J., Angius, L., Kidgell, D., Frazer, A., Hicks, K. M., Durbaba, R., Howatson, G., Goodall, S., & Thomas, K. (2020). Task-specific strength increases after lower-limb compound resistance training occurred in the absence of corticospinal changes in vastus lateralis. *Experimental Physiology*, *105*(7), 1132–1150. https://doi.org/10.1113/EP088629

Balagopal, P., Rooyackers, O. E., Adey, D. B., Ades, P. A., & Nair, K. S. (1997). Effects of aging on in vivo synthesis of skeletal muscle myosin heavy-chain and sarcoplasmic protein in humans. *The American Journal of Physiology*, *273*(4), E790-800. https://doi.org/10.1152/ajpendo.1997.273.4.E790

Barker, A. T., Jalinous, R., & Freeston, I. L. (1985). Non-invasive magnetic stimulation of human motor cortex. *The Lancet*, *325*(8437), 1106–1107. https://doi.org/10.1016/S0140-6736(85)92413-4

Bashir, S., Perez, J. M., Horvath, J. C., Pena-Gomez, C., Vernet, M., Capia, A., Alonso-Alonso, M., & Pascual-Leone, A. (2014). Differential effects of

motor cortical excitability and plasticity in young and old individuals: A Transcranial Magnetic Stimulation (TMS) study. *Frontiers in Aging Neuroscience*, *6*, 111. https://doi.org/10.3389/fnagi.2014.00111

Bastani, A., & Jaberzadeh, S. (2012). A higher number of TMS-elicited MEP from a combined hotspot improves intra- and inter-session reliability of the upper limb muscles in healthy individuals. *PloS One*, *7*(10), e47582. https://doi.org/10.1371/journal.pone.0047582

Baudry, S., Maerz, A. H., & Enoka, R. M. (2010). Presynaptic Modulation of Ia Afferents in Young and Old Adults When Performing Force and Position Control. *Journal of Neurophysiology*, *103*(2), 623–631. https://doi.org/10.1152/jn.00839.2009

Bawa, P., & Lemon, R. N. (1993). Recruitment of motor units in response to transcranial magnetic stimulation in man. *The Journal of Physiology*, *471*(1), 445–464. https://doi.org/10.1113/jphysiol.1993.sp019909

Bazzocchi, A., Diano, D., Ponti, F., Andreone, A., Sassi, C., Albisinni, U., Marchesini, G., & Battista, G. (2013). Health and ageing: A cross-sectional study of body composition. *Clinical Nutrition*, *32*(4), 569–578. https://doi.org/10.1016/j.clnu.2012.10.004

Bear, M., Connors, B., & Paradiso, M. A. (2015). *Neuroscience: Exploring the Brain*. (4th Edition). Wolters Kluwer.

Bemben, M. G., Massey, B. H., Bemben, D. A., Misner, J. E., & Boileau, R. A. (1991). Isometric muscle force production as a function of age in healthy 20- to 74-yr-old men. *Medicine and Science in Sports and Exercise*, *23*(11), 1302–1310.

Bhandari, A., Radhu, N., Farzan, F., Mulsant, B. H., Rajji, T. K., Daskalakis, Z. J., & Blumberger, D. M. (2016). A Meta-Analysis of the Effects of Aging on Motor Cortex Neurophysiology Assessed by Transcranial Magnetic Stimulation. *Clinical Neurophysiology : Official Journal of the International Federation of Clinical Neurophysiology*, *127*(8), 2834–2845. https://doi.org/10.1016/j.clinph.2016.05.363

Borde, R., Hortobágyi, T., & Granacher, U. (2015). Dose-Response Relationships of Resistance Training in Healthy Old Adults: A Systematic Review and Meta-Analysis. *Sports Medicine*, *45*(12), 1693–1720. https://doi.org/10.1007/s40279-015-0385-9

Boyd, S. G., Rothwell, J. C., Cowan, J. M., Webb, P. J., Morley, T., Asselman, P., & Marsden, C. D. (1986). A method of monitoring function in corticospinal pathways during scoliosis surgery with a note on motor conduction velocities. *Journal of Neurology, Neurosurgery, and Psychiatry*, *49*(3), 251–257. https://doi.org/10.1136/jnnp.49.3.251

Branski, L. K., Norbury, W. B., Herndon, D. N., Chinkes, D. L., Cochran, A., Suman, O., Benjamin, D., & Jeschke, M. G. (2010). Measurement of Body Composition: Is there a Gold Standard?. *Journal of Parenteral and Enteral Nutrition*, *34*(1), 55–63. https://doi.org/10.1177/0148607109336601

Brodmann, K. (1909). *Vergleichende Lokalisationslehre der Grosshirnrinde in ihren Prinzipien dargestellt auf Grund des Zellenbaues*. Leipzig : Barth. http://archive.org/details/b28062449

Brownstein, C. G., Ansdell, P., Škarabot, J., Howatson, G., Goodall, S., & Thomas, K. (2018). An optimal protocol for measurement of corticospinal excitability, short intracortical inhibition and intracortical facilitation in the *rectus femoris*. *Journal of the Neurological Sciences*, *394*, 45–56. https://doi.org/10.1016/j.jns.2018.09.001

Brownstein, C. G., Espeit, L., Royer, N., Ansdell, P., Škarabot, J., Souron, R., Lapole, T., & Millet, G. Y. (2021). Reductions in motoneuron excitability during sustained isometric contractions are dependent on stimulus and contraction intensity. *Journal of Neurophysiology*, *125*(5), 1636–1646. https://doi.org/10.1152/jn.00070.2021

Brownstein, C. G., Souron, R., Royer, N., Singh, B., Lapole, T., & Millet, G. Y. (2020). Disparate kinetics of change in responses to electrical stimulation at the thoracic and lumbar level during fatiguing isometric knee extension. *Journal of Applied Physiology (1985)*, *128*(1), 159–167. https://doi.org/10.1152/japplphysiol.00635.2019

Bryndum, B., & Marquardsen, J. (1964). The tendon reflexes in old age. *Gerontologia Clinica*, *6*, 257–265. https://doi.org/10.1159/000244838

Buckner, S. L., Jessee, M. B., Mattocks, K. T., Mouser, J. G., Counts, B. R., Dankel, S. J., & Loenneke, J. P. (2017). Determining Strength: A Case for Multiple Methods of Measurement. *Sports Medicine*, *47*(2), 193–195. https://doi.org/10.1007/s40279-016-0580-3

Burke, D., Hicks, R. G., & Stephen, J. P. (1990). Corticospinal volleys evoked by anodal and cathodal stimulation of the human motor cortex. *The Journal of Physiology*, *425*, 283–299. https://doi.org/10.1113/jphysiol.1990.sp018103

Burke, D., Hicks, R., Gandevia, S. C., Stephen, J., Woodforth, I., & Crawford, M. (1993). Direct comparison of corticospinal volleys in human subjects to transcranial magnetic and electrical stimulation. *The Journal of Physiology*, *470*(1), 383–393. https://doi.org/10.1113/jphysiol.1993.sp019864

Burke, R. E. (1967). Motor unit types of cat triceps surae muscle. *The Journal of Physiology*, *193*(1), 141–160.

Burke, R. E. (1968). Firing patterns of gastrocnemius motor units in the decerebrate cat. *The Journal of Physiology*, *196*(3), 631–654. https://doi.org/10.1113/jphysiol.1968.sp008527

Burke, R. E., Levine, D. N., Tsairis, P., & Zajac, F. E. (1973). Physiological types and histochemical profiles in motor units of the cat gastrocnemius. *The Journal of Physiology*, *234*(3), 723–748. https://doi.org/10.1113/jphysiol.1973.sp010369

Butler, J. E., Petersen, N. C., Herbert, R. D., Gandevia, S. C., & Taylor, J. L. (2012). Origin of the low-level EMG during the silent period following transcranial magnetic stimulation. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, *123*(7), 1409–1414. https://doi.org/10.1016/j.clinph.2011.11.034

Butler, J. E., Taylor, J. L., & Gandevia, S. C. (2003). Responses of human motoneurons to corticospinal stimulation during maximal voluntary contractions and ischemia. *The Journal of Neuroscience: The Official Journal*

of the Society for Neuroscience, *23*(32), 10224–10230. https://doi.org/10.1523/JNEUROSCI.23-32-10224.2003

Buxton, D. F., & Goodman, D. C. (1967). Motor function and the corticospinal tracts in the dog and raccoon. *The Journal of Comparative Neurology*, *129*(4), 341–360. https://doi.org/10.1002/cne.901290405

Cajal, S. (1906). Morfología de la célula nerviosa. *Archivos de Pedagogía y Ciencias Afines*, *1*(1), 92–106. http://www.memoria.fahce.unlp.edu.ar/art_revistas/pr.1352/pr.1352.pdf

Cajal, S. (1894). Histología. Consideraciones generales sobre la morfología de la célula nerviosa. *La veterinaria Española*.

Calancie, B., Nordin, M., Wallin, U., & Hagbarth, K. E. (1987). Motor-unit responses in human wrist flexor and extensor muscles to transcranial cortical stimuli. *Journal of Neurophysiology*, *58*(5), 1168–1185. https://doi.org/10.1152/jn.1987.58.5.1168

Campos, G. E. R., Luecke, T. J., Wendeln, H. K., Toma, K., Hagerman, F. C., Murray, T. F., Ragg, K. E., Ratamess, N. A., Kraemer, W. J., & Staron, R. S. (2002). Muscular adaptations in response to three different resistance-training regimens: Specificity of repetition maximum training zones. *European Journal of Applied Physiology*, *88*(1–2), 50–60. https://doi.org/10.1007/s00421-002-0681-6

Cantello, R., Gianelli, M., Civardi, C., & Mutani, R. (1992). Magnetic brain stimulation: The silent period after the motor evoked potential. *Neurology*, *42*(10), 1951–1959. https://doi.org/10.1212/wnl.42.10.1951

Cavaleri, R., Schabrun, S. M., & Chipchase, L. S. (2017). The number of stimuli required to reliably assess corticomotor excitability and primary motor cortical representations using transcranial magnetic stimulation (TMS): A systematic review and meta-analysis. *Systematic Reviews*, *6*(1), 48. https://doi.org/10.1186/s13643-017-0440-8

Christie, A., & Kamen, G. (2014). Cortical inhibition is reduced following short-term training in young and older adults. *Age*, *36*(2), 749–758. https://doi.org/10.1007/s11357-013-9577-0

Cirillo, J., Todd, G., & Semmler, J. G. (2011). Corticomotor excitability and plasticity following complex visuomotor training in young and old adults. *The European Journal of Neuroscience*, *34*(11), 1847–1856. https://doi.org/10.1111/j.1460-9568.2011.07870.x

Clark, B. C., & Taylor, J. L. (2011). Age-Related Changes in Motor Cortical Properties and Voluntary Activation of Skeletal Muscle. *Current Aging Science*, *4*(3), 192. https://doi.org/10.2174/1874609811104030192

Coombs, T. A., Frazer, A. K., Horvath, D. M., Pearce, A. J., Howatson, G., & Kidgell, D. J. (2016). Cross-education of wrist extensor strength is not influenced by non-dominant training in right-handers. *European Journal of Applied Physiology*, *116*(9), 1757–1769. https://doi.org/10.1007/s00421-016-3436-5

Cruz-Jentoft, A. J., Bahat, G., Bauer, J., Boirie, Y., Bruyère, O., Cederholm, T., Cooper, C., Landi, F., Rolland, Y., Sayer, A. A., Schneider, S. M., Sieber, C. C., Topinkova, E., Vandewoude, M., Visser, M., & Zamboni, M. (2019). Sarcopenia: Revised European consensus on definition and diagnosis. *Age and Ageing*, *48*(1), 16–31. https://doi.org/10.1093/ageing/afy169

Cruz-Sánchez, F. F., Moral, A., Tolosa, E., de Belleroche, J., & Rossi, M. L. (1998). Evaluation of neuronal loss, astrocytosis and abnormalities of cytoskeletal components of large motor neurons in the human anterior horn in aging. *Journal of Neural Transmission, 105*(6–7), 689–701. https://doi.org/10.1007/s007020050088

Csapo, R., & Alegre, L. M. (2016). Effects of resistance training with moderate vs heavy loads on muscle mass and strength in the elderly: A meta-analysis. *Scandinavian Journal of Medicine & Science in Sports*, *26*(9), 995–1006. https://doi.org/10.1111/sms.12536

Damas, F., Libardi, C. A., & Ugrinowitsch, C. (2018). The development of skeletal muscle hypertrophy through resistance training: The role of muscle damage and muscle protein synthesis. *European Journal of Applied Physiology*, *118*(3), 485–500. https://doi.org/10.1007/s00421-017-3792-9

Damas, F., Phillips, S. M., Lixandrão, M. E., Vechin, F. C., Libardi, C. A., Roschel, H., Tricoli, V., & Ugrinowitsch, C. (2016). Early resistance training-induced increases in muscle cross-sectional area are concomitant with edema-induced muscle swelling. *European Journal of Applied Physiology*, *116*(1), 49–56. https://doi.org/10.1007/s00421-015-3243-4

Damas, F., Phillips, S., Vechin, F. C., & Ugrinowitsch, C. (2015). A review of resistance training-induced changes in skeletal muscle protein synthesis and their contribution to hypertrophy. *Sports Medicine*, *45*(6), 801–807. https://doi.org/10.1007/s40279-015-0320-0

Damron, L. A., Dearth, D. J., Hoffman, R. L., & Clark, B. C. (2008). Quantification of the corticospinal silent period evoked via transcranial magnetic stimulation. *Journal of Neuroscience Methods*, *173*(1), 121–128. https://doi.org/10.1016/j.jneumeth.2008.06.001

Day, B. L., Dressler, D., Maertens de Noordhout, A., Marsden, C. D., Nakashima, K., Rothwell, J. C., & Thompson, P. D. (1989). Electric and magnetic stimulation of human motor cortex: Surface EMG and single motor unit responses. *The Journal of Physiology*, *412*(1), 449–473. https://doi.org/10.1113/jphysiol.1989.sp017626

Day, B. L., Rothwell, J. C., Thompson, P. D., Dick, J. P., Cowan, J. M., Berardelli, A., & Marsden, C. D. (1987). Motor cortex stimulation in intact man. 2. Multiple descending volleys. *Brain: A Journal of Neurology*, *110(5)*, 1191–1209. https://doi.org/10.1093/brain/110.5.1191

Day, B. L., Rothwell, J. C., Thompson, P. D., Maertens de Noordhout, A., Nakashima, K., Shannon, K., & Marsden, C. D. (1989). Delay in the execution of voluntary movement by electrical or magnetic brain stimulation in intact man. Evidence for the storage of motor programs in

the brain. *Brain: A Journal of Neurology*, *112(3)*, 649–663. https://doi.org/10.1093/brain/112.3.649

DeFreitas, J. M., Beck, T. W., Stock, M. S., Dillon, M. A., & Kasishke, P. R. (2011). An examination of the time course of training-induced skeletal muscle hypertrophy. *European Journal of Applied Physiology*, *111*(11), 2785–2790. https://doi.org/10.1007/s00421-011-1905-4

Di Lazzaro, V., Oliviero, A., Pilato, F., Saturno, E., Dileone, M., Mazzone, P., Insola, A., Tonali, P. A., & Rothwell, J. C. (2004). The physiological basis of transcranial motor cortex stimulation in conscious humans. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, *115*(2), 255–266. https://doi.org/10.1016/j.clinph.2003.10.009

Di Lazzaro, V., Oliviero, A., Saturno, E., Pilato, F., Insola, A., Mazzone, P., Profice, P., Tonali, P., & Rothwell, J. C. (2001). The effect on corticospinal volleys of reversing the direction of current induced in the motor cortex by transcranial magnetic stimulation. *Experimental Brain Research*, *138*(2), 268–273. https://doi.org/10.1007/s002210100722

Di Lazzaro, V., & Rothwell, J. C. (2014). Corticospinal activity evoked and modulated by non-invasive stimulation of the intact human motor cortex. *The Journal of Physiology*, *592*(19), 4115–4128. https://doi.org/10.1113/jphysiol.2014.274316

Di Virgilio, T. G., Ietswaart, M., Selvamoorthy, R., & Hunter, A. M. (2022). The Reliability of Transcranial Magnetic Stimulation-Derived Corticomotor Inhibition as a Brain Health Evaluation Tool in Soccer Players. *Sports Medicine - Open*, *8*(1), 7. https://doi.org/10.1186/s40798-021-00399-3

Dimitrijević, M. R., Kofler, M., McKay, W. B., Sherwood, A. M., Van der Linden, C., & Lissens, M. A. (1992). Early and late lower limb motor evoked potentials elicited by transcranial magnetic motor cortex stimulation. *Electroencephalography and Clinical Neurophysiology*, *85*(6), 365–373. https://doi.org/10.1016/0168-5597(92)90049-h

Eccles, J. C., Schmidt, R. F., & Willis, W. D. (1962). Presynaptic inhibition of the spinal monosynaptic reflex pathway. *The Journal of Physiology*, *161*(2), 282–297.

Enoka, R. M. (2008). *Neuromechanics of Human Movement* (4th Edition). Human Kinetics.

Faraday, M. (1839). *Experimental Researches in Electricity. Fifteenth Series*. Royal Society of London. http://archive.org/details/philtrans09391630

Farrow, M., Biglands, J., Tanner, S. F., Clegg, A., Brown, L., Hensor, E. M. A., O'Connor, P., Emery, P., & Tan, A. L. (2021). The effect of ageing on skeletal muscle as assessed by quantitative MR imaging: An association with frailty and muscle strength. *Aging Clinical and Experimental Research*, *33*(2), 291–301. https://doi.org/10.1007/s40520-020-01530-2

Fathi, D., Ueki, Y., Mima, T., Koganemaru, S., Nagamine, T., Tawfik, A., & Fukuyama, H. (2010). Effects of aging on the human motor cortical plasticity studied by paired associative stimulation. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical*

Neurophysiology, *121*(1), 90–93. https://doi.org/10.1016/j.clinph.2009.07.048

Fields, R. D. (2010). Change in the Brain's White Matter. *Science*, *330*(6005), 768–769. https://doi.org/10.1126/science.1199139

Finn, H. T., Rouffet, D. M., Kennedy, D. S., Green, S., & Taylor, J. L. (2018). Motoneuron excitability of the quadriceps decreases during a fatiguing submaximal isometric contraction. *Journal of Applied Physiology (1985)*, *124*(4), 970–979. https://doi.org/10.1152/japplphysiol.00739.2017

Folland, J. P., & Williams, A. G. (2007). The adaptations to strength training: Morphological and neurological contributions to increased strength. *Sports Medicine*, *37*(2), 145–168. https://doi.org/10.2165/00007256-200737020-00004

Fragala, M. S., Cadore, E. L., Dorgo, S., Izquierdo, M., Kraemer, W. J., Peterson, M. D., & Ryan, E. D. (2019). Resistance Training for Older Adults: Position Statement From the National Strength and Conditioning Association. *Journal of Strength and Conditioning Research*, *33*(8), 2019–2052. https://doi.org/10.1519/JSC.0000000000003230

Frijns, C. J., Laman, D. M., van Duijn, M. A., & van Duijn, H. (1997). Normal values of patellar and ankle tendon reflex latencies. *Clinical Neurology and Neurosurgery*, *99*(1), 31–36. https://doi.org/10.1016/s0303-8467(96)00593-8

Fry, A. C. (2004). The role of resistance exercise intensity on muscle fibre adaptations. *Sports Medicine*, *34*(10), 663–679. https://doi.org/10.2165/00007256-200434100-00004

Fuhr, P., Agostino, R., & Hallett, M. (1991). Spinal motor neuron excitability during the silent period after cortical stimulation. *Electroencephalography and Clinical Neurophysiology*, *81*(4), 257–262. https://doi.org/10.1016/0168-5597(91)90011-l

Fujiyama, H., Hinder, M. R., Schmidt, M. W., Garry, M. I., & Summers, J. J. (2012). Age-related differences in corticospinal excitability and inhibition during coordination of upper and lower limbs. *Neurobiology of Aging*, *33*(7), 1484.e1-14. https://doi.org/10.1016/j.neurobiolaging.2011.12.019

Fulton, J. F. (1935). A note on the definition of the "motor" and "premotor" areas. *Brain*, *58*(2), 311–316. https://doi.org/10.1093/brain/58.2.311

Gandevia, S. C., Petersen, N., Butler, J. E., & Taylor, J. L. (1999). Impaired response of human motoneurones to corticospinal stimulation after voluntary exercise. *The Journal of Physiology*, *521*(3), 749–759. https://doi.org/10.1111/j.1469-7793.1999.00749.x

Gao, F., Edden, R. A. E., Li, M., Puts, N. A. J., Wang, G., Liu, C., Zhao, B., Wang, H., Bai, X., Zhao, C., Wang, X., & Barker, P. B. (2013). Edited Magnetic Resonance Spectroscopy Detects an Age-related Decline in Brain GABA Levels. *NeuroImage*, *78*, 75–82. https://doi.org/10.1016/j.neuroimage.2013.04.012

Garfinkel, S., & Cafarelli, E. (1992). Relative changes in maximal force, EMG, and muscle cross-sectional area after isometric training. *Medicine and Science in Sports and Exercise*, *24*(11), 1220–1227.

Geertsen, S. S., Willerslev-Olsen, M., Lorentzen, J., & Nielsen, J. B. (2017). Development and aging of human spinal cord circuitries. *Journal of Neurophysiology*, *118*(2), 1133–1140. https://doi.org/10.1152/jn.00103.2017

Giesebrecht, S., Martin, P. G., Gandevia, S. C., & Taylor, J. L. (2010). Facilitation and inhibition of tibialis anterior responses to corticospinal stimulation after maximal voluntary contractions. *Journal of Neurophysiology*, *103*(3), 1350–1356. https://doi.org/10.1152/jn.00879.2009

Gómez-Feria, J., Martín-Rodríguez, J. F., & Mir, P. (2023). Corticospinal adaptations following resistance training and its relationship with strength: A systematic review and multivariate meta-analysis. *Neuroscience & Biobehavioral Reviews*, *152*, 105289. https://doi.org/10.1016/j.neubiorev.2023.105289

Goodall, S., Romer, L. M., & Ross, E. Z. (2009). Voluntary activation of human knee extensors measured using transcranial magnetic stimulation. *Experimental Physiology*, *94*(9), 995–1004. https://doi.org/10.1113/expphysiol.2009.047902

Goodwill, A. M., Pearce, A. J., & Kidgell, D. J. (2012). Corticomotor plasticity following unilateral strength training. *Muscle & Nerve*, *46*(3), 384–393. https://doi.org/10.1002/mus.23316

Gordon, E. M., Chauvin, R. J., Van, A. N., Rajesh, A., Nielsen, A., Newbold, D. J., Lynch, C. J., Seider, N. A., Krimmel, S. R., Scheidter, K. M., Monk, J., Miller, R. L., Metoki, A., Montez, D. F., Zheng, A., Elbau, I., Madison, T., Nishino, T., Myers, M. J., … Dosenbach, N. U. F. (2023). A somato-cognitive action network alternates with effector regions in motor cortex. *Nature*, *617*(7960), 351–359. https://doi.org/10.1038/s41586-023-05964-2

Granit, R., Haase, J., & Rutledge, L. T. (1960). Recurrent inhibition in relation to frequency of firing and limitation of discharge rate of extensor motoneurones. *The Journal of Physiology*, *154*(2), 308–328. https://doi.org/10.1113/jphysiol.1960.sp006581

Groppa, S., Oliviero, A., Eisen, A., Quartarone, A., Cohen, L. G., Mall, V., Kaelin-Lang, A., Mima, T., Rossi, S., Thickbroom, G. W., Rossini, P. M., Ziemann, U., Valls-Solé, J., & Siebner, H. R. (2012). A practical guide to diagnostic transcranial magnetic stimulation: Report of an IFCN committee. *Clinical Neurophysiology : Official Journal of the International Federation of Clinical Neurophysiology*, *123*(5), 858–882. https://doi.org/10.1016/j.clinph.2012.01.010

Gualtierotti, T., & Paterson, A. S. (1954). Electrical stimulation of the unexposed cerebral cortex. *The Journal of Physiology*, *125*(2), 278–291.

Guralnik, J. M., Ferrucci, L., Simonsick, E. M., Salive, M. E., & Wallace, R. B. (1995). Lower-extremity function in persons over the age of 70 years as a predictor of subsequent disability. *The New England Journal of Medicine*, *332*(9), 556–561. https://doi.org/10.1056/NEJM199503023320902

Häkkinen, K., Alen, M., Kallinen, M., Newton, R. U., & Kraemer, W. J. (2000). Neuromuscular adaptation during prolonged strength training, detraining and re-strength-training in middle-aged and elderly people. *European Journal of Applied Physiology*, *83*(1), 51–62. https://doi.org/10.1007/s004210000248

Hannah, R., & Rothwell, J. C. (2017). Pulse Duration as Well as Current Direction Determines the Specificity of Transcranial Magnetic Stimulation of Motor Cortex during Contraction. *Brain Stimulation*, *10*(1), 106–115. https://doi.org/10.1016/j.brs.2016.09.008

Hasey, G. M. (1999). Transcranial magnetic stimulation: Using a law of physics to treat psychopathology. *Journal of Psychiatry and Neuroscience*, *24*(2), 97–101.

Hassanlouei, H., Sundberg, C. W., Smith, A. E., Kuplic, A., & Hunter, S. K. (2017). Physical activity modulates corticospinal excitability of the lower limb in young and old adults. *Journal of Applied Physiology*, *123*(2), 364–374. https://doi.org/10.1152/japplphysiol.01078.2016

Haun, C. T., Vann, C. G., Roberts, B. M., Vigotsky, A. D., Schoenfeld, B. J., & Roberts, M. D. (2019). A Critical Evaluation of the Biological Construct Skeletal Muscle Hypertrophy: Size Matters but So Does the Measurement. *Frontiers in Physiology*, *10*, 247. https://doi.org/10.3389/fphys.2019.00247

Heise, K.-F., Zimerman, M., Hoppe, J., Gerloff, C., Wegscheider, K., & Hummel, F. C. (2013). The aging motor system as a model for plastic changes of GABA-mediated intracortical inhibition and their behavioral relevance. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *33*(21), 9039–9049. https://doi.org/10.1523/JNEUROSCI.4094-12.2013

Henneman, E. (1957). Relation between size of neurons and their susceptibility to discharge. *Science*, *126*(3287), 1345–1347. https://doi.org/10.1126/science.126.3287.1345

Hermens, H., Freriks, B., Merletti, R., Stegeman, D., Blok, J., Rau, G., Disselhorst-Klug, C., Hägg, G., Hermens, I. H. J. B., & Freriks. (1999). *European recommendations for surface electromyography: Results of the SENIAM Project*. https://www.semanticscholar.org/paper/European-recommendations-for-surface-Results-of-the-Hermens-Freriks/1ab28b8afcb1216cab1b2f8da0de246c3d5ed6e8

Hess, C. W., Mills, K. R., & Murray, N. M. (1986). Magnetic stimulation of the human brain: Facilitation of motor responses by voluntary contraction of ipsilateral and contralateral muscles with additional observations on an amputee. *Neuroscience Letters*, *71*(2), 235–240. https://doi.org/10.1016/0304-3940(86)90565-3

Hess, G., & Donoghue, J. P. (1994). Long-term potentiation of horizontal connections provides a mechanism to reorganize cortical motor maps. *Journal of Neurophysiology*, *71*(6), 2543–2547. https://doi.org/10.1152/jn.1994.71.6.2543

Hofstoetter, U. S., Freundl, B., Binder, H., & Minassian, K. (2018). Common neural structures activated by epidural and transcutaneous lumbar spinal

cord stimulation: Elicitation of posterior root-muscle reflexes. *PloS One*, *13*(1), e0192013. https://doi.org/10.1371/journal.pone.0192013

Hofstoetter, U. S., Minassian, K., Hofer, C., Mayr, W., Rattay, F., & Dimitrijevic, M. R. (2008). Modification of reflex responses to lumbar posterior root stimulation by motor tasks in healthy subjects. *Artificial Organs*, *32*(8), 644–648. https://doi.org/10.1111/j.1525-1594.2008.00616.x

Holmgren, H., Larsson, L. E., & Pedersen, S. (1990). Late muscular responses to transcranial cortical stimulation in man. *Electroencephalography and Clinical Neurophysiology*, *75*(3), 161–172. https://doi.org/10.1016/0013-4694(90)90170-o

Holtermann, A., Roeleveld, K., Engstrøm, M., & Sand, T. (2007). Enhanced H-reflex with resistance training is related to increased rate of force development. *European Journal of Applied Physiology*, *101*(3), 301–312. https://doi.org/10.1007/s00421-007-0503-y

Hortobágyi, T., & Devita, P. (2006). Mechanisms responsible for the age-associated increase in coactivation of antagonist muscles. *Exercise and Sport Sciences Reviews*, *34*(1), 29–35. https://doi.org/10.1097/00003677-200601000-00007

Houk, J. C., Singer, J. J., & Goldman, M. R. (1970). An evaluation of length and force feedback to soleus muscles of decerebrate cats. *Journal of Neurophysiology*, *33*(6), 784–811. https://doi.org/10.1152/jn.1970.33.6.784

Hultborn, H., Jankowska, E., & Lindström, S. (1971). Recurrent inhibition from motor axon collaterals of transmission in the Ia inhibitory pathway to motoneurones. *The Journal of Physiology*, *215*(3), 591–612. https://doi.org/10.1113/jphysiol.1971.sp009488

Hultborn, H., Lindström, S., & Wigström, H. (1979). On the function of recurrent inhibition in the spinal cord. *Experimental Brain Research*, *37*(2), 399–403. https://doi.org/10.1007/BF00237722

Hultborn, H., & Pierrot-Deseilligny, E. (1979). Changes in recurrent inhibition during voluntary soleus contractions in man studied by an H-reflex technique. *The Journal of Physiology*, *297*(1), 229–251. https://doi.org/10.1113/jphysiol.1979.sp013037

Hunter, S. K., Pereira, H. M., & Keenan, K. G. (2016). The aging neuromuscular system and motor performance. *Journal of Applied Physiology (1985)*, *121*(4), 982–995. https://doi.org/10.1152/japplphysiol.00475.2016

Hupfeld, K. E., Swanson, C. W., Fling, B. W., & Seidler, R. D. (2020). TMS-induced silent periods: A review of methods and call for consistency. *Journal of Neuroscience Methods*, *346*, 108950. https://doi.org/10.1016/j.jneumeth.2020.108950

Inghilleri, M., Berardelli, A., Cruccu, G., & Manfredi, M. (1993). Silent period evoked by transcranial stimulation of the human cortex and cervicomedullary junction. *The Journal of Physiology*, *466*, 521–534. https://doi.org/10.1113/jphysiol.1993.sp019732

Isaka, M., Sugimoto, K., Akasaka, H., Yasunobe, Y., Takahashi, T., Xie, K., Onishi, Y., Yoshida, S., Minami, T., Yamamoto, K., Kamide, K., & Rakugi, H. (2022).

The Muscle Thickness Assessment Using Ultrasonography is a Useful Alternative to Skeletal Muscle Mass by Bioelectrical Impedance Analysis. *Clinical Interventions in Aging*, *17*, 1851–1861. https://doi.org/10.2147/CIA.S385469

Izumi, S. I., Furukawa, T., Koyama, Y., & Ishida, A. (2001). The nature of facilitation of leg muscle motor evoked potentials by knee flexion. *Somatosensory & Motor Research*, *18*(4), 322–329. https://doi.org/10.1080/01421590120089000

Jensen, J. L., Marstrand, P. C. D., & Nielsen, J. B. (2005). Motor skill training and strength training are associated with different plastic changes in the central nervous system. *Journal of Applied Physiology (1985)*, *99*(4), 1558–1568. https://doi.org/10.1152/japplphysiol.01408.2004

Kaiser, L. G., Schuff, N., Cashdollar, N., & Weiner, M. W. (2005). Age-related glutamate and glutamine concentration changes in normal human brain: 1H MR spectroscopy study at 4 T. *Neurobiology of Aging*, *26*(5), 665–672. https://doi.org/10.1016/j.neurobiolaging.2004.07.001

Kamen, G., & Knight, C. A. (2004). Training-related adaptations in motor unit discharge rate in young and older adults. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, *59*(12), 1334–1338. https://doi.org/10.1093/gerona/59.12.1334

Kandel, E. R., Koester, J. D., Mack, S. H., & Siegelbaum, S. A. (2021). *Principles of Neural Science* (6th Edition). McGraw Hill Professional.

Kaneko, K., Kawai, S., Fuchigami, Y., Morita, H., & Ofuji, A. (1996). The effect of current direction induced by transcranial magnetic stimulation on the corticospinal excitability in human brain. *Electroencephalography and Clinical Neurophysiology*, *101*(6), 478–482. https://doi.org/10.1016/s0013-4694(96)96021-x

Kawamura, Y., O'Brien, P., Okazaki, H., & Dyck, P. J. (1977). Lumbar motoneurons of man II: The number and diameter distribution of large- and intermediate-diameter cytons in 'motoneuron columns' of spinal cord of man. *Journal of Neuropathology and Experimental Neurology*, *36*(5), 861–870. https://doi.org/10.1097/00005072-197709000-00010

Kidgell, D. J., Bonanno, D. R., Frazer, A. K., Howatson, G., & Pearce, A. J. (2017). Corticospinal responses following strength training: A systematic review and meta-analysis. *The European Journal of Neuroscience*, *46*(11), 2648–2661. https://doi.org/10.1111/ejn.13710

Kidgell, D. J., Stokes, M. A., & Pearce, A. J. (2011). Strength training of one limb increases corticomotor excitability projecting to the contralateral homologous limb. *Motor Control*, *15*(2), 247–266. https://doi.org/10.1123/mcj.15.2.247

Kido, A., Tanaka, N., & Stein, R. B. (2004). Spinal excitation and inhibition decrease as humans age. *Canadian Journal of Physiology and Pharmacology*, *82*(4), 238–248. https://doi.org/10.1139/y04-017

Kobayashi, M., & Pascual-Leone, A. (2003). Transcranial magnetic stimulation in neurology. *The Lancet. Neurology*, *2*(3), 145–156. https://doi.org/10.1016/s1474-4422(03)00321-1

Kurokawa, K., Mimori, Y., Tanaka, E., Kohriyama, T., & Nakamura, S. (1999). Age-related change in peripheral nerve conduction: Compound muscle action potential duration and dispersion. *Gerontology*, *45*(3), 168–173. https://doi.org/10.1159/000022081

Kuypers, H. G., & Brinkman, J. (1970). Precentral projections to different parts of the spinal intermediate zone in therhesus monkey. *Brain Research*, *24*(1), 29–48. https://doi.org/10.1016/0006-8993(70)90272-6

Kyriazis, M. (2020). Aging as "Time-Related Dysfunction": A Perspective. *Frontiers in Medicine*, *7*, 371. https://doi.org/10.3389/fmed.2020.00371

Laidlaw, D. H., Bilodeau, M., & Enoka, R. M. (2000). Steadiness is reduced and motor unit discharge is more variable in old adults. *Muscle & Nerve*, *23*(4), 600–612. https://doi.org/10.1002/(sici)1097-4598(200004)23:4<600::aid-mus20>3.0.co;2-d

Landin, D., Thompson, M., & Reid, M. (2016). Actions of Two Bi-Articular Muscles of the Lower Extremity: A Review. *Journal of Clinical Medicine Research*, *8*(7), 489–494. https://doi.org/10.14740/jocmr2478w

Larkum, M. E., Nevian, T., Sandler, M., Polsky, A., & Schiller, J. (2009). Synaptic integration in tuft dendrites of layer 5 pyramidal neurons: A new unifying principle. *Science*, *325*(5941), 756–760. https://doi.org/10.1126/science.1171958

Lefebvre, R., Pépin, A., Louis, P.-F., & Boucher, J. P. (2004). Reliability of the motor evoked potentials elicited through magnetic stimulation at three sites. *Journal of Manipulative and Physiological Therapeutics*, *27*(2), 97–102. https://doi.org/10.1016/j.jmpt.2003.12.004

Leung, H., Latella, C., Lamon, S., & Hendy, A. M. (2018). The Reliability of Neurological Measurement in the Vastus Medialis: Implications for Research and Practice. *Frontiers in Psychology*, *9*, 1857. https://doi.org/10.3389/fpsyg.2018.01857

Leung, M., Rantalainen, T., Teo, W.-P., & Kidgell, D. (2017). The corticospinal responses of metronome-paced, but not self-paced strength training are similar to motor skill training. *European Journal of Applied Physiology*, *117*(12), 2479–2492. https://doi.org/10.1007/s00421-017-3736-4

Levin, O., Fujiyama, H., Boisgontier, M. P., Swinnen, S. P., & Summers, J. J. (2014). Aging and motor inhibition: A converging perspective provided by brain stimulation and imaging approaches. *Neuroscience and Biobehavioral Reviews*, *43*, 100–117. https://doi.org/10.1016/j.neubiorev.2014.04.001

Lillie, E. M., Urban, J. E., Lynch, S. K., Weaver, A. A., & Stitzel, J. D. (2016). Evaluation of Skull Cortical Thickness Changes With Age and Sex From Computed Tomography Scans. *Journal of Bone and Mineral Research: The Official Journal of the American Society for Bone and Mineral Research*, *31*(2), 299–307. https://doi.org/10.1002/jbmr.2613

Lontis, E. R., Voigt, M., & Struijk, J. J. (2006). Focality assessment in transcranial magnetic stimulation with double and cone coils. *Journal of Clinical Neurophysiology: Official Publication of the American Electroencephalographic Society*, *23*(5), 462–471. https://doi.org/10.1097/01.wnp.0000229944.63011.a1

López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M., & Kroemer, G. (2023). Hallmarks of aging: An expanding universe. *Cell*, *186*(2), 243–278. https://doi.org/10.1016/j.cell.2022.11.001

Lu, M., & Ueno, S. (2017). Comparison of the induced fields using different coil configurations during deep transcranial magnetic stimulation. *PLOS ONE*, *12*(6), e0178422. https://doi.org/10.1371/journal.pone.0178422

Magistris, M. R., Rösler, K. M., Truffert, A., & Myers, J. P. (1998). Transcranial stimulation excites virtually all motor neurons supplying the target muscle. A demonstration and a method improving the study of motor evoked potentials. *Brain: A Journal of Neurology*, *121(3)*, 437–450. https://doi.org/10.1093/brain/121.3.437

Malcolm, R., Cooper, S., Folland, J., Tyler, C., Hannah, R., & Sunderland, C. (2021). Reliability of transcranial magnetic stimulation measurements of maximum activation of the knee extensors in young adult males. *Human Movement Science*, *78*, 102828. https://doi.org/10.1016/j.humov.2021.102828

Manca, A., Ginatempo, F., Cabboi, M. P., Mercante, B., Ortu, E., Dragone, D., De Natale, E. R., Dvir, Z., Rothwell, J. C., & Deriu, F. (2016). No evidence of neural adaptations following chronic unilateral isometric training of the intrinsic muscles of the hand: A randomized controlled study. *European Journal of Applied Physiology*, *116*(10), 1993–2005. https://doi.org/10.1007/s00421-016-3451-6

Manning, C. D., & Bawa, P. (2011). Heteronymous reflex connections in human upper limb muscles in response to stretch of forearm muscles. *Journal of Neurophysiology*, *106*(3), 1489–1499. https://doi.org/10.1152/jn.00084.2011

Marner, L., Nyengaard, J. R., Tang, Y., & Pakkenberg, B. (2003). Marked loss of myelinated nerve fibers in the human brain with age. *The Journal of Comparative Neurology*, *462*(2), 144–152. https://doi.org/10.1002/cne.10714

Martin, P. G., Butler, J. E., Gandevia, S. C., & Taylor, J. L. (2008). Noninvasive stimulation of human corticospinal axons innervating leg muscles. *Journal of Neurophysiology*, *100*(2), 1080–1086. https://doi.org/10.1152/jn.90380.2008

Martin, P. G., Gandevia, S. C., & Taylor, J. L. (2006). Output of human motoneuron pools to corticospinal inputs during voluntary contractions. *Journal of Neurophysiology*, *95*(6), 3512–3518. https://doi.org/10.1152/jn.01230.2005

McNeil, C. J., Butler, J. E., Taylor, J. L., & Gandevia, S. C. (2013). Testing the excitability of human motoneurons. *Frontiers in Human Neuroscience*, *7*, 152. https://doi.org/10.3389/fnhum.2013.00152

McNeil, C. J., Martin, P. G., Gandevia, S. C., & Taylor, J. L. (2009). The response to paired motor cortical stimuli is abolished at a spinal level during human muscle fatigue. *The Journal of Physiology*, *587*(23), 5601–5612. https://doi.org/10.1113/jphysiol.2009.180968

Merton, P. A., & Morton, H. B. (1980). Stimulation of the cerebral cortex in the intact human subject. *Nature*, *285*(5762), 227. https://doi.org/10.1038/285227a0

Millington, P. J., Myklebust, B. M., & Shambes, G. M. (1992). Biomechanical analysis of the sit-to-stand motion in elderly persons. *Archives of Physical Medicine and Rehabilitation*, *73*(7), 609–617.

Mills, K. R. (1988). Excitatory and inhibitory effects on human spinal motoneurones from magnetic brain stimulation. *Neuroscience Letters*, *94*(3), 297–302. https://doi.org/10.1016/0304-3940(88)90034-1

Milne, J. S., & Williamson, J. (1972). The ankle jerk in older people. *Gerontologia Clinica*, *14*(2), 86–88. https://doi.org/10.1159/000245378

Mogk, J., Rogers, L., Murray, W., Perreault, E., & Stinear, J. (2014). Corticomotor excitability of arm muscles modulates according to static position and orientation of the upper limb. *Clinical Neurophysiology : Official Journal of the International Federation of Clinical Neurophysiology*, *125*(10). https://doi.org/10.1016/j.clinph.2014.02.007

Moritani, T., & deVries, H. A. (1979). Neural factors versus hypertrophy in the time course of muscle strength gain. *American Journal of Physical Medicine*, *58*(3), 115–130.

Nakamura, H., Kitagawa, H., Kawaguchi, Y., & Tsuji, H. (1996). Direct and indirect activation of human corticospinal neurons by transcranial magnetic and electrical stimulation. *Neuroscience Letters*, *210*(1), 45–48. https://doi.org/10.1016/0304-3940(96)12659-8

Nichols, T. R. (2018). Distributed force feedback in the spinal cord and the regulation of limb mechanics. *Journal of Neurophysiology*, *119*(3), 1186–1200. https://doi.org/10.1152/jn.00216.2017

Nistri, A., & Constanti, A. (1979). Pharmacological characterization of different types of GABA and glutamate receptors in vertebrates and invertebrates. *Progress in Neurobiology*, *13*(2), 117–235. https://doi.org/10.1016/0301-0082(79)90016-9

Noback, C. R., Ruggiero, D. A., Strominger, N. L., & Demarest, R. J. (2005). *The Human Nervous System: Structure and Function* (5th Edition). Springer Science & Business Media.

Nuzzo, J. L., Kennedy, D. S., Finn, H. T., & Taylor, J. L. (2021). Voluntary activation of knee extensor muscles with transcranial magnetic stimulation. *Journal of Applied Physiology*, *130*(3), 589–604. https://doi.org/10.1152/japplphysiol.00717.2020

O'Leary, T. J., Morris, M. G., Collett, J., & Howells, K. (2015). Reliability of single and paired-pulse transcranial magnetic stimulation in the vastus lateralis muscle. *Muscle & Nerve*, *52*(4), 605–615. https://doi.org/10.1002/mus.24584

Oliviero, A., Profice, P., Tonali, P. A., Pilato, F., Saturno, E., Dileone, M., Ranieri, F., & Di Lazzaro, V. (2006). Effects of aging on motor cortex excitability. *Neuroscience Research*, *55*(1), 74–77. https://doi.org/10.1016/j.neures.2006.02.002

Orth, M., & Rothwell, J. C. (2004). The cortical silent period: Intrinsic variability and relation to the waveform of the transcranial magnetic stimulation pulse. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, *115*(5), 1076–1082. https://doi.org/10.1016/j.clinph.2003.12.025

Oya, T., Hoffman, B. W., & Cresswell, A. G. (2008). Corticospinal-evoked responses in lower limb muscles during voluntary contractions at varying strengths. *Journal of Applied Physiology (1985)*, *105*(5), 1527–1532. https://doi.org/10.1152/japplphysiol.90586.2008

Pagan, J. I., Harmon, K. K., Girts, R. M., MacLennan, R. J., Beausejour, J. P., Hernandez-Sarabia, J. A., Coker, N. A., Carr, J. C., Ye, X., DeFreitas, J. M., & Stock, M. S. (2023). Sex-Specific Reliability of Lower-Limb Corticospinal Excitability and Silent Periods. *Journal of Strength and Conditioning Research*, *37*(9), 1882–1887. https://doi.org/10.1519/JSC.0000000000004525

Pagni, C. A., Naddeo, M., & Mascari, C. (1988). History of Evoked Potential Recording in Humans. In B. L. Grundy & R. M. Villani (Eds.), *Evoked Potentials: Intraoperative and ICU Monitoring* (pp. 17–44). Springer. https://doi.org/10.1007/978-3-7091-4431-2_3

Pakkenberg, B., & Gundersen, H. J. (1997). Neocortical neuron number in humans: Effect of sex and age. *The Journal of Comparative Neurology*, *384*(2), 312–320.

Pascual-Leone, A., Nguyet, D., Cohen, L. G., Brasil-Neto, J. P., Cammarota, A., & Hallett, M. (1995). Modulation of muscle responses evoked by transcranial magnetic stimulation during the acquisition of new fine motor skills. *Journal of Neurophysiology*, *74*(3), 1037–1045. https://doi.org/10.1152/jn.1995.74.3.1037

Penfield, W., & Boldery, E. (1937). Somatic motor and sensory representation in the cerebral cortex of man as studied by electrical stimulation. *Brain*, *60*(4), 389–443. https://doi.org/10.1093/brain/60.4.389

Petersen, N. T., Taylor, J. L., & Gandevia, S. C. (2002). The effect of electrical stimulation of the corticospinal tract on motor units of the human biceps brachii. *The Journal of Physiology*, *544*(1), 277–284. https://doi.org/10.1113/jphysiol.2002.024539

Petrofsky, J. (2008). The effect of the subcutaneous fat on the transfer of current through skin and into muscle. *Medical Engineering & Physics*, *30*(9), 1168–1176. https://doi.org/10.1016/j.medengphy.2008.02.009

Piekarz, K. M., Bhaskaran, S., Sataranatarajan, K., Street, K., Premkumar, P., Saunders, D., Zalles, M., Gulej, R., Khademi, S., Laurin, J., Peelor, R., Miller, B. F., Towner, R., & Van Remmen, H. (2020). Molecular changes associated with spinal cord aging. *GeroScience*, *42*(2), 765–784. https://doi.org/10.1007/s11357-020-00172-6

Pierrot-Deseilligny, E., & Burke, D. (2005). *The Circuitry of the Human Spinal Cord: Its Role in Motor Control and Movement Disorders*. Cambridge University Press. https://doi.org/10.1017/CBO9780511545047

Piotrkiewicz, M., Kudina, L., Mierzejewska, J., Jakubiec, M., & Hausmanowa-Petrusewicz, I. (2007). Age-related change in duration of afterhyperpolarization of human motoneurones. *The Journal of Physiology*, *585*(2), 483–490. https://doi.org/10.1113/jphysiol.2007.142356

Pitcher, J. B., Ogston, K. M., & Miles, T. S. (2003). Age and sex differences in human motor cortex input-output characteristics. *The Journal of Physiology*, *546*(2), 605–613. https://doi.org/10.1113/jphysiol.2002.029454

Purves, D., Augustine, G., Fitzpatrick, D., Hall, W. C., LaMantia, A., Mooney, R., & White, L. E. (2018). *Neuroscience*. Sinauer.

Raz, N., Lindenberger, U., Rodrigue, K. M., Kennedy, K. M., Head, D., Williamson, A., Dahle, C., Gerstorf, D., & Acker, J. D. (2005). Regional brain changes in aging healthy adults: General trends, individual differences and modifiers. *Cerebral Cortex*, *15*(11), 1676–1689. https://doi.org/10.1093/cercor/bhi044

Reijonen, J., Pitkänen, M., Kallioniemi, E., Mohammadi, A., Ilmoniemi, R. J., & Julkunen, P. (2020). Spatial extent of cortical motor hotspot in navigated transcranial magnetic stimulation. *Journal of Neuroscience Methods*, *346*, 108893. https://doi.org/10.1016/j.jneumeth.2020.108893

Renshaw, B. (1946). Central effects of centripetal impulses in axons of spinal ventral roots. *Journal of Neurophysiology*, *9*, 191–204. https://doi.org/10.1152/jn.1946.9.3.191

Rexed, B. (1952). The cytoarchitectonic organization of the spinal cord in the cat. *The Journal of Comparative Neurology*, *96*(3), 414–495. https://doi.org/10.1002/cne.900960303

Rodriguez-Falces, J., & Place, N. (2018). Determinants, analysis and interpretation of the muscle compound action potential (M wave) in humans: Implications for the study of muscle fatigue. *European Journal of Applied Physiology*, *118*(3), 501–521. https://doi.org/10.1007/s00421-017-3788-5

Roos, M. R., Rice, C. L., & Vandervoort, A. A. (1997). Age-related changes in motor unit function. *Muscle & Nerve*, *20*(6), 679–690. https://doi.org/10.1002/(SICI)1097-4598(199706)20:6<679::AID-MUS4>3.0.CO;2-5

Rosenberg, I. H. (1997). Sarcopenia: Origins and clinical relevance. *The Journal of Nutrition*, *127*(5 Suppl), 990S-991S. https://doi.org/10.1093/jn/127.5.990S

Rossi, S., Hallett, M., Rossini, P. M., Pascual-Leone, A., & Safety of TMS Consensus Group. (2009). Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, *120*(12), 2008–2039. https://doi.org/10.1016/j.clinph.2009.08.016

Rossini, P. M., Barker, A. T., Berardelli, A., Caramia, M. D., Caruso, G., Cracco, R. Q., Dimitrijević, M. R., Hallett, M., Katayama, Y., & Lücking, C. H. (1994). Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: Basic principles and procedures for routine clinical application. Report of an IFCN committee. *Electroencephalography and Clinical Neurophysiology*, *91*(2), 79–92. https://doi.org/10.1016/0013-4694(94)90029-9

Rotenberg, A., Horvath, J. C., & Pascual-Leone, A. (2016). *Transcranial Magnetic Stimulation*. Springer New York.

Roth, Y., Zangen, A., & Hallett, M. (2002). A coil design for transcranial magnetic stimulation of deep brain regions. *Journal of Clinical Neurophysiology: Official Publication of the American Electroencephalographic Society*, *19*(4), 361–370. https://doi.org/10.1097/00004691-200208000-00008

Rothwell, J. C., Hallett, M., Berardelli, A., Eisen, A., Rossini, P., & Paulus, W. (1999). Magnetic stimulation: Motor evoked potentials. The International Federation of Clinical Neurophysiology. *Electroencephalography and Clinical Neurophysiology. Supplement*, *52*, 97–103.

Roy, F. D., Gibson, G., & Stein, R. B. (2012). Effect of percutaneous stimulation at different spinal levels on the activation of sensory and motor roots. *Experimental Brain Research*, *223*(2), 281–289. https://doi.org/10.1007/s00221-012-3258-6

Rozand, V., Senefeld, J. W., Sundberg, C. W., Smith, A. E., & Hunter, S. K. (2019). Differential effects of aging and physical activity on corticospinal excitability of upper and lower limb muscles. *Journal of Neurophysiology*, *122*(1), 241–250. https://doi.org/10.1152/jn.00077.2019

Ruge, D., Muggleton, N., Hoad, D., Caronni, A., & Rothwell, J. C. (2014). An unavoidable modulation? Sensory attention and human primary motor cortex excitability. *The European Journal of Neuroscience*, *40*(5), 2850–2858. https://doi.org/10.1111/ejn.12651

Russell, B., Motlagh, D., & Ashley, W. W. (2000). Form follows function: How muscle shape is regulated by work. *Journal of Applied Physiology (1985)*, *88*(3), 1127–1132. https://doi.org/10.1152/jappl.2000.88.3.1127

Rustani, K., Kundisova, L., Capecchi, P. L., Nante, N., & Bicchi, M. (2019). Ultrasound measurement of rectus femoris muscle thickness as a quick screening test for sarcopenia assessment. *Archives of Gerontology and Geriatrics*, *83*, 151–154. https://doi.org/10.1016/j.archger.2019.03.021

Säisänen, L., Pirinen, E., Teitti, S., Könönen, M., Julkunen, P., Määttä, S., & Karhu, J. (2008). Factors influencing cortical silent period: Optimized stimulus location, intensity and muscle contraction. *Journal of Neuroscience Methods*, *169*(1), 231–238. https://doi.org/10.1016/j.jneumeth.2007.12.005

Salat, D. H., Buckner, R. L., Snyder, A. Z., Greve, D. N., Desikan, R. S. R., Busa, E., Morris, J. C., Dale, A. M., & Fischl, B. (2004). Thinning of the cerebral cortex in aging. *Cerebral Cortex*, *14*(7), 721–730. https://doi.org/10.1093/cercor/bhh032

Sale, D. G. (1988). Neural adaptation to resistance training. *Medicine and Science in Sports and Exercise*, *20*(5 Suppl), S135-145. https://doi.org/10.1249/00005768-198810001-00009

Sale, M. V., & Semmler, J. G. (2005). Age-related differences in corticospinal control during functional isometric contractions in left and right hands. *Journal of Applied Physiology (1985)*, *99*(4), 1483–1493. https://doi.org/10.1152/japplphysiol.00371.2005

Scaglioni, G., Ferri, A., Minetti, A. E., Martin, A., Van Hoecke, J., Capodaglio, P., Sartorio, A., & Narici, M. V. (2002). Plantar flexor activation capacity and H reflex in older adults: Adaptations to strength training. *Journal of Applied Physiology (1985)*, *92*(6), 2292–2302. https://doi.org/10.1152/japplphysiol.00367.2001

Scaglioni, G., Narici, M. V., Maffiuletti, N. A., Pensini, M., & Martin, A. (2003). Effect of ageing on the electrical and mechanical properties of human soleus motor units activated by the H reflex and M wave. *The Journal of Physiology*, *548*(2), 649–661. https://doi.org/10.1113/jphysiol.2002.032763

Schilling, R., Schmidt, S. C. E., Fiedler, J., & Woll, A. (2023). Associations between physical activity, physical fitness, and body composition in adults living in Germany: A cross-sectional study. *PLOS ONE*, *18*(10), e0293555. https://doi.org/10.1371/journal.pone.0293555

Schoenfeld, B. J., Grgic, J., Ogborn, D., & Krieger, J. W. (2017). Strength and Hypertrophy Adaptations Between Low- vs. High-Load Resistance Training: A Systematic Review and Meta-analysis. *Journal of Strength and Conditioning Research*, *31*(12), 3508–3523. https://doi.org/10.1519/JSC.0000000000002200

Sherrington, C. S. (1925). Remarks on some aspects of reflex inhibition. *Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character*, *97*(686), 519–545. https://doi.org/10.1098/rspb.1925.0017

Siddique, U., Frazer, A. K., Avela, J., Walker, S., Ahtiainen, J. P., Howatson, G., Tallent, J., & Kidgell, D. J. (2022). Determining the cortical, spinal and muscular adaptations to strength-training in older adults: A systematic review and meta-analysis. *Ageing Research Reviews*, *82*, 101746. https://doi.org/10.1016/j.arr.2022.101746

Siddique, U., Rahman, S., Frazer, A. K., Pearce, A. J., Howatson, G., & Kidgell, D. J. (2020). Determining the Sites of Neural Adaptations to Resistance Training: A Systematic Review and Meta-analysis. *Sports Medicine*, *50*(6), 1107–1128. https://doi.org/10.1007/s40279-020-01258-z

Sidhu, S. K., Bentley, D. J., & Carroll, T. J. (2009). Cortical voluntary activation of the human knee extensors can be reliably estimated using transcranial magnetic stimulation. *Muscle & Nerve*, *39*(2), 186–196. https://doi.org/10.1002/mus.21064

Sidhu, S. K., Cresswell, A. G., & Carroll, T. J. (2013). Corticospinal responses to sustained locomotor exercises: Moving beyond single-joint studies of central fatigue. *Sports Medicine*, *43*(6), 437–449. https://doi.org/10.1007/s40279-013-0020-6

Siebner, H. R., Dressnandt, J., Auer, C., & Conrad, B. (1998). Continuous intrathecal baclofen infusions induced a marked increase of the transcranially evoked silent period in a patient with generalized dystonia. *Muscle & Nerve*, *21*(9), 1209–1212. https://doi.org/10.1002/(sici)1097-4598(199809)21:9<1209::aid-mus15>3.0.co;2-m

Sivilotti, L., & Nistri, A. (1991). GABA receptor mechanisms in the central nervous system. *Progress in Neurobiology*, *36*(1), 35–92. https://doi.org/10.1016/0301-0082(91)90036-z

Škarabot, J., Ansdell, P., Brownstein, C. G., Hicks, K. M., Howatson, G., Goodall, S., & Durbaba, R. (2019). Reduced corticospinal responses in older compared with younger adults during submaximal isometric, shortening, and lengthening contractions. *Journal of Applied Physiology (1985)*, *126*(4), 1015–1031. https://doi.org/10.1152/japplphysiol.00987.2018

Škarabot, J., Ansdell, P., Brownstein, C. G., Thomas, K., Howatson, G., Goodall, S., & Durbaba, R. (2019). Electrical stimulation of human corticospinal axons at the level of the lumbar spinal segments. *The European Journal of Neuroscience*, *49*(10), 1254–1267. https://doi.org/10.1111/ejn.14321

Škarabot, J., Mesquita, R. N. O., Brownstein, C. G., & Ansdell, P. (2019). Myths and Methodologies: How loud is the story told by the transcranial magnetic stimulation-evoked silent period? *Experimental Physiology*, *104*(5), 635–642. https://doi.org/10.1113/EP087557

Škarabot, J., Tallent, J., Goodall, S., Durbaba, R., & Howatson, G. (2018). Corticospinal excitability during shortening and lengthening actions with incremental torque output. *Experimental Physiology*, *103*(12), 1586–1592. https://doi.org/10.1113/EP087347

Stachowski, N. J., & Dougherty, K. J. (2021). Spinal Inhibitory Interneurons: Gatekeepers of Sensorimotor Pathways. *International Journal of Molecular Sciences*, *22*(5), 2667. https://doi.org/10.3390/ijms22052667

Stock, M. S., Mota, J. A., DeFranco, R. N., Grue, K. A., Jacobo, A. U., Chung, E., Moon, J. R., DeFreitas, J. M., & Beck, T. W. (2017). The time course of short-term hypertrophy in the absence of eccentric muscle damage. *European Journal of Applied Physiology*, *117*(5), 989–1004. https://doi.org/10.1007/s00421-017-3587-z

Stone, M., Plisk, S., & Collins, D. (2002). Training principles: Evaluation of modes and methods of resistance training--a coaching perspective. *Sports Biomechanics*, *1*(1), 79–103. https://doi.org/10.1080/14763140208522788

Talelli, P., Waddingham, W., Ewas, A., Rothwell, J. C., & Ward, N. S. (2008). The effect of age on task-related modulation of interhemispheric balance. *Experimental Brain Research*, *186*(1), 59–66. https://doi.org/10.1007/s00221-007-1205-8

Tan, S., Faull, R. L. M., & Curtis, M. A. (2023). The tracts, cytoarchitecture, and neurochemistry of the spinal cord. *Anatomical Record*, *306*(4), 777–819. https://doi.org/10.1002/ar.25079

Taylor, J. L. (2006). Stimulation at the cervicomedullary junction in human subjects. *Journal of Electromyography and Kinesiology*, *16*(3), 215–223. https://doi.org/10.1016/j.jelekin.2005.07.001

Taylor, J. L., Allen, G. M., Butler, J. E., & Gandevia, S. C. (1997). Effect of contraction strength on responses in biceps brachii and adductor pollicis to transcranial magnetic stimulation. *Experimental Brain Research*, *117*(3), 472–478. https://doi.org/10.1007/s002210050243

Taylor, J. L., Butler, J. E., Allen, G. M., & Gandevia, S. C. (1996). Changes in motor cortical excitability during human muscle fatigue. *The Journal of Physiology*, *490*(2), 519–528.

Taylor, J. L., Butler, J. E., & Gandevia, S. C. (1999). Altered responses of human elbow flexors to peripheral-nerve and cortical stimulation during a sustained maximal voluntary contraction. *Experimental Brain Research*, *127*(1), 108–115. https://doi.org/10.1007/s002210050779

Taylor, J. L., & Gandevia, S. C. (2004). Noninvasive stimulation of the human corticospinal tract. *Journal of Applied Physiology (1985)*, *96*(4), 1496–1503. https://doi.org/10.1152/japplphysiol.01116.2003

Taylor, J. L., Petersen, N. T., Butler, J. E., & Gandevia, S. C. (2002). Interaction of transcranial magnetic stimulation and electrical transmastoid stimulation in human subjects. *The Journal of Physiology*, *541*(3), 949–958. https://doi.org/10.1113/jphysiol.2002.016782

Tecchio, F., Zappasodi, F., Pasqualetti, P., Gennaro, L. D., Pellicciari, M. C., Ercolani, M., Squitti, R., & Rossini, P. M. (2008). Age dependence of primary motor cortex plasticity induced by paired associative stimulation. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, *119*(3), 675–682. https://doi.org/10.1016/j.clinph.2007.10.023

Temesi, J., Gruet, M., Rupp, T., Verges, S., & Millet, G. Y. (2014). Resting and active motor thresholds versus stimulus–response curves to determine transcranial magnetic stimulation intensity in quadriceps femoris. *Journal of NeuroEngineering and Rehabilitation*, *11*(1), 40. https://doi.org/10.1186/1743-0003-11-40

Temesi, J., Ly, S. N., & Millet, G. Y. (2017). Reliability of single- and paired-pulse transcranial magnetic stimulation for the assessment of knee extensor muscle function. *Journal of the Neurological Sciences*, *375*, 442–449. https://doi.org/10.1016/j.jns.2017.02.037

Todd, G., Taylor, J. L., & Gandevia, S. C. (2003). Measurement of voluntary activation of fresh and fatigued human muscles using transcranial magnetic stimulation. *The Journal of Physiology*, *551*(2), 661–671. https://doi.org/10.1113/jphysiol.2003.044099

Tokimura, H., Ridding, M. C., Tokimura, Y., Amassian, V. E., & Rothwell, J. C. (1996). Short latency facilitation between pairs of threshold magnetic stimuli applied to human motor cortex. *Electroencephalography and Clinical Neurophysiology*, *101*(4), 263–272. https://doi.org/10.1016/0924-980x(96)95664-7

Tomlinson, B. E., & Irving, D. (1977). The numbers of limb motor neurons in the human lumbosacral cord throughout life. *Journal of the Neurological Sciences*, *34*(2), 213–219. https://doi.org/10.1016/0022-510x(77)90069-7

Triggs, W. J., Cros, D., Macdonell, R. A., Chiappa, K. H., Fang, J., & Day, B. J. (1993). Cortical and spinal motor excitability during the transcranial magnetic stimulation silent period in humans. *Brain Research*, *628*(1–2), 39–48. https://doi.org/10.1016/0006-8993(93)90935-g

Ueno, S., Matsuda, T., & Fujiki, M. (1990). Functional mapping of the human motor cortex obtained by focal and vectorial magnetic stimulation of the brain. *IEEE Transactions on Magnetics*, *26*(5), 1539–1544. https://doi.org/10.1109/20.104438

Ueno, S., Tashiro, T., & Harada, K. (1988). Localized stimulation of neural tissues in the brain by means of a paired configuration of time-varying magnetic fields. *Journal of Applied Physics*, *64*(10), 5862–5864. https://doi.org/10.1063/1.342181

Ugawa, Y., Genba-Shimizu, K., & Kanazawa, I. (1995). Electrical stimulation of the human descending motor tracts at several levels. *The Canadian Journal of Neurological Sciences. Le Journal Canadien Des Sciences Neurologiques*, *22*(1), 36–42. https://doi.org/10.1017/s0317167100040476

Ugawa, Y., Rothwell, J. C., Day, B. L., Thompson, P. D., & Marsden, C. D. (1991). Percutaneous electrical stimulation of corticospinal pathways at the level of the pyramidal decussation in humans. *Annals of Neurology*, *29*(4), 418–427. https://doi.org/10.1002/ana.410290413

Ugawa, Y., Terao, Y., Hanajima, R., Sakai, K., & Kanazawa, I. (1995). Facilitatory effect of tonic voluntary contraction on responses to motor cortex stimulation. *Electroencephalography and Clinical Neurophysiology/Electromyography and Motor Control*, *97*(6), 451–454. https://doi.org/10.1016/0924-980X(95)00214-6

Unhjem, R., Tøien, T., Kvellestad, A. C. G., Øren, T. S., & Wang, E. (2021). External Resistance Is Imperative for Training-Induced Efferent Neural Drive Enhancement in Older Adults. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, *76*(2), 224–232. https://doi.org/10.1093/gerona/glaa160

van Melick, N., Meddeler, B. M., Hoogeboom, T. J., Nijhuis-van der Sanden, M. W. G., & van Cingel, R. E. H. (2017). How to determine leg dominance: The agreement between self-reported and observed performance in healthy adults. *PLoS ONE*, *12*(12), e0189876. https://doi.org/10.1371/journal.pone.0189876

Vernillo, G., Barbi, C., Temesi, J., Giuriato, G., Giuseppe Laginestra, F., Martignon, C., Schena, F., & Venturelli, M. (2022). Reliability of relaxation properties of knee-extensor muscles induced by transcranial magnetic stimulation. *Neuroscience Letters*, *782*, 136694. https://doi.org/10.1016/j.neulet.2022.136694

Vila-Chã, C., Falla, D., Correia, M. V., & Farina, D. (2012). Changes in H reflex and V wave following short-term endurance and strength training. *Journal*

of Applied Physiology (1985), *112*(1), 54–63. https://doi.org/10.1152/japplphysiol.00802.2011

Vucic, S., Ziemann, U., Eisen, A., Hallett, M., & Kiernan, M. C. (2013). Transcranial magnetic stimulation and amyotrophic lateral sclerosis: Pathophysiological insights. *Journal of Neurology, Neurosurgery, and Psychiatry*, *84*(10), 1161–1170. https://doi.org/10.1136/jnnp-2012-304019

Walker, S., Blazevich, A. J., Haff, G. G., Tufano, J. J., Newton, R. U., & Häkkinen, K. (2016). Greater Strength Gains after Training with Accentuated Eccentric than Traditional Isoinertial Loads in Already Strength-Trained Men. *Frontiers in Physiology*, *7*, 149. https://doi.org/10.3389/fphys.2016.00149

Walker, S., & Häkkinen, K. (2014). Similar Increases in Strength After Short-Term Resistance Training Due to Different Neuromuscular Adaptations in Young and Older Men. *The Journal of Strength & Conditioning Research*, *28*(11), 3041. https://doi.org/10.1519/JSC.0000000000000381

Wassermann, E. M. (2002). Variation in the response to transcranial magnetic brain stimulation in the general population. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, *113*(7), 1165–1171. https://doi.org/10.1016/s1388-2457(02)00144-x

Watanabe, K., Holobar, A., Kouzaki, M., Ogawa, M., Akima, H., & Moritani, T. (2016). Age-related changes in motor unit firing pattern of vastus lateralis muscle during low-moderate contraction. *Age*, *38*(3), 48. https://doi.org/10.1007/s11357-016-9915-0

Weavil, J. C., Sidhu, S. K., Mangum, T. S., Richardson, R. S., & Amann, M. (2016). Fatigue diminishes motoneuronal excitability during cycling exercise. *Journal of Neurophysiology*, *116*(4), 1743–1751. https://doi.org/10.1152/jn.00300.2016

Weier, A. T., Pearce, A. J., & Kidgell, D. J. (2012). Strength training reduces intracortical inhibition. *Acta Physiologica*, *206*(2), 109–119. https://doi.org/10.1111/j.1748-1716.2012.02454.x

Wilmink, R. J. H., & Nichols, T. R. (2003). Distribution of heterogenic reflexes among the quadriceps and triceps surae muscles of the cat hind limb. *Journal of Neurophysiology*, *90*(4), 2310–2324. https://doi.org/10.1152/jn.00833.2002

Wong, Y.-M., & Ng, G. Y. F. (2006). Surface electrode placement affects the EMG recordings of the quadriceps muscles. *Physical Therapy in Sport*, *7*(3), 122–127. https://doi.org/10.1016/j.ptsp.2006.03.006

Yacyshyn, A. F., Woo, E. J., Price, M. C., & McNeil, C. J. (2016). Motoneuron responsiveness to corticospinal tract stimulation during the silent period induced by transcranial magnetic stimulation. *Experimental Brain Research*, *234*(12), 3457–3463. https://doi.org/10.1007/s00221-016-4742-1

Young-Bernier, M., Kamil, Y., Tremblay, F., & Davidson, P. S. (2012). Associations between a neurophysiological marker of central cholinergic activity and cognitive functions in young and older adults. *Behavioral and Brain Functions : BBF*, *8*, 17. https://doi.org/10.1186/1744-9081-8-17

Zehr, E. P., Collins, D. F., & Chua, R. (2001). Human interlimb reflexes evoked by electrical stimulation of cutaneous nerves innervating the hand and foot. *Experimental Brain Research*, *140*(4), 495–504. https://doi.org/10.1007/s002210100857

Zehr, E. P., & Kido, A. (2001). Neural control of rhythmic, cyclical human arm movement: Task dependency, nerve specificity and phase modulation of cutaneous reflexes. *The Journal of Physiology*, *537*(3), 1033–1045. https://doi.org/10.1111/j.1469-7793.2001.01033.x

Zhou, M., Goto, N., Zhang, C., & Tang, W. (1996). Aging process of the human lumbar spinal cord: A morphometric analysis. *Neuropathology*, *16*(2), 106–111. https://doi.org/10.1111/j.1440-1789.1996.tb00164.x

Ziemann, U., Netz, J., Szelényi, A., & Hömberg, V. (1993). Spinal and supraspinal mechanisms contribute to the silent period in the contracting soleus muscle after transcranial magnetic stimulation of human motor cortex. *Neuroscience Letters*, *156*(1–2), 167–171. https://doi.org/10.1016/0304-3940(93)90464-v

Ziemann, U., Rothwell, J. C., & Ridding, M. C. (1996). Interaction between intracortical inhibition and facilitation in human motor cortex. *The Journal of Physiology*, *496*(3), 873–881. https://doi.org/10.1113/jphysiol.1996.sp021734

Ziemann, U., Tergau, F., Wassermann, E. M., Wischer, S., Hildebrandt, J., & Paulus, W. (1998). Demonstration of facilitatory I wave interaction in the human motor cortex by paired transcranial magnetic stimulation. *The Journal of Physiology*, *511(1)*, 181–190. https://doi.org/10.1111/j.1469-7793.1998.181bi.x

Zilles, K. (2018). Brodmann: A pioneer of human brain mapping—his impact on concepts of cortical organization. *Brain*, *141*(11), 3262–3278. https://doi.org/10.1093/brain/awy273

ORIGINAL PUBLICATIONS

I

TEST-RETEST RELIABILITY OF CORTICO- SPINAL MEASUREMENTS IN THE RECTUS FEMORIS AT DIFFERENT CONTRACTION LEVELS

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Test–retest reliability of cortico-spinal measurements in the rectus femoris at different contraction levels

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Single-pulse Transcranial Magnetic Stimulation (TMS) and, very recently, lumbar stimulation (LS) have been used to measure cortico-spinal excitability from various interventions using maximal or submaximal contractions in the lower limbs. However, reliability studies have overlooked a wide range of contraction intensities for MEPs, and no reliability data is available for LEPs. This study investigated the reliability of motor evoked potentials and lumbar evoked potentials at different stimulation intensities and contraction levels in m.rectus femoris. Twenty-two participants performed non-fatiguing isometric knee extensions at 20 and 60% of maximum voluntary contraction (MVC). LS induced a lumbar-evoked potential (LEP) of 25 and 50% resting maximal compound action potential (M-max). TMS stimulator output was adjusted to 120, 140, and 160% of active motor threshold (aMT). In each contraction, a single MEP or LEP was delivered. Ten contractions were performed at each stimulator intensity and contraction level in random order. Moderate-to-good reliability was found when LEP was normalized to M-max/Root Mean Square in all conditions (ICC:0.74–0.85). Excellent reliability was found when MEP was normalized to Mmax for all conditions (ICC > 0.90) at 60% of MVC. Good reliability was found for the rest of the TMS conditions. Moderate-to-good reliability was found for silent period (SP) elicited by LS (ICC: 0.71–0.83). Good-to-excellent reliability was found for SP elicited by TMS (ICC > 0.82). MEPs and LEPs elicited in m.rectus femoris appear to be reliable to assess changes at different segments of the cortico-spinal tract during different contraction levels and stimulator output intensities. Furthermore, the TMS- and LS- elicited SP was a reliable tool considered to reflect inhibitory processes at spinal and cortical levels.

KEYWORDS

reliability, lumbar stimulation, spinal excitability, silent period, cortico-spinal tract, lower limb, knee extensors

Introduction

Transcranial magnetic stimulation (TMS) is a safe and non-invasive technique used over the skull to elicit a response in a specific target. A single-pulse stimulus over the contralateral motor cortex of a specific muscle will induce a descending volley, by transynaptically activating pyramidal cells, creating a muscle action potential recorded by electromyography (EMG) in the

muscle targeted (Barker et al., 1985). The action potential generated by TMS is known as a motor evoked potential (MEP) and changes in its size provides information about cortico-spinal excitability (Day et al., 1989; Kobayashi and Pascual-Leone, 2003). However, MEPs cannot dissociate between changes at cortical or spinal level excitability (Taylor, 2006). Dissociating between cortical and spinal excitability changes could lead to a better understanding of the nervous system and how different segments of the nervous system respond to different interventions (Butler et al., 2003; Taylor, 2006; McNeil et al., 2009). Therefore, other methodologies, using transcutaneous electrical stimulation, have been validated to assess spinal excitability (Ugawa et al., 1991; Petersen et al., 2002; Taylor et al., 2002; Martin et al., 2008; Škarabot et al., 2019b).

Transcutaneous electrical stimulation at the spinal level has been used in the literature to assess spinal excitability at different levels (Ugawa et al., 1995; Gandevia et al., 1999; Škarabot et al., 2019a; Brownstein et al., 2020). Cervicomedullary stimulation has been used to elicit a monosynaptic response in upper and lower limb muscles through activation of cortico-spinal tract neurons. The induced action potential is known as a cervicomedullary motor evoked potential (CMEP). However, high intensities are required to elicit a CMEP in the lower limbs, which may be only visible in some participants (Ugawa et al., 1991, 1995). Given the importance of understanding the neural mechanisms in the lower limbs for health and performance, some authors have performed spinal stimulations to other segments of the spine (Martin et al., 2008; Škarabot et al., 2019b). Martin et al. (2008) and Škarabot et al. (2019b) validated thoracic stimulation and lumbar stimulation, respectively, by demonstrating that both stimulations can activate axons of the cortico-spinal tract without activating ventral or dorsal roots. The action potential elicited by these stimulations are known as a thoracic motor evoked potential (TMEP) and a lumbar evoked potential (LEP) (Martin et al., 2008; Škarabot et al., 2019b). Nevertheless, the site of thoracic stimulation (T3 and T4) is far from lower limb motor-neurons (L3–L5) as opposed to lumbar stimulation, where the center of stimulation is approximately at L1 (Martin et al., 2008; Škarabot et al., 2019b). This difference leads to higher stimulation intensities in thoracic than lumbar stimulation, which makes the thoracic method more unpleasant (Brownstein et al., 2020), similar to cervicomedullary stimulations. Thus, lumbar stimulation may be considered a more appropriate method to target the lower limbs. In addition the silent period (SP), considered as an interruption of the EMG during voluntary muscle contraction after TMS, can also be observed after lumbar stimulation (LS), and has been reported as a measure of inhibition at the spine (Merton, 1951; Inghilleri et al., 1993). Despite validation studies and the increased use of these methodologies in clinical and sport science settings, there is a lack of reliability studies examining both LEP amplitude and its SP.

Further, even though a number of studies have reported MEP reliability (O'Leary et al., 2015; Beaulieu et al., 2017; Peri et al., 2017; Temesi et al., 2017; Houde et al., 2018; Leung et al., 2018), a limited number of studies have examined reliability during maximal (Malcolm et al., 2021) or submaximal contractions in the knee extensors (at 10% of MVC: O'Leary et al., 2015; Brownstein et al., 2018; Leung et al., 2018; Pagan et al., 2023; and at 20% of MVC: Temesi et al., 2017). Interestingly, MEPs showed good-to-excellent (ICC: 0.78–0.90) reliability during low submaximal contractions (10–20% of MVC) (O'Leary et al., 2015; Temesi et al., 2017; Brownstein et al., 2018), but poor reliability (ICC = 0.56) was found during maximal contractions intensities (100% of MVC) (Malcolm et al., 2021). Moreover, MEPs and CMEPs increased similarly during a sustained task at 50% of MVC on the biceps brachii (Lévénez et al., 2008), whereas MEPs increased to a greater extent than CMEPs during a 30% of MVC in the plantar flexors (Hoffman et al., 2009). Such findings demonstrate the possible impact of various contraction intensities on electrophysiological data. In addition, strength training and acute fatigue studies have used a wide range of contraction intensities (20–100% of MVC) to assess cortical and/or spinal excitability (Butler et al., 2003; Lévénez et al., 2008; Goodall et al., 2009; Hoffman et al., 2009; Sidhu et al., 2009; Tallent et al., 2017), for which prior reliability has not been established. Thus, there is a need to determine reliability of MEP and LEP data from lower limbs to allow full evaluation of previous and future intervention studies.

Examining the contributing factors of cortical or spinal excitability in the locomotor muscles is important for determining exercise-induced alterations in nervous system function throughout the spectrum of health, exercise and disease (Sidhu et al., 2013). Furthermore, m.rectus femoris (RF) is involved in lower limb swing actions and stability (Landin et al., 2016), playing an important role in locomotion. Therefore, the aim of this study is to assess the test–retest reliability of MEP and LEP at different stimulator output intensities and different submaximal contraction levels in RF in a wide age range of asymptomatic adults.

Materials and methods

Participants

Twenty-seven participants volunteered for the study (14 female). Five participants were removed during the offline analysis due to possible activation of ventral roots (see Lumbar-evoked potentials). Therefore, the data presented here are representative of the 22 (12 female) volunteers fulfilling all study requirements (47 ± 23 years; height: 171 ± 10 cm; body mass: 80 ± 20 kg). All included participants were free from neurological illness and musculoskeletal injury in the lower-limbs for the last 6 months, were not taking any medications known to affect the nervous system and had no contraindications to transcranial magnetic stimulation (TMS), which was assessed via a health questionnaire (modified from Rossi et al., 2011). Before testing, all participants were fully informed of the procedures and possible risks, and each participant provided written inform consent. The Ethical committee of the University of Jyväskylä provided a statement for the study (857/13.00.04.00/2021) and the study was conducted in accordance with the ethical standards establish in the *Declaration of Helsinki* (2013).

Experimental set-up

Participants visited the laboratory on five occasions. The first session was a familiarization session, where the participants were introduced to all instructions and stimulations that were given during the testing sessions. Furthermore, this session was used for preliminary assessment of the lumbar stimulation electrode placement and transcranial magnetic stimulation intensity for motor threshold determination. The other four sessions were

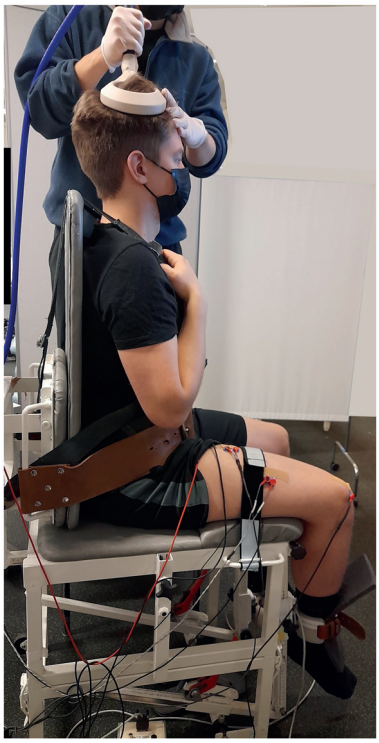

FIGURE 1
Representation of participants set-up during the testing session.

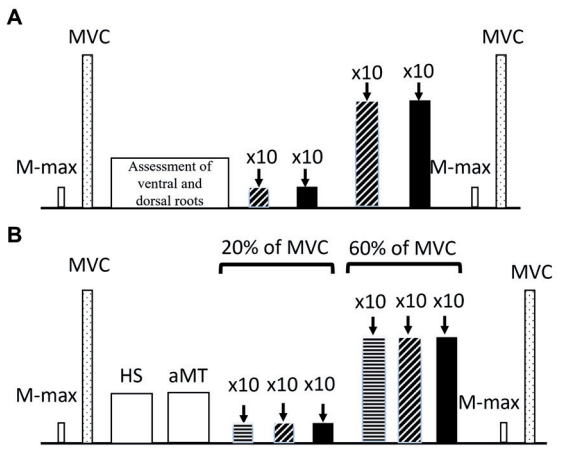

FIGURE 2
Representation of **(A)** lumbar session settings, low intensity (diagonal stripes) and high intensity (filled up bars). and **(B)** TMS session settings with 120% aMT (horizontal stripes), 140% aMT (diagonal stripes) and 160% aMT (filled bars). M-max: maximal compound action potential, MVC: Maximal voluntary contraction, HS, hotspot; aMT, active motor threshold.

testing sessions: two of them were dedicated for Lumbar Stimulation (LS) and the other two for TMS stimulation. One session of each stimulation method was performed 10–14 days prior to the second one. For each participant, sessions were performed at the same time of day. TMS was performed at least 48 h after LS.

To assess responses in RF, participants sat in a custom-built chair with a calibrated load cell (Faculty of Sport and Health Sciences, University of Jyväskylä, Finland) with hip and knee at 90° flexion and the shin strapped by a non-elastic restraint ~2 cm superior to the ankle malleoli (Figure 1). The voltage signal originating from the load cell was calibrated and converted into torque (N·m). All measures were performed on the right (i.e., dominant) leg.

Every session followed the same structure. Once the participant was secured to the dynamometer, the maximum compound action potential (M-max) was assessed in a relaxed condition (i.e., M-maxpre). Two maximal voluntary contraction (MVC) trials were performed 60 s apart (i.e., MVCpre). Prior to the MVC, two contractions at ~50% and ~ 80% of estimated MVC were performed as a warm-up. Verbal encouragement and visual feedback were provided to motivate participants to produce maximal effort and torque was recorded.

In every testing session, visual feedback was provided to the participants to produce the required submaximal torque and then a single TMS/LS stimulus was delivered manually. Contractions at 20 and 60% of MVC were held for 5–8 s. Sets of 10 stimulations were given per condition and per contraction level as a single block, giving a total of 40 LS and 60 TMS stimulations. To avoid fatigue, 30 s and 45 s rest was given between contractions during 20 and 60% of MVC, respectively, and 60 s and 180 s rest was given between the sets of 10 contractions. At the end of the protocol, M-max (M-maxpost) and MVC (MVCpost) were re-assessed (Figure 2).

Peripheral nerve stimulation

Transcutaneous electrical stimulation of the femoral nerve (32 mm cathode/anode arrangement; Polar Neurostimulation Electrodes, Espoo, Finland) was performed to elicit M-max in RF (1 ms pulse duration; Digitimer DS7AH, Hertfordshire, United Kingdom). Electrodes were placed 2 cm apart and placed at each side of the femoral nerve, located by palpation and identification of the femoral artery (Walker et al., 2016). M-max was elicited by gradually increasing stimulator output intensity until the EMG response plateaued. To ensure supramaximality, this intensity was further increased by 50% (Table 1).

Transcranial magnetic stimulation

Single TMS pulses were delivered using a Magstim 200^2 magnetic stimulator (Magstim Co., Ltd., Whitland, United Kingdom) connected to a concave double-cone coil, positioned over the left cortical hemisphere for RF with a posterior-to-anterior current orientation, to elicit MEPs. The hotspot was defined, at rest, as the position eliciting the largest visible MEP recorded in the EMG using the same intensity (approx. 50–70% stimulator output). Once the hotspot was found, the coil position was marked on the scalp to maintain the same position throughout the protocol. Active motor threshold (aMT) was determined by increasing stimulator intensity in 5% steps, starting at 30% of the stimulator output. Thereafter, stimulator intensity was decreased in steps of 1% until clear MEPs (>100 μV) were elicited in 3 out of 5 stimulations during 10% of MVC. Sets of 10 single TMS

TABLE 1 Mean and standard deviation values of torque, peripheral nerve stimulation, TMS and LS parameters from the participants during test sessions 1 and 2.

		Test 1	SD	Test 2	SD	Value of *p*	95% CI	Hedges' *g*
	MVC (n.m)	175	60	179	62	0.286	[−10, 3]	−0.06
Peripheral								
	Peripheral							
	M-max (mV)	1.99	1.17	1.91	0.88	0.694	[−0.17, 0.30]	0.08
	Peripheral Stim. intensity (mA)	220	93	229	79	0.698	[−61, 42]	−0.10
TMS								
	aMT (%)	35	9	35	8	0.555	[−2, 1]	0.00
LS								
	25% M-max Stim. intensity (mA)	240	98	231	92	0.577	[−18, 31]	0.09
	LEP onset 25% M-max latency (ms)	8.5	1.4	8.5	1.8	0.685	[−3.5, 5.2]	0.00
	50% M-max Stim. intensity (mA)	274	104	273	104	0.958	[−36, 34]	0.01
	LEP onset 50% M-max latencies (ms)	8.4	1.6	8.3	1.8	0.647	[−3.5, 5.5]	0.06

SD, standard deviation; CI, confidence interval; MVC, maximal voluntary contraction; TMS, transcranial magnetic stimulation; LS, lumbar stimulation; M-max, maximal compound action potential; aMT, active motor threshold; LEP, lumbar evoked potential; Stim., stimulation.

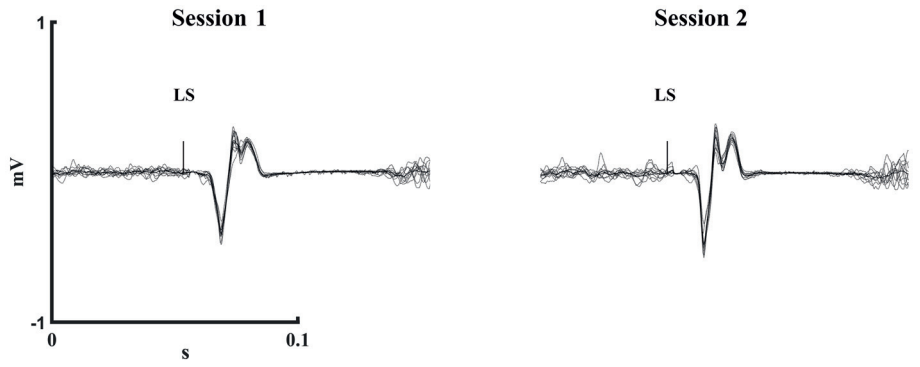

FIGURE 3
One participant's mean (solid) and individual (dashed) trials of low intensity LEPs at 20% of MVC during test session 1 and 2. LS, Lumbar stimulation.

stimulations were delivered in a random order for each of the assigned conditions (i.e., 120, 140, and 160% aMT).

Lumbar stimulation

Transcutaneous electrical lumbar stimulation was used to elicit LEPs with a constant-current stimulator (1 ms pulse duration; Digitimer DS7AH, Hertfordshire, United Kingdom) via self-adhesive electrodes (Polar Neurostimulation Electrodes, Espoo, Finland). The cathode (5 × 10 cm) was centered over the first lumbar vertebra (L_1) and the anode (circular shape; 5 cm diameter) was placed on the midline of the vertebral column ~5 cm above the top edge of the cathode as described by Škarabot et al. (2019a).

The intensity of stimulation was standardized to 25% or 50% of the M-max evoked in the resting position (Table 1). Potential activation of ventral roots was assessed by examining the onset latency of the LEP with an increase in stimulator intensity (Petersen et al., 2002) and tracking LEP amplitude during increased voluntary contraction (Taylor et al., 2002). Onset latency would be expected to shorten when increasing stimulation intensity and LEP amplitude would have remained consistent during higher contraction intensities should the ventral roots be activated (Petersen et al., 2002; Taylor et al., 2002, 2006; Škarabot et al., 2019b). To ensure the placement was the same in both sessions, the distance from the 7th cervical vertebra (C7) to the anode and from the bottom of the anode to the top of the cathode (i.e., inter-electrode distance) were taken. Five out of the 27 participants showed no increase in LEP amplitude with an increase in voluntary torque, and they were, therefore, removed from further analyses.

Dorsal root activation was assessed via paired LS with a 50 ms time delay, where the second LEP amplitude was compared to the first. Evidence of dorsal root activation would be a decrease in the second LEP compared to the first due to post-activation depression at the motor-neuron pool from the first stimulus to the second (Hofstoetter et al., 2018). All remaining participants showed no sign of the responses described and reported that they found LS to be tolerable. Once the placement was confirmed, stimulator intensity was adjusted to that which produced a LEP of 25% (Low intensity) or 50% (High intensity) of the M-max at rest, and these stimulation intensities were used throughout the experiment (Figure 3).

Bipolar surface electromyography and torque

Muscle activity was recorded using adhesive Ag/AgCl electrodes (30 × 20mm, BlueSensor N, Ambu, Penang, Malaysia) from m.Bicep Femoris (BF) and RF according to SENIAM guidelines (Hermens et al., 2000). Skin was shaved, abraded with sandpaper, and wiped with alcohol before setting the electrodes in bipolar arrangement with 2 cm center-to-center distance. Impedance was set <2 kΩ, and the reference electrode was positioned above the patella. EMG data were sampled online at 3000 Hz, amplified (1000×) and bandpass filtered (16–1,000 Hz; Neurolog System, Digitimer Ltd., United Kingdom) using CED Power1401-3 (Cambridge Electronic Design Ltd., Cambridge, United Kingdom).

Torque was sampled at 1000 Hz, amplified by a custom-built amplifier (ForAmps 1 v1.2, University of Jyväskylä, Finland) and converted by a 16-bit A/D board (CED Power1401-3, Cambridge Electronics Design, Cambridge, United Kingdom) in combination with Spike2 software (version 6.10, Cambridge Electronic Design, Cambridge, United Kingdom).

Data and statistical analyses

Offline analyses were performed with Spike2 software (version 6.10, Cambridge Electronic Design, Cambridge, United Kingdom) to manually obtain M-max amplitude, MVC, and unconditioned LEP onset latencies. The other outcome measures were analyzed by a customized MATLAB script (version R2020b, The MathWorks, Inc., Natick, United States). Peak-to-peak amplitude of MEPs and LEPs were analyzed automatically between latencies-of-interest following TMS or LS (Taylor et al., 1999), respectively. Silent period and onset latencies were analyzed semi-automatically, by visually selecting the end of the SP and the onset for MEPs and LEPs. The median values for each set of 10 MEPs, LEPS, MEP SPs and LEP SPs were calculated, as the median is less sensitive to outliers. Torque and Root Mean Square (RMS) of the EMG were averaged over the 100 ms before the stimulator artefact (Škarabot et al., 2019c). SP duration was determined, through visual inspection, as the time from the stimulator artefact to the return of voluntary EMG (Damron et al., 2008). Normalization of MEP and LEP amplitude was performed by normalizing to M-max (Single *N*) or to M-max and then RMS (Double *N*). Double *N* is typically performed to avoid the possibility that the background EMG level might modify the MEP or LEP (Sidhu et al., 2013; Škarabot et al., 2019a).

SPSS software (version 26.0, SPSS Inc., Chicago, United States) was used for all statistical methods. Means and standard deviation (SD) were calculated and reported throughout. Normality of the data was tested with the Shapiro–Wilk test and confirmed by z-score with an acceptance of +2 to −2 (e.g., skewness score/skewness score$_{SE}$ and kurtosis score/kurtosis score$_{SE}$). Data that did not fulfil those requirements: 20% of MVC Single N at low intensity, 120% aMT and 160% aMT; double N at low intensity, high intensity, 120% aMT, 140% aMT and 160% aMT; SP at low intensity, 140% aMT and 160% aMT; 60% of MVC SP at 160% aMT were Log10 transformed, which then fulfilled the requirements for normality. Paired *t*-tests were used to examine difference between means trials of Single N and Double N for MEP and LEP amplitude and SP. Statistical significance was accepted at alpha <0.05. Between-group effect sizes are represented as Hedge's *g* for the relative changes over time (g = small: <0.3, medium: 0.3–0.8, large: >0.8). Relative reliability, as the degree to which individuals maintain their position in a sample with repeated measurements, of TMS and LS were assessed using Intraclass Correlation coefficient (ICC). Absolute reliability, as the degree to which repeated measurements vary within individuals, was assessed using typical error (TE), coefficient of variance (CV) and standard error of the measurement (SEM) calculated as: averaged SD of test 1 and test 2 $\times \sqrt{1 - \text{ICC}}$ (Portney, 2020) expressed in ratio (Single N or Double N) or time (SP) for MEPs and LEPs (Atkinson and Nevill, 1998; Portney, 2020). The minimal detectable change (MDC) was calculated as SEM $\times$ 1.96 $\times \sqrt{2}$. Reliability, based on ICCs and their 95% CIs, was categorized as poor (ICC < 0.5), moderate (ICC: > 0.5 - < 0.75), good (ICC: > 0.75 - < 0.9) and excellent (ICC: > 0.9) (Koo and Li, 2016). Bland–Altman plots of LEPs and MEPs in all conditions were used to assess the agreement between the two sessions.

Results

Control measurements

As shown in Table 1, stimulation variables that could potentially influence changes at cortical and spinal levels were assessed for potential differences between session 1 and session 2. None of the variables assessed were statistically significant and, thus, were stable from one test session to the next.

Reliability of lumbar evoked potentials at different submaximal contraction levels

There were no significant changes in LEP amplitude elicited at any intensity from test 1 to test 2, regardless of whether Single *N* or Double *N* was used, at any contraction level (Table 2). All reliability values for LS can be found in Table 3. Good reliability was found in Double N for LEPs elicited at high intensity during 20% of MVC (ICC = 0.847) and at low intensity during 60% of MVC (ICC = 0.828), while moderate reliability was found for the rest of the conditions (Table 3). CVs for Single N was 23% for lower intensities, independent of the contraction level, whereas at high intensities CVs of 29 and 33% were observed. SEM for Single *N* was between 0.07–0.17 and MDC was between 0.20–0.47. CV for Double N was between 30–39%. SEM was between 2–10 and MDC was between 6–27. Bland–Altman plots showed a good agreement between test 1 and test 2 for all LS conditions (Figures 4A–D). Low intensity stimulation during 20% of MVC showed a mean bias of −0.002 and 95% limits of agreement [−0.24, 0.24] (Figure 4A).

Reliability of motor evoked potentials at different contraction levels

MEP amplitude elicited at all intensities did not show any changes ($p > 0.05$) from test 1 to test 2, regardless of which normalization or contraction level was used (Table 4). Excellent reliability was found in

TABLE 2 Mean and standard deviation, 95% confidence intervals, effect sizes and results of paired *t*-test analyses for Single *N* and Double *N* LEP amplitudes and LEP SP comparisons between test sessions 1 and 2.

		Test 1	SD	Test 2	SD	value of p	95% CI	Hedges' *g*
20% of MVC								
	Low intensity							
	Single *N* (LEP/M-max)	0.352	0.106	0.354	0.135	0.95	[−0.056, 0.053]	−0.02
	Double *N* (LEP/M-max/RMS)	19.1	13.7	17.8	13.1	0.45	[−2.1, 4.6]	0.09
	Silent period (ms)	79	12	77	12	0.26	[−2, 6]	0.16
	High intensity							
	Single *N* (LEP/M-max)	0.597	0.219	0.566	0.226	0.48	[−0.060, 0.122]	0.14
	Double *N* (LEP/M-max/RMS)	29.0	22.5	29.0	27.2	0.99	[−4.2, 4.3]	0.00
	Silent period (ms)	87	15	86	12	0.62	[−3, 5]	0.07
60% of MVC								
	Low intensity							
	Single *N* (LEP/M-max)	0.51	0.184	0.454	0.189	0.115	[−0.012, 0.102]	0.29
	Double *N* (LEP/M-max/RMS)	7.9	6.2	7.0	4.8	0.110	[−0.3, 2.6]	0.15
	Silent period (ms)	69	14	67	16	0.348	[−2, 7]	0.13
	High intensity							
	Single *N* (LEP/M-max)	0.717	0.26	0.655	0.311	0.193	[−0.031, 0.143]	0.21
	Double *N* (LEP/M-max/RMS)	11.4	9.2	10.1	6.7	0.300	[−1.8, 5.5]	0.16
	Silent period (ms)	68	10	67	9	0.528	[−2, 4]	0.10

SD, standard deviation; CI, confidence interval; LS, lumbar stimulation; LEP, lumbar evoked potential; M-max, maximal compound action potential; RMS, root mean square.

TABLE 3 Between-session test–retest reliability for Single *N* and Double *N* LEP amplitudes and LEP SP with ICC, TE, SEM, and MDC.

		TE [95%CI]		CV% [95% CI]		ICC [95% CI]		SEM	MDC
20% of MVC									
	Low intensity								
	Single *N* (LEP/M-max)	0.09	[0.07–0.12]	23.0	[17.3–34.5]	0.632	[0.29–0.83]	0.07	0.20
	Double *N* (LEP/M-max/RMS)	5.3	[4.1–7.7]	38.5	[28.5–59.3]	0.737	[0.46–0.88]	6.86	19.02
	Silent period (ms)	5.9	[4.6–8.5]	7.5	[5.7–10.9]	0.713	[0.42–0.87]	6.43	17.82
	High intensity								
	Single *N* (LEP/M-max)	0.13	[0.10–0.20]	33.4	[24.3–53.2]	0.520	[0.09–0.79]	0.15	0.43
	Double *N* (LEP/M-max/RMS)	6.3	[4.7–9.3]	30.0	[22.0–47.5]	0.847	[0.64–0.94]	9.71	26.90
	Silent period (ms)	5.4	[4.1–8.1]	6.7	[5.0–10.0]	0.830	[0.60–0.93]	5.57	15.43
60% of MVC									
	Low intensity								
	Single *N* (LEP/M-max)	0.09	[0.07–0.13]	22.8	[17.1–34.1]	0.749	[0.48–0.89]	0.09	0.26
	Double *N* (LEP/M-max/RMS)	2.3	[1.8–3.3]	31.9	[23.8–48.5]	0.828	[0.62–0.93]	2.27	6.30
	Silent period (ms)	7.3	[5.7–10.5]	11.8	[9.0–17.3]	0.710	[0.41–0.87]	8.08	22.39
	High intensity								
	Single *N* (LEP/M-max)	0.13	[0.10–0.19]	28.8	[21.1–45.4]	0.643	[0.24–0.82]	0.17	0.47
	Double *N* (LEP/M-max/RMS)	5.4	[4.1–7.9]	39.5	[28.6–63.7]	0.742	[0.43–0.90]	4.04	11.20
	Silent period (ms)	5.1	[3.9–7.6]	7.9	[5.9–11.9]	0.750	[0.44–0.90]	4.75	13.17

TE, typical error; CI, confidence interval; CV, coefficient of variance; ICC, intra-class correlation; SEM, standard error of the measurement; MDC, minimal detectable change; MVC, maximal voluntary contraction; LS, lumbar stimulation; LEP, lumbar evoked potential; M-max, maximal compound action potential; RMS, root mean square.

Single N for all the TMS conditions (ICC > 0.900) and at 140% aMT for Double N (ICC = 0.926) at 60% of MVC. Good reliability was found for the rest of the conditions (Table 5). CVs for Single N was between 20–26% during 20% of MVC, whereas 60% of MVC showed CVs of 14–18%. SEM for Single N was between 0.09–0.13 and MDC was between 0.24–0.36. CV for Double N was between 29–35% for

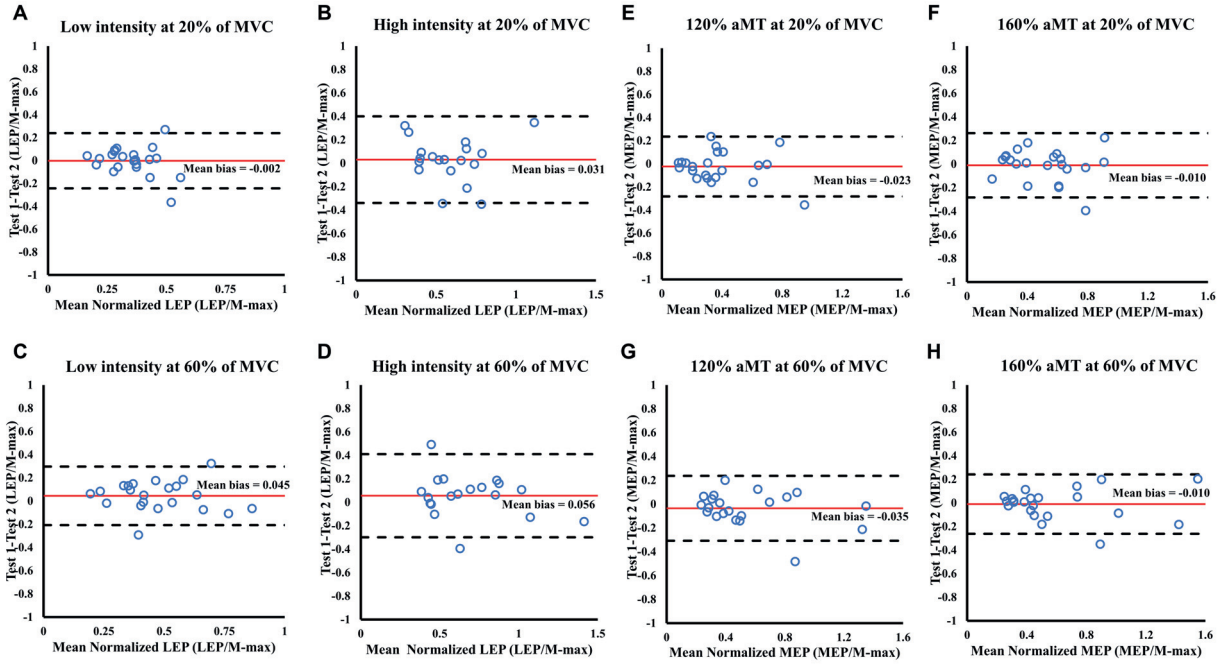

FIGURE 4
Bland Altman plots showing the level of agreement for LEP **(A–D)** and MEP **(E–H)** amplitude during 20 and 60% of MVC between test sessions 1 and 2. Each panel shows the differences as a function of the average of the two testing sessions indicating the mean bias (solid line) and the 95% limits of agreement (dashed lines).

TABLE 4 Mean and standard deviation, 95% confidence intervals, effect sizes and results of paired *t*-test analyses for Single *N* and Double *N* MEP amplitudes and MEP SP comparisons between test sessions 1 and 2.

		Test 1	SD	Test 2	SD	value of p	95% CI	Hedges' *g*
20% of MVC								
	120% aMT							
	Single *N* (MEP/M-max)	0.366	0.223	0.389	0.48	0.421	[−0.082, 0.035]	−0.06
	Double *N* (MEP/M-max/RMS)	19.0	19.4	18.1	10.5	0.656	[−3.4, 5.3]	0.06
	Silent period (ms)	107	19	112	16	0.031	[−9, −1]	−0.28
	140% aMT							
	Single *N* (MEP/M-max)	0.472	0.195	0.479	0.224	0.812	[−0.062, 0.049]	−0.03
	Double *N* (MEP/M-max/RMS)	29.6	30.2	27.0	23.3	0.405	[−3.7, 8.7]	0.09
	Silent period (ms)	127	27	131	27	0.106	[−11, 1]	−0.15
	160% aMT							
	Single *N* (MEP/M-max)	0.509	0.224	0.519	0.240	0.742	[−0.072, 0.052]	−0.04
	Double *N* (MEP/M-max/RMS)	28.0	25.2	26.9	21.0	0.653	[−3.9, 6.2]	0.05
	Silent period (ms)	143	31	144	28	0.468	[−7, 3]	−0.03
60% of MVC								
	120% aMT							
	Single *N* (MEP/M-max)	0.54	0.317	0.558	0.351	0.246	[−0.097, 0.026]	−0.05
	Double *N* (MEP/M-max/RMS)	9.4	9.3	8.8	7.3	0.520	[−1.4, 2.7]	0.08
	Silent period (ms)	106	22	107	17	0.463	[−7, 3]	−0.05
	140% aMT							
	Single *N* (MEP/M-max)	0.552	0.321	0.582	0.366	0.399	[−0.101, 0.042]	−0.09
	Double *N* (MEP/M-max/RMS)	9.3	8.3	10.0	8.8	0.442	[−2.3–1.1]	−0.07
	Silent period (ms)	122	23	124	19	0.245	[−8, 2]	−0.09
	160% aMT							
	Single *N* (MEP/M-max)	0.587	0.368	0.597	0.372	0.723	[−0.067, 0.047]	−0.03
	Double *N* (MEP/M-max/RMS)	10.0	9.1	9.8	8.2	0.810	[−1.7, 2.1]	0.03
	Silent period (ms)	140	35	145	34	0.081	[−10, 1]	−0.14

SD, standard deviation; CI, confidence interval; TMS, transcranial magnetic stimulation; M-max, maximal compound action potential; RMS, root mean square; aMT, active motor threshold; MEP, motor evoked potential.

TABLE 5 Between-session test–retest reliability for Single *N* and Double *N* MEP amplitudes and MEPSP with ICC, TE, SEM, and MDC.

		TE [95%CI]		CV% [95% CI]		ICC [95% CI]		SEM	MDC
20% of MVC									
	120% aMT								
	Single *N* (MEP/M-max)	0.09	[0.07–0.13]	26.1	[19.5–39.3]	0.861	[0.69–0.94]	0.13	0.36
	Double *N* (MEP/M-max/RMS)	7.0	[5.4–10.0]	34.8	[25.9–53.3]	0.816	[0.60–0.92]	6.40	17.74
	Silent period (ms)	7.2	[5.5–10.3]	7.2	[5.5–10.4]	0.820	[0.61–0.92]	7.42	20.58
	140% aMT								
	Single *N* (MEP/M-max)	0.09	[0.07–0.13]	20.6	[15.5–30.7]	0.831	[0.63–0.93]	0.09	0.24
	Double *N* (MEP/M-max/RMS)	9.9	[7.6–14.2]	29.2	[21.8–44.3]	0.891	[0.75–0.95]	8.83	24.47
	Silent period (ms)	9.3	[7.2–13.3]	7.9	[6.0–11.5]	0.840	[0.64–0.93]	10.80	29.94
	160% aMT								
	Single *N* (MEP/M-max)	0.10	[0.08–0.14]	24.0	[17.9–35.9]	0.821	[0.61–0.92]	0.10	0.27
	Double *N* (MEP/M-max/RMS)	8.1	[6.2–11.5]	29.9	[22.3–45.3]	0.851	[0.67–0.94]	8.93	24.74
	Silent period (ms)	7.7	[6.0–11.1]	5.5	[4.2–8.0]	0.920	[0.81–0.97]	8.34	23.13
60% of MVC									
	120% aMT								
	Single *N* (MEP/M-max)	0.1	[0.08–0.14]	18.4	[13.9–27.4]	0.901	[0.77–0.96]	0.11	0.29
	Double *N* (MEP/M-max/RMS)	3.3	[2.5–4.7]	28.0	[20.9–42.2]	0.896	[0.76–0.96]	2.67	7.40
	Silent Period (ms)	8.2	[6.3–11.7]	8.2	[6.2–11.9]	0.820	[0.60–0.92]	8.27	22.93
	140% aMT								
	Single *N* (MEP/M-max)	0.11	[0.09–0.16]	15.5	[11.7–22.9]	0.922	[0.82–0.97]	0.10	0.27
	Double *N* (MEP/M-max/RMS)	2.7	[2.1–3.9]	22.5	[16.9–33.7]	0.926	[0.83–0.97]	2.33	6.45
	Silent period (ms)	7.9	[6.1–11.3]	7.0	[5.3–10.1]	0.835	[0.64–0.93]	8.53	23.64
	160% aMT								
	Single *N* (MEP/M-max)	0.09	[0.70–0.13]	14.0	[10.6–20.6]	0.941	[0.86–0.98]	0.09	0.25
	Double *N* (MEP/M-max/RMS)	3.03	[2.3–4.3]	27.1	[20.3–40.9]	0.898	[0.77–0.96]	2.76	7.65
	Silent period (ms)	8.5	[6.6–12.2]	6.5	[4.9–9.4]	0.920	[0.81–0.97]	9.76	27.05

TE, typical error; CI, confidence interval; CV, coefficient of variance; ICC, intra-class correlation; SEM, standard error of the measurement; MDC, minimal detectable change; MVC, maximal voluntary contraction; M-max, maximal compound action potential; RMS, root mean square; TMS, transcranial magnetic stimulation; aMT, active motor threshold; MEP, motor evoked potential.

lower contraction levels. SEM was between 6–9 and MDC was between 6–25. Bland–Altman plots showed good agreement between test 1 and 2, with a low ratio (MEP/M-max) for the mean bias (−0.010) and data within the 95% limits of agreement (Figures 4E–H).

Reliability of silent period durations at different torque levels

SP showed a statistically significant difference at 120% aMT during 20% of MVC ($p = 0.031$) between test 1 and test 2, although the effect size was small (Hedges' $g = -0.28$). No other condition showed any significant changes (Tables 2, 4). Moderate reliability was observed for low intensity LS at all contraction levels (Table 3). Excellent reliability was found for SP elicited by TMS at 160% aMT during 20 and 60% of MVC (ICC: 0.920 for both). Good reliability was found for high intensity LS and the rest of the TMS conditions and at any contraction level. CV for LS was between 7–12% and CV was between 6–8% for TMS. SEM for LS was between 13–22 and MDC was between 13–18. SEMs for TMS were between 7–10 and MDCs were 21–30. Bland–Altman plots showed good agreement between test 1 and test 2 regardless of the stimulation method, intensity, or contraction level (Figure 5).

Discussion

The aim of the present study was to assess the reliability of MEP and LEP measures of cortico-spinal excitability during different submaximal contraction levels in the RF muscle of healthy adults. Our findings indicate that the use of MEP amplitudes normalized to M-max (Single *N*) and M-max/RMS (Double *N*) are reliable methods. In addition, Single *N* and Double *N* LEP amplitudes showed moderate reliability. Furthermore, MEP and LEP silent periods showed good-to-very good reliability. Moreover, small magnitude systematic bias demonstrated that MEPs, LEPs and their SPs are reliable tools to measure the cortico-spinal tract.

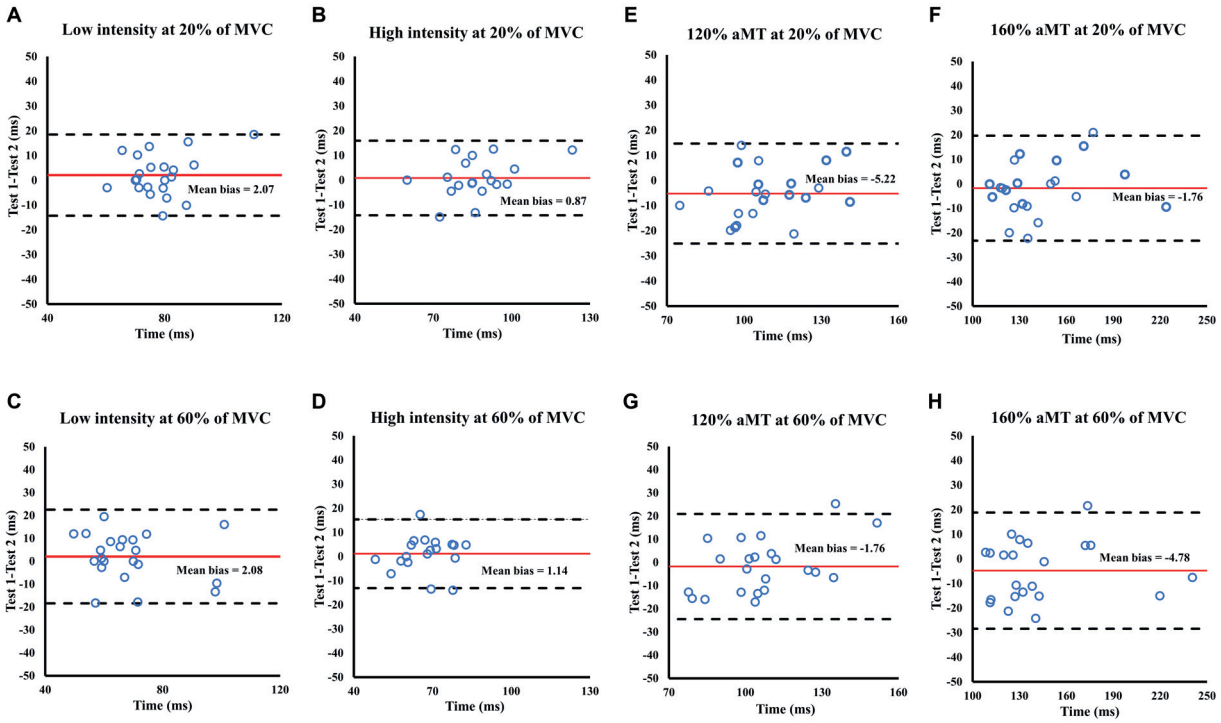

FIGURE 5
Bland Altman plots showing the level of agreement for LEP **(A–D)** and MEP **(E–H)** SPs during 20 and 60% of MVC between test sessions 1 and test 2. Each panel shows the differences as a function of the average of the two testing sessions indicating the mean bias (solid line) and the 95% limits of agreement (dashed lines).

Lumbar stimulation

This is the first study reporting LEP reliability during different submaximal contraction levels. LS can elicit a LEP in the target muscle, which represents the excitability of the motor-neuron (Škarabot et al., 2019b; Brownstein et al., 2021). Our results suggest moderate-to-good reliability of LEPs with 10 stimulations during different contraction levels and stimulator output intensities, independent of the normalization procedure. Despite the moderate reliability shown for some conditions (low intensity during 20% of MVC with Single N), these LEP values are within the range reported by previous MEP reliability studies investigating the lower limbs (ICC = 0.6–0.9) (Brownstein et al., 2018; Leung et al., 2018; Malcolm et al., 2021). Furthermore, the CV reported in the present study are lower than ones reported previously (e.g., 59% CV in Ansdell et al., 2020) for LEPs normalized to M-max. It is, however, important to mention that MDC was more than 100% in some conditions with Double *N*. Thus, LS could be used as a tool to understand spinal excitability in the lower limbs in various experiments typical in clinical neurophysiology or exercise science fields (e.g., pharmacological treatment, training intervention, fatigue intervention, balance perturbation).

Transcranial magnetic stimulation reliability

MEPs elicited by TMS showed very good-to-excellent reliability (0.82–0.94), depending on the normalization procedure and the contraction level. In our study, reliability was good during 20% of MVC and excellent during 60% of MVC for Single N. Interestingly, Brownstein et al. (2018) reported ICC of 0.77–0.87 in RF during 10% of MVC, Temesi et al. (2017) reported ICC > 0.80 for MEPs elicited in RF during 20% of MVC but poor reliability (ICC = 0.59) was observed by Malcolm et al. (2021) who used maximal voluntary contractions. Malcolm et al. (2021) proposed some factors for their poor reliability at high contractions intensities, such as high variability of individual EMG between measurement sessions, motor units synchronization and signal cancelation, and intrinsic fluctuation in cortical and spinal excitability. Particularly during higher contraction intensities (>75% of MVC), the firing rate of motor-neurons increases, and with an increase in refractory period that could reduce the magnitude of the MEP (Todd et al., 2003; Goodall et al., 2009). MEPs increase their size with increasing contraction intensity seemingly up to 50–75% of MVC (Martin et al., 2006; Oya et al., 2008; Goodall et al., 2009; Škarabot et al., 2019b) depending on the muscle. In the present study, 60% of MVC was used. Therefore, the MEP reliability could have benefited from testing at this contraction intensity and the lower level of motor-neuron activation compared to the maximal voluntary contraction used in Malcolm et al. (2021). Consequently, these factors could have led to a reduction in variability and higher ICC values in the present study than those reported by Malcolm et al. (2021).

Furthermore, our results have similar or even lower CV than those reported during 10% (CV = 18–20%) (Brownstein et al., 2018; Leung et al., 2018) and 20% (CV = 21%) (O'Leary et al., 2015) of MVC. Moreover, the values for systematic bias reported in the present study suggest that TMS-elicited responses during 20 and 60% of MVC are a reliable tool to measure the cortico-spinal tract, for example, in

studies expecting changes in the magnitude of 0.24–0.36 for Single *N* and 6–25 for Double *N* (Leung et al., 2018).

Silent period reliability

The duration of the silent period can provide information about the inhibition at the cortical or spinal level (Inghilleri et al., 1993). Reliability of LS-elicited SP were moderate and good, which were slightly higher than the TMS-elicited SP reported by Di Virgilio et al. (2022). Furthermore, CV were similar to those reported previous (CV = 7–15%) (O'Leary et al., 2015; Leung et al., 2018; Di Virgilio et al., 2022). Moreover, reliability for TMS-elicited SP at different stimulation intensities and contraction level were good and excellent, respectively. Our results were in concordance with other reported in by other groups (O'Leary et al., 2015; Leung et al., 2018; Pagan et al., 2023). Furthermore, the CV of the TMS-elicited SP was within the ranges mentioned above. Therefore, our results suggest that SP could be used to understand inhibitory process at cortical and spinal segments by utilizing both TMS and LS concurrently.

Strength and limitations

This study is the first to provide reliability statistics for two methods to assess cortico-spinal and spinal excitability during different submaximal contraction levels and stimulation intensities. Although previous studies have reported the reliability of MEPs at low submaximal contraction levels, this is the first that provides reliability for submaximal contraction levels higher than 20% of MVC (Temesi et al., 2017; Brownstein et al., 2018; Leung et al., 2018). Moreover, this is the first study reporting reliability of LEPs at different submaximal contraction levels. This study also provides reliability data of a normalization technique for MEPs and LEPs that aims to take into account the possible effect of EMG background activity on the induced responses (Sidhu et al., 2013; Škarabot et al., 2019c).

In terms of limitations, the number of stimuli might have been a possible factor for the LEPs moderate reliability. Although studies that have reported LEPs have used 10 stimuli, there is evidence from MEP reliability studies reporting that an increase in number of stimuli (>15) could improve reliability of MEPs (Bastani and Jaberzadeh, 2012; Cavaleri et al., 2017; Brownstein et al., 2018).

In conclusion, the results suggest that MEPs and LEPs are reliable tools to assess different segments of the cortico-spinal tract during different contraction levels and stimulator output intensities, independent of the normalization procedure. Thus, it may not be necessary to account for background EMG during TMS or LS stimulation when normalized to a valid maximal compound action potential. Furthermore, the TMS- and LS-elicited SP has also shown to be a reliable tool considered to reflect inhibitory processes at cortical and spinal levels.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the Petteri Niemi University of Jyväskylä. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

GG-G, JA, and SW: conceptualization. GG-G, ME, EH, PA, and GH: piloting and lab set up. GG-G, ME, EH, PA, and SW: data collection and data analysis. GG and SW: writing original draft. GG-G, PA, GH, JA, and SW: writing-reviewing-editing. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2023.1239982/full#supplementary-material

References

Ansdell, P., Brownstein, C. G., Škarabot, J., Angius, L., Kidgell, D., Frazer, A., et al. (2020). Task-specific strength increases after lower-limb compound resistance training occurred in the absence of corticospinal changes in vastus lateralis. *Exp. Physiol.* 105, 1132–1150. doi: 10.1113/EP088629

Atkinson, G., and Nevill, A. M. (1998). Measurement error (reliability) in variables relevant to. *Sports Med.* 26, 217–238. doi: 10.2165/00007256-199826040-00002

Barker, A. T., Jalinous, R., and Freeston, I. L. (1985). Non-invasive magnetic stimulation of human motor cortex. *Lancet* 325, 1106–1107. doi: 10.1016/S0140-6736(85)92413-4

Bastani, A., and Jaberzadeh, S. (2012). A higher number of TMS-elicited MEP from a combined hotspot improves intra- and inter-session reliability of the upper limb muscles in healthy individuals. *PLoS One* 7:e47582. doi: 10.1371/journal.pone.0047582

Beaulieu, L. D., Flamand, V. H., Massé-Alarie, H., and Schneider, C. (2017). Reliability and minimal detectable change of transcranial magnetic stimulation outcomes in healthy adults: a systematic review. *Brain Stimul.* 10, 196–213. doi: 10.1016/j.brs.2016.12.008

Brownstein, C. G., Ansdell, P., Škarabot, J., Howatson, G., Goodall, S., and Thomas, K. (2018). An optimal protocol for measurement of corticospinal excitability, short intracortical inhibition and intracortical facilitation in the rectus femoris. *J. Neurol. Sci.* 394, 45–56. doi: 10.1016/j.jns.2018.09.001

Brownstein, C. G., Espeit, L., Royer, N., Ansdell, P., Škarabot, J., Souron, R., et al. (2021). Reductions in motoneuron excitability during sustained isometric contractions are dependent on stimulus and contraction intensity. *J. Neurophysiol.* 125, 1636–1646. doi: 10.1152/jn.00070.2021

Brownstein, C. G., Souron, R., Royer, N., Singh, B., Lapole, T., and Millet, G. Y. (2020). Disparate kinetics of change in responses to electrical stimulation at the thoracic and lumbar level during fatiguing isometric knee extension. *J. Appl. Physiol.* 128, 159–167. doi: 10.1152/japplphysiol.00635.2019

Butler, J. E., Taylor, J. L., and Gandevia, S. C. (2003). Responses of human Motoneurons to corticospinal stimulation during maximal voluntary contractions and ischemia. *J. Neurosci.* 23, 10224–10230. doi: 10.1523/JNEUROSCI.23-32-10224.2003

Cavaleri, R., Schabrun, S. M., and Chipchase, L. S. (2017). The number of stimuli required to reliably assess corticomotor excitability and primary motor cortical representations using transcranial magnetic stimulation (TMS): a systematic review and meta-analysis. *Syst. Rev.* 6, 1–11. doi: 10.1186/s13643-017-0440-8

Damron, L. A., Dearth, D. J., Hoffman, R. L., and Clark, B. C. (2008). Quantification of the corticospinal silent period evoked via transcranial magnetic stimulation. *J. Neurosci. Methods* 173, 121–128. doi: 10.1016/j.jneumeth.2008.06.001

Day, B. L., Dressler, D., Maertens de Noordhout, A., Marsden, C. D., Nakashima, K., Rothwell, J. C., et al. (1989). Electric and magnetic stimulation of human motor cortex: surface EMG and single motor unit responses. *J. Physiol.* 412, 449–473. doi: 10.1113/jphysiol.1989.sp017626

Di Virgilio, T. G., Ietswaart, M., Selvamoorthy, R., and Hunter, A. M. (2022). The reliability of transcranial magnetic stimulation-derived Corticomotor inhibition as a brain health evaluation tool in soccer players. *Sport. Med. Open* 8:7. doi: 10.1186/s40798-021-00399-3

Gandevia, S. C., Petersen, N., Butler, J. E., and Taylor, J. L. (1999). Impaired response of human motoneurones to corticospinal stimulation after voluntary exercise. *J. Physiol.* 521, 749–759. doi: 10.1111/j.1469-7793.1999.00749.x

Goodall, S., Romer, L. M., and Ross, E. Z. (2009). Voluntary activation of human knee extensors measured using transcranial magnetic stimulation. *Exp. Physiol.* 94, 995–1004. doi: 10.1113/expphysiol.2009.047902

Hermens, H. J., Freriks, B., Disselhorst-Klug, C., and Rau, G. (2000). Developent of recommendations for SEMG sensors and sensor placement procedures. *J. Electromyogr. Kinesiol.* 10, 361–374. doi: 10.1016/S1050-6411(00)00027-4

Hoffman, B. W., Oya, T., Carroll, T. J., and Cresswell, A. G. (2009). Increases in corticospinal responsiveness during a sustained submaximal plantar flexion. *J. Appl. Physiol.* 107, 112–120. doi: 10.1152/japplphysiol.91541.2008

Hofstoetter, U. S., Freundl, B., Binder, H., and Minassian, K. (2018). Common neural structures activated by epidural and transcutaneous lumbar spinal cord stimulation: elicitation of posterior root-muscle reflexes. *PLoS One* 13, 1–22. doi: 10.1371/journal.pone.0192013

Houde, F., Leonard, G., Harvey, M.-P., Laroche, S., Thivierge, V., Olivares-Marchant, A., et al. (2018). Transcranial magnetic stimulation measures in the elderly: reliability, smallest detectable change and the potential influence of lifestyle habits. *Front. Aging Neurosci.* 10, 1–12. doi: 10.3389/fnagi.2018.00379

Inghilleri, M., Berardelli, A., Cruccu, G., and Manfredi, M. (1993). Silent period evoked by transcranial magnetic stimulation of the human cortex and Cervicomedullary junction. *J. Physiol.* 466, 521–534.

Kobayashi, M., and Pascual-Leone, A. (2003). Basic principles of magnetic stimulation. *Lancet* 2, 145–156. doi: 10.1016/S1474-4422(03)00321-1

Koo, T. K., and Li, M. Y. (2016). A guideline of selecting and reporting Intraclass correlation coefficients for reliability research. *J. Chiropr. Med.* 15, 155–163. doi: 10.1016/j.jcm.2016.02.012

Landin, D., Thompson, M., and Reid, M. (2016). Actions of two bi-articular muscles of the lower extremity: a review article. *J. Clin. Med. Res.* 8, 489–494. doi: 10.14740/jocmr2478w

Leung, H., Latella, C., Lamon, S., and Hendy, A. M. (2018). The reliability of neurological measurement in the vastus Medialis: implications for research and practice. *Front. Psychol.* 9:1857. doi: 10.3389/fpsyg.2018.01857

Lévénez, M., Garland, S. J., Klass, M., and Duchateau, J. (2008). Cortical and spinal modulation of antagonist coactivation during a submaximal fatiguing contraction in humans. *J. Neurophysiol.* 99, 554–563. doi: 10.1152/jn.00963.2007

Malcolm, R., Cooper, S., Folland, J., Tyler, C., Hannah, R., and Sunderland, C. (2021). Reliability of transcranial magnetic stimulation measurements of maximum activation of the knee extensors in young adult males. *Hum. Mov. Sci.* 78:102828. doi: 10.1016/j.humov.2021.102828

Martin, P. G., Butler, J. E., Gandevia, S. C., and Taylor, J. L. (2008). Noninvasive stimulation of human corticospinal axons innervating leg muscles. *J. Neurophysiol.* 100, 1080–1086. doi: 10.1152/jn.90380.2008

Martin, P. G., Gandevia, S. C., and Taylor, J. L. (2006). Output of human Motoneuron pools to corticospinal inputs during voluntary contractions. *J. Neurophysiol.* 95, 3512–3518. doi: 10.1152/jn.01230.2005

McNeil, C. J., Martin, P. G., Gandevia, S. C., and Taylor, J. L. (2009). The response to paired motor cortical stimuli is abolished at a spinal level during human muscle fatigue. *J. Physiol.* 587, 5601–5612. doi: 10.1113/jphysiol.2009.180968

Merton, P. A. (1951). The silent period in a muscle of the human hand. *J. Physiol.* 114, 183–198. doi: 10.1113/jphysiol.1951.sp004610

O'Leary, T. J., Morris, M. G., Collett, J., and Howells, K. (2015). Reliability of single and paired-pulse transcranial magnetic stimulation in the vastus lateralis muscle. *Muscle Nerve* 52, 605–615. doi: 10.1002/mus.24584

Oya, T., Hoffman, B. W., and Cresswell, A. G. (2008). Corticospinal-evoked responses in lower limb muscles during voluntary contractions at varying strengths. *J. Appl. Physiol.* 105, 1527–1532. doi: 10.1152/japplphysiol.90586.2008

Pagan, J. I., Harmon, K. K., Girts, R. M., Maclennan, R. J., Beausejour, J. P., Hernandez-Sarabia, J. A., et al. (2023). Sex-specific reliability of lower-limb corticospinal excitability and silent periods. *J. Strength Cond. Res.* 37, 1882–1887. doi: 10.1519/JSC.0000000000004525

Peri, E., Ambrosini, E., Colombo, V. M., van de Ruit, M. L., Grey, M. J., Monticone, M., et al. (2017). Intra and inter-session reliability of rapid transcranial magnetic stimulation stimulus-response curves of tibialis anterior muscle in healthy older adults. *PLoS One* 12:e0184828. doi: 10.1371/journal.pone.0184828

Petersen, N. T., Taylor, J. L., and Gandevia, S. C. (2002). The effect of electrical stimulation of the corticospinal tract on motor units of the human biceps brachii. *J. Physiol.* 544, 277–284. doi: 10.1113/jphysiol.2002.024539

Portney, L. G. (2020). *Foundations of clinical research: applications to evidence-based practice. 4th Edn*. ed. F. A. Davis.

Rossi, S., Hallett, M., Rossini, P. M., and Pascual-Leone, A. (2011). Screening questionnaire before TMS: an update. *Clin. Neurophysiol.* 122:1686. doi: 10.1016/j.clinph.2010.12.037

Sidhu, S. K., Bentley, D. J., and Carroll, T. J. (2009). Cortical voluntary activation of the human knee extensors can be reliably estimated using transcranial magnetic stimulation. *Muscle Nerve* 39, 186–196. doi: 10.1002/mus.21064

Sidhu, S. K., Cresswell, A. G., and Carroll, T. J. (2013). Corticospinal responses to sustained locomotor exercises: moving beyond single-joint studies of central fatigue. *Sport. Med.* 43, 437–449. doi: 10.1007/s40279-013-0020-6

Škarabot, J., Ansdell, P., Brownstein, C. G., Hicks, K. M., Howatson, G., Goodall, S., et al. (2019a). Reduced corticospinal responses in older compared with younger adults during submaximal isometric, shortening, and lengthening contractions. *J. Appl. Physiol.* 126, 1015–1031. doi: 10.1152/japplphysiol.00987.2018

Škarabot, J., Ansdell, P., Brownstein, C. G., Thomas, K., Howatson, G., Goodall, S., et al. (2019b). Electrical stimulation of human corticospinal axons at the level of the lumbar spinal segments. *Eur. J. Neurosci.* 49, 1254–1267. doi: 10.1111/ejn.14321

Škarabot, J., Tallent, J., Goodall, S., Durbaba, R., and Howatson, G. (2019c). Corticospinal excitability during shortening and lengthening actions with incremental torque output. *Exp. Physiol.* 103, 1586–1592. doi: 10.1113/EP087347

Tallent, J., Goodall, S., Gibbon, K. C., Hortobágyi, T., and Howatson, G. (2017). Enhanced corticospinal excitability and volitional drive in response to shortening and lengthening strength training and changes following detraining. *Front. Physiol.* 8, 1–12. doi: 10.3389/fphys.2017.00057

Taylor, J. L. (2006). Stimulation at the cervicomedullary junction in human subjects. *J. Electromyogr. Kinesiol.* 16, 215–223. doi: 10.1016/j.jelekin.2005.07.001

Taylor, J. L., Butler, J. E., and Gandevia, S. C. (1999). Altered responses of human elbow flexors to peripheral-nerve and cortical stimulation during a sustained maximal voluntary contraction. *Exp. Brain Res.* 127, 108–115. doi: 10.1007/s002210050779

Taylor, J. L., Petersen, N. T., Butler, B., and Gandevia, S. C. (2002). Interaction of transcranial magnetic stimulation and electrical transmastoid stimulation in human subjects. *J. Physiol.* 541, 949–958. doi: 10.1113/jphysiol.2002.016782

Taylor, J. L., Todd, G., and Gandevia, S. C. (2006). Evidence for a supraspinal contribution to human muscle fatigue. *Clin. Exp. Pharmacol. Physiol.* 33, 400–405. doi: 10.1111/j.1440-1681.2006.04363.x

Temesi, J., Ly, S. N., and Millet, G. Y. (2017). Reliability of single- and paired-pulse transcranial magnetic stimulation for the assessment of knee extensor muscle function. *J. Neurol. Sci.* 375, 442–449. doi: 10.1016/j.jns.2017.02.037

Todd, G., Taylor, J. L., and Gandevia, S. C. (2003). Measurement of voluntary activation of fresh and fatigued human muscles using transcranial magnetic stimulation. *J. Physiol.* 551, 661–671. doi: 10.1113/jphysiol.2003.044099

Ugawa, Y., Genba-Shimizu, K., and Kanazawa, I. (1995). Electrical stimulation of the human descending motor tracts at several levels. *Can. J. Neurol. Sci.* 22, 36–42. doi: 10.1017/S0317167100040476

Ugawa, Y., Rothwell, J. C., Day, B. L., Thompson, P. D., and Marsden, C. D. (1991). Percutaneous electrical stimulation of corticospinal pathways at the level of the pyramidal decussation in humans. *Ann. Neurol.* 29, 418–427. doi: 10.1002/ana.410290413

Walker, S., Blazevich, A. J., Haff, G. G., Tufano, J. J., Newton, R. U., and Häkkinen, K. (2016). Greater strength gains after training with accentuated eccentric than traditional isoinertial loads in already strength-trained men. *Front. Physiol.* 7, 1–12. doi: 10.3389/fphys.2016.00149

II

CONTRACTION INTENSITY MODULATES SPINAL EXCITABILITY DURING TRANSCRANIAL MAGNETIC STIMULATION-EVOKED SILENT PERIOD IN RECTUS FEMORIS MUSCLE

by

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ORIGINAL ARTICLE

Contraction intensity modulates spinal excitability during transcranial magnetic stimulation-evoked silent period in rectus femoris muscle

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Abstract

Purpose Reduced spinal excitability during the transcranial magnetic stimulation (TMS) silent period (SP) has recently been shown to last longer than previously thought in the upper limbs, as assessed via spinal electrical stimulation. Further, there is reason to expect that contraction intensity affects the duration of the reduced spinal excitability.

Methods This study investigated spinal excitability at different time delays within the TMS-evoked SP in m.rectus femoris. Fifteen participants performed non-fatiguing isometric knee extensions at 25%, 50% and 75% of maximum voluntary contraction (MVC). Lumbar stimulation (LS) induced a lumbar-evoked potential (LEP) of 50% resting M-max. TMS stimulator output induced a SP lasting ~ 200 ms. In each contraction, a LEP (unconditioned) was delivered ~ 2–3 s prior to TMS, which was followed by a second LEP (conditioned) 60, 90, 120 or 150 ms into the silent period. Five contractions were performed at each contraction intensity and for each time delay in random order.

Results Compared to the unconditioned LEP, the conditioned LEP amplitude was reduced ($-28 \pm 34\%$, $p = 0.007$) only at 60 ms during 25% of MVC. Conditioned LEP amplitudes during 50% and 75% of MVC were reduced at 60 ms ($-37 \pm 47\%$, $p = 0.009$ and $-37 \pm 42\%$, $p = 0.005$, respectively) and 150 ms ($-30\% \pm 37\%$, $p = 0.0083$ and $-37 \pm 43\%$, $p = 0.005$, respectively). LEP amplitude at 90 ms during 50% of MVC also reduced ($-25 \pm 35\%$, $p = 0.013$).

Conclusion Reduced spinal excitability is extended during 50% and 75% of MVC. In future, paired TMS-LS could be a potential method to understand changes in spinal excitability during SP (at different contraction intensities) when testing various neurophysiological phenomena.

Keywords Lumbar stimulation · Spinal inhibition · Lower limbs · Force production · Cortico-spinal tract

Abbreviations

AHP	Afterhyperpolarization
ANOVA	Analysis of variance
BF	Bicep femoris
CMEP	Cervicomedullary-evoked potential
EMG	Electromyography
GTO	Golgi tendon organ
H-reflex	Hoffmann's reflex
kΩ	Kiloohm
L_1	First lumbar vertebra
LEP	Lumbar-evoked potential
LS	Lumbar stimulation
MEP	Motor-evoked potential
M-max	Maximum compound action potential
Ms	Milliseconds
MVC	Maximal voluntary contraction
RC	Renshaw cells
RF	Rectus femoris
RI	Recurrent inhibition
s	Seconds
SOL	Soleus muscle
SORE	Stimulation offset to return of electromyography
SP	Silent period
TMEP	Thoracic motor-evoked potential
TMS	Transcranial magnetic stimulation

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Introduction

Transcranial Magnetic Stimulation (TMS) applied over the contralateral motor cortex of the muscle targeted, in relaxed and active conditions, produces a muscle action potential that can be recorded by electromyography (EMG) and a muscle twitch. The muscle action potential is referred to as the motor-evoked potential (MEP) and provides information about cortico-spinal excitability (Barker et al. 1985; Day et al. 1989a). In addition, when TMS is applied during voluntary muscle contraction there is an interruption of the background EMG activity after the MEP (Mills 1988; Day et al. 1989b). This interruption is known as the TMS-evoked silent period (SP) and its duration provides information about inhibition of the cortico-spinal tract (Inghilleri et al. 1993; Triggs et al. 1993; Taylor et al. 1996).

For some time, changes in the length of SP have been considered as an indicator of altered intracortical inhibition (Kidgell et al. 2013; Ruotsalainen et al. 2014; Manca et al. 2016; Latella et al. 2017). However, while reduced MEP amplitude, as an indicator of intracortical inhibition, has indeed been shown during the TMS-evoked SP, studies have consistently shown concomitant decreases in spinal excitability 50–100 ms after TMS that evokes a ~ 200 ms SP (Fuhr et al. 1991; Inghilleri et al. 1993; McDonnell et al. 2006; McNeil et al. 2009). Reduced spinal excitability is possibly due to motor-neuron afterhyperpolarization (AHP) and/or recurrent inhibition (RI) via Renshaw cells (RC), as well as Ia interneuron unloading through reciprocal inhibition (Mills 1988; Fuhr et al. 1991; Ziemann et al. 1993). Interestingly, a recent study showed reduced spinal excitability up to 150 ms in the upper limbs after TMS, which was argued to be attributed to an increase in Golgi tendon organ (GTO) activity and muscle spindle unloading (Yacyshyn et al. 2016). Thus, emerging evidence suggests that spinal excitability is modulated over a longer proportion of SP than previously thought.

One experimental consideration is that traditional H-reflex methodology used in previous studies (Fuhr et al. 1991; Ziemann et al. 1993) limits the assessment of modified spinal excitability < 100 ms, as the measure reflects modified pre-synaptic inhibition. In contrast, direct percutaneous activation of the spinal cord predominantly activates monosynaptic cortico-spinal tract axons (Taylor 2006; McNeil et al. 2013) and can be applied during both submaximal and maximal contractions (Petersen et al. 2002; Škarabot et al. 2019a). It would, therefore, be appropriate to test whether there is reduced spinal excitability at time delays greater than 100 ms (Yacyshyn et al. 2016) in the lower-limbs, since previous studies have relied on H-reflex methodology (Ziemann et al. 1993). While spinal responses can be elicited at cervical (cervicomedullary-evoked potential (CMEP)) and thoracic (thoracic motor-evoked potential (TMEP)) (Martin et al. 2008) segments of the spine, recent studies suggested that lumbar stimulation (lumbar-evoked potentials (LEP)) are a valid (Škarabot et al. 2019a) and more tolerable (Brownstein et al. 2020) method to study spinal excitability of the lower-limbs.

One final consideration is that contraction intensity could affect the duration of the reduced spinal excitability during the TMS-evoked SP. Increases in voluntary torque production increase the tension of the tendon and, consequently, increase GTO activity (Houk et al. 1970). In addition, muscle relaxation rate following TMS is greater with increased torque, which could activate muscle spindles as the sarcomeres lengthen (Vernillo et al. 2022). As such, afferent feedback mechanisms may be modified by increased torque level and potentially influence spinal excitability during SP. In the knee extensors, contractions of 25% of maximal voluntary contraction (MVC) resulted in the unconditioned TMEP being the same amplitude as the subsequent (TMS-) conditioned TMEP evoked at a time delay of 100 ms (Finn et al. 2018). In another study, the conditioned TMEP amplitude at a time delay of 100 ms was decreased when contracting to 50% of MVC (Brownstein et al. 2020). These results suggest contrasting responses between 25 and 50% of MVC.

Examining the contributing factors to the SP in locomotor muscles is important for determining exercise-induced alterations in nervous system function throughout the spectrum of health, exercise and disease (Sidhu et al. 2013). Consequently, there is a need to directly examine the duration of spinal inhibition within the TMS-evoked SP in the lower-limbs across different contraction intensities. The purpose of the study was to assess spinal excitability at different time delays (60, 90, 120 and 150 ms) within the TMS-evoked SP in the rectus femoris (RF) muscle with lumbar stimulation (LS) at different contraction intensities (25, 50, and 75% of MVC). It was hypothesized that reduced spinal excitability would be observed at longer time delays within the SP at increasing contraction intensities.

Material and methods

Participants

Twenty-two healthy adults (8 female) volunteered for the study. Seven participants were not considered due to possible activation of ventral roots (see Lumbar-evoked potentials). Therefore, the data presented here are representative of the 15 (4 female) volunteers fulfilling all study requirements (males: 11 subjects, 31 ± 6 years, height 178 ± 6 cm, weight 82 ± 8 kg; females: 4 subjects, 28 ± 1 years, height 166 ± 8 cm, weight 64 ± 7 kg). All included participants

were free from neurological illness and musculoskeletal injury in the lower-limbs for the last 6 months, were not taking any medications known to affect the nervous system and had no contraindications to transcranial magnetic stimulation (TMS), which was assessed via a health questionnaire (modified from Rossi et al. (2009). Before testing, all participants were fully informed of the procedures and possible risks, and each participant provided written inform consent. The study was approved by the Ethical committee of the University of Jyväskylä (10.01.2020) and was conducted with accordance with the *Declaration of Helsinki* (2013).

An a priori sample size estimation was conducted using G*Power software (version 3.1, University of Dusseldorf, Germany), based on data presented by Yacyshyn et al. (2016) for $\alpha = 0.05$ and power = 0.80. The estimated sample size needed was 18 participants to assess torque × time delay interaction between unconditioned and conditioned LEPs.

Experimental set-up

Detailed description of Torque, M-max, TMS, Lumbar stimulation and EMG can be found in the subsections below.

Participants visited the laboratory on one occasion. To assess responses in the RF muscle, participants were sat in a custom-built chair with a calibrated load cell (Faculty of Sport and Health Sciences, University of Jyväskylä, Finland) with hip and knee at 90° flexion and the shin strapped with a non-elastic restraint ~ 2 cm superior to the ankle malleoli. The voltage signal originating from the load cell was calibrated and converted into torque (N·m). All measures were performed on the right (i.e., dominant) leg, assessed by self-report of which foot they primarily kick a ball (van Melick et al. 2017).

Once the participant was secured to the dynamometer, the maximum compound action potential (M-max) was assessed in a relaxed condition. Two maximal voluntary contraction (MVC) trials were performed 60 s apart. Prior to the MVC, two contractions at ~ 50% and ~ 80% of estimated MVC were performed as a warm-up. Verbal encouragement and visual feedback were provided to motivate participants to produce maximal effort. Thereafter, target contraction intensities (25%, 50% and 75% of MVC) were displayed on the screen as visual feedback for the participant.

Placement of the lumbar stimulation electrodes was assessed to avoid activating spinal nerve roots (see Lumbar-evoked potentials). Thereafter, stimulator intensity was adjusted to produce a LEP of 50% of the M-max at rest, and this stimulation intensity was used throughout the experiment. TMS coil placement was defined as the location producing the largest MEP in the RF, and stimulator output intensity was standardized to evoke ~ 200 ms SP from the stimulator artefact to the resumption of the voluntary EMG signal, during brief voluntary contractions at each torque.

During the session, unconditioned and conditioned LEPs were delivered during the same voluntary contraction. Unconditioned LEP consisted of a single stimulation delivered at the lumbar level. Conditioned LEPs consisted of a paired stimulation of TMS followed by lumbar stimulation separated by predetermined and randomly ordered time delays (60, 90, 120 and 150 ms). Participants were instructed to contract to, and briefly hold, one of the three different contraction intensities (25, 50 and 75% of MVC) in a randomized order. Once the participant reached the required level, an unconditioned LEP was delivered followed by a conditioned LEP at one of the different time delays (Fig. 1). The contractions were held for 5–8 s and stimuli were delivered 2–3 s apart. Sets of five unconditioned, followed by conditioned LEPs, were given per time delay and per torque level as a single block, giving a total of 60 unconditioned and conditioned stimuli. To avoid fatigue (see Results), 30, 45 and 60 s rest was given between contractions at 25%, 50% and 75% of MVC, respectively, and 60, 120 and 180 s rest was given between the sets of 5 contractions. At the end of the protocol, M-max and MVC were reassessed.

Peripheral nerve stimulation

Percutaneous electrical stimulation of the femoral nerve (3.2 cm cathode/anode arrangement; Polar Neurostimulation Electrodes, Espoo, Finland) was performed to elicit M-max in RF (1 ms square pulse duration; Digitimer DS7AH, Hertfordshire, UK). Electrodes were placed 2 cm apart and placed at each side of the femoral nerve, located by palpation and identification of the femoral artery (Walker et al. 2016). M-max was elicited by gradually increasing stimulator output intensity until the EMG response plateaued. To ensure supramaximality, this intensity was further increased by 50% (mean ± standard deviation intensity: 257 ± 151 mA).

Transcranial magnetic stimulation

Single TMS pulses were delivered using a Magstim 200^2 magnetic stimulator (Magstim Co., Ltd., Whitland, UK) connected to a concave double-cone coil, positioned over the left cortical hemisphere for RF with a posterior-to-anterior current orientation. The hotspot was defined, at rest, as the position eliciting the largest MEP recorded in the EMG using the same intensity (i.e., 50–70% stimulator output) producing a visible MEP. The coil position was marked on the scalp, once the hotspot was found, to maintain the same position throughout the protocol. Stimulus intensities were set to evoke a silent period of ~ 200 ms for all contraction intensities (Table 1).

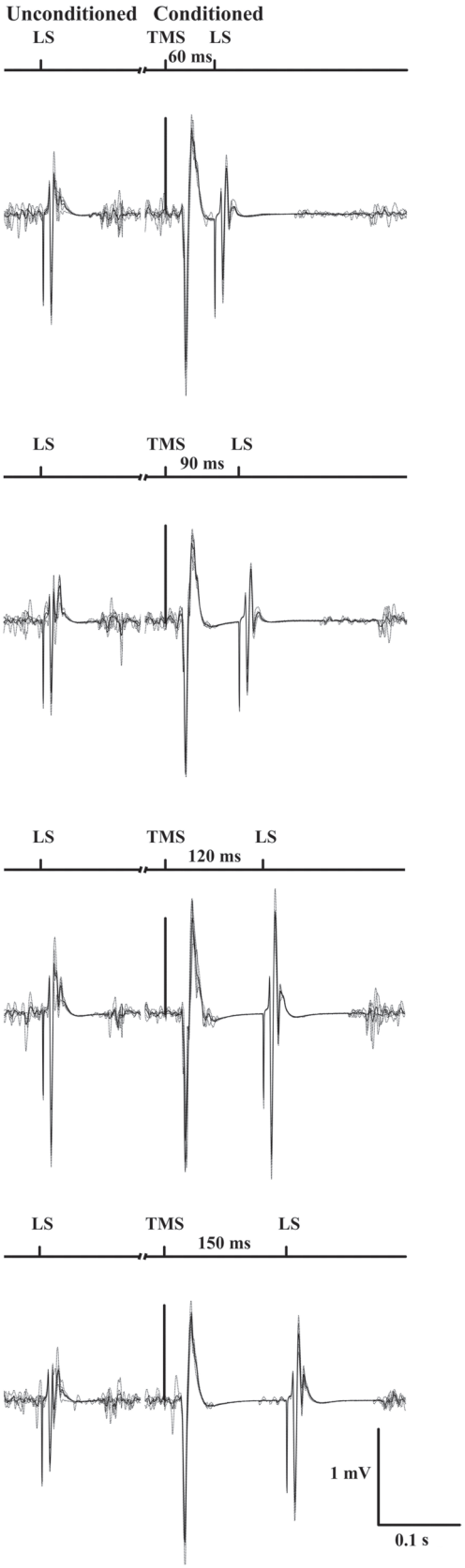

Fig. 1 One participant's mean (solid) and individual (dashed) trials ▶ that represent the experimental design of one set of unconditioned and conditioned lumbar stimulation at different time delays taken from 25% MVC trials. *TMS* transcranial magnetic stimulation, *LS* lumbar stimulation

Lumbar-evoked potentials

LEPs were elicited with a constant-current stimulator (1 ms square pulse duration; Digitimer DS7AH, Hertfordshire, UK) via self-adhesive electrodes (Polar Neurostimulation Electrodes, Espoo, Finland). The cathode (5 × 10 cm) was centered over the first lumbar vertebra (L_1) and the anode (circular shape; 3.2 cm diameter) was placed on the midline of the vertebral column ~ 5 cm above the top edge of the cathode as described by Škarabot et al. (2019a).

The intensity of stimulation (309 ± 108 mA) was standardized to 50% of the M-max evoked in the resting position. Potential activation of ventral roots was assessed by examining the onset latency of the LEP with an increase in stimulator intensity (Petersen et al. 2002) and tracking LEP amplitude during increased voluntary contraction (Taylor et al. 2002). Should the ventral roots be activated by the stimulation procedures, onset latency would have shortened with an increase in stimulator intensity and LEP amplitude would have been the same during increased voluntary contraction (Petersen et al. 2002; Taylor et al. 2002, 2006; Škarabot et al. 2019a).

Dorsal root activation was assessed via paired LS with 50 ms time delay (Fig. 2), where the amplitude of the second LEP was compared to the first. Evidence of dorsal root activation would be a decrease in the second LEP due to post-activation depression at the motor-neuron pool (Hofstoetter et al. 2018). All remaining participants showed no sign of the responses described and reported that they found LS to be tolerable.

Bipolar surface electromyography and torque

Muscle activity was recorded using adhesive Ag/AgCl electrodes (3 × 2 cm, BlueSensor N, Ambu, Penang, Malaysia) from m.Bicep Femoris (BF) and RF according to SENIAM Guidelines (Hermens et al. 2000). Skin was shaved, abraded with sandpaper, and wiped with alcohol before setting the electrodes in bipolar arrangement with 2 cm center-to-center distance. Impedance was set < 2kΩ, and the reference electrode was positioned above the patella. EMG data were amplified (1000 ×), bandpass filtered (16–1000 Hz; Neurolog System, Digitimer Ltd, UK)) and sampled online at 3000 Hz using CED Power1401-3 (Cambridge Electronic Design Ltd, Cambridge, UK).

Torque was sampled at 1000 Hz, amplified by a custom-built amplifier (ForAmps 1 v1.2, University of Jyväskylä,

Table 1 Mean and standard deviation values of MEP, lumbar stimulation and involuntary EMG activity parameters from the participants at different submaximal torque levels

	25% MVC	50% MVC	75% MVC
TMS stimulator output (%)	66 ± 16	64 ± 12	65 ± 14
MEP SP: SORE (ms)	216 ± 15	210 ± 10	216 ± 14
MEP (mV)	2.16 ± 1.35	2.02 ± 1.10	1.79 ± 0.84
LEP latency (ms)	6.3 ± 0.7	6.6 ± 0.7	6.6 ± 0.5
Involuntary EMG activity amplitude (mV)	0.11 ± 0.07	0.14 ± 0.09	0.20 ± 0.14

These values represent the standardization of the measurement

MVC maximal voluntary contraction, *TMS* transcranial magnetic stimulation, *MEP* motor evoked potential, *SP* silent period, *SORE* stimulation offset to return of electromyography, *LEP* lumbar evoked potential

Finland) and converted by a 16-bit A/D board (CED Power1401-3, Cambridge Electronics Design, Cambridge, UK) in combination with Spike2 software (version 6.10, Cambridge Electronic Design, Cambridge, UK).

Data and statistical analyses

Offline analyses were performed with Spike software (version 6.10, Cambridge Electronic Design, Cambridge, UK) to manually obtain M-max amplitude, MVC, MEP Silent Period and unconditioned LEP onset latencies. The other outcome measures were analyzed by a customized MATLAB script (version R2020b, The MathWorks, Inc., Natick, USA). Peak-to-peak amplitude of LEPs and MEPs was analyzed automatically between latencies-of-interest following peripheral nerve stimulation, lumbar stimulation or TMS (Taylor et al. 1999), respectively. Torque was averaged over the 100 ms before the stimulator artefact. SP duration was determined, through visual inspection, as the time from the stimulator artefact to the return of voluntary EMG (Damron et al. 2008).

SPSS software (version 26.0, SPSS Inc., Chicago, USA) was used for all statistical methods. Means and standard deviation (SD) were calculated and reported throughout. Normality of the data was tested with the Shapiro–Wilk test and confirmed by z-score with an acceptance of + 2 to -2 (e.g. skewness score/skewness score$_{SE}$ and kurtosis score/kurtosis score$_{SE}$). Data that did not fulfil those requirements were Log10 transformed, which then fulfilled the requirements for Normality. Paired t-tests were used to assess possible effects of fatigue between M-maxpre and M-maxpost, MVCpre and MVCpost, and to evaluate unconditioned LEP amplitude at different torque levels in the control measurements (shown in Fig. 3). One-way analysis of variance (ANOVA) was used to assess potential differences between the three contraction intensities in control measures: Unconditioned LEP latencies, MEP amplitude and MEP Silent Period (shown in Table 1). To determine whether Normalized [Conditioned/Unconditioned LEP*100] LEPs responded differently at the tested time delays between the three different torque levels, two-way repeated measures ANOVA was employed. When sphericity assumptions were violated, Greenhouse–Geisser corrections were used. Post-hoc Bonferroni adjustments were used when significant main effects were found. When comparing Unconditioned and Conditioned LEP at each time delay, the Benjamin–Hochberg test corrected for multiple paired *t* test comparisons with a 10% false discovery rate. Effect sizes are represented as partial eta-squared values (η_p^2 = small: 0.01, medium: 0.06, large: 0.14) for the factors of the ANOVA and as Hedge's *g* for between-group effect sizes for these relative

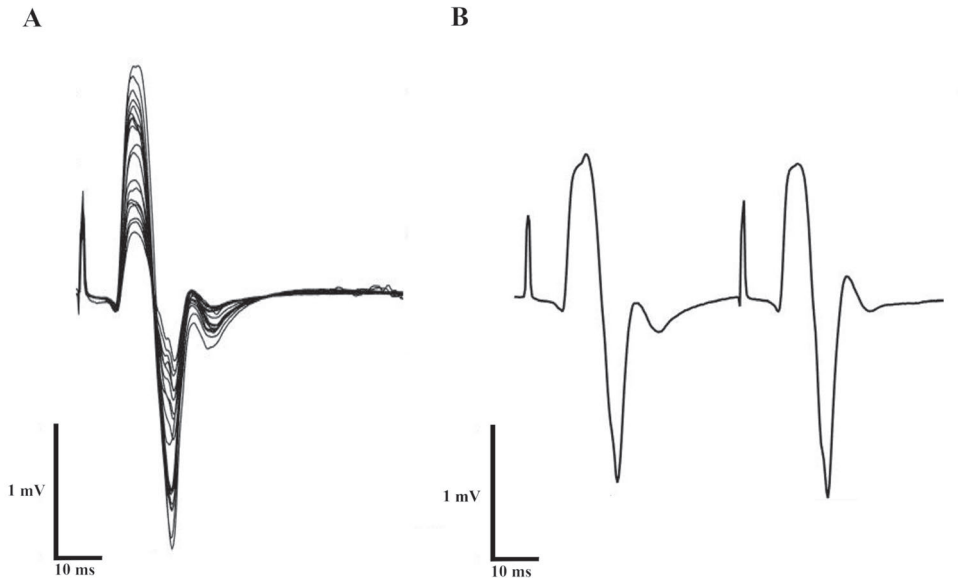

Fig. 2 Data extracted from one participant showing that spinal root activation did not occur. **A** When increasing the intensity of stimulator output there was no reduction in latency. **B** A lumbar stimulated doublet with 50 ms interval, showing similar amplitudes between the stimulations

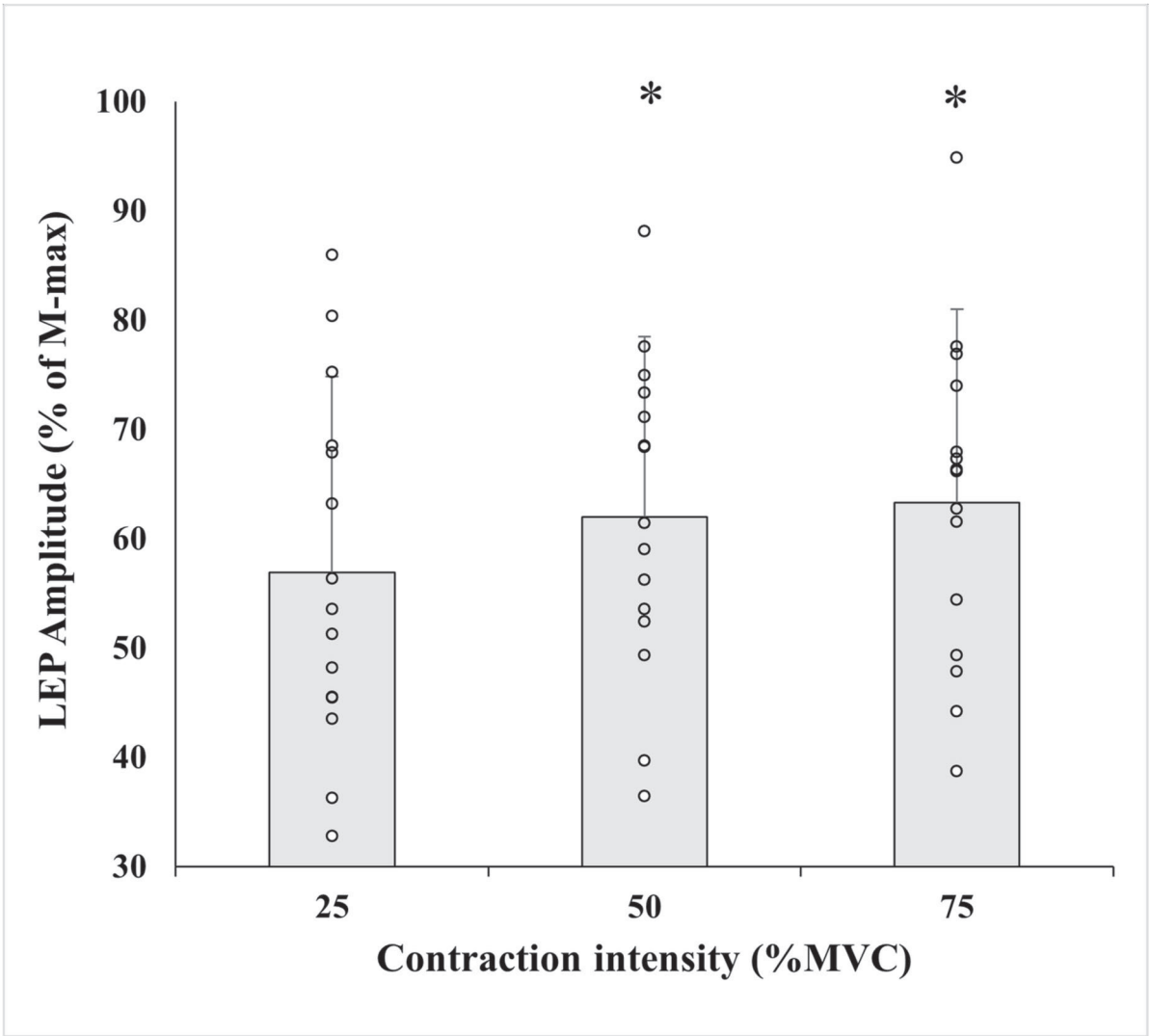

Fig. 3 Mean (±SD) and individual values of unconditioned LEP response normalized to M-max at different contraction intensities. Increases in LEP amplitude with increases in torque shows that the stimulation was evoked trans-synaptically

changes (g = small: < 0.3, medium: 0.3–0.8, large: > 0.8). Alpha was set at 0.05.

Results

Control measurements

There were no statistically significant differences between time delays for MEP amplitude during 25% of MVC ($F_{(3, 56)} = 0.033$, $p = 0.992$), during 50% of MVC ($F_{(3, 56)} = 0.024$, $p = 0.995$), or during 75% of MVC ($F_{(3, 56)} = 0.191$, $p = 0.902$). Additionally, there were no statistical differences between SP duration at any contraction intensity ($F_{(2\,42)} = 1.110$, $p = 0.339$), indicating standardized conditions throughout the experiment to examine spinal excitability.

There were no statistically significant differences between M-maxpre and M-maxpost (M-maxpre = 3.27 ± 1.13 mV, M-maxpost = 2.96 ± 1.04 mV, $p = 0.054$, 95% CI [− 0.01, 0.62], Hedges' $g = 0.27$) nor between MVCpre and MVC-post (MVCpre = 221 ± 60 N·m; MVCpost = 214 ± 54 N·m, $p = 0.106$, 95% CI [− 1.74, 15.25], Hedges' $g = 0.12$).

LEP latencies did not show statistical difference between time delays during 25% of MVC ($F_{(3, 56)} = 0.106$, $p = 0.956$), during 50% of MVC ($F_{(3, 56)} = 0.016$, $p = 0.997$) or during 75% of MVC ($F_{(3, 56)} = 0.153$, $p = 0.902$). There was a statistically significant difference between unconditioned LEP amplitude during 25% vs 50% of MVC ($p < 0.001$, 95% CI [− 1.74, 15.25], Hedges' $g = -0.26$) and 25% vs 75% ($p = 0.001$, 95% CI [− 0.21, − 0.06], Hedges' $g = -0.27$) of

MVC, although no statistical difference was found between 50% of MVC and 75% of MVC ($p = 0.956$, 95% CI [− 0.05, 0.05], Hedges' $g = -0.01$) (Fig. 3). Collectively, these findings indicate that LS activated the cortico-spinal tract.

Effects of torque on spinal excitability at different time delays

Two-way repeated measures ANOVA showed a significant main effect between time delays ($F_{(2,5,\ 102.4)} = 6.542$, $p = 0.001$, $\eta_p^2 = 0.135$) and torque × time delay interaction ($F_{(4.9,\ 102.4)} = 2.953$, $p = 0.016$, $\eta_p^2 = 0.123$) for the normalized LEP. Post hoc analyses revealed significant difference in LEP amplitude between 60 ms (0.73 ± 0.27) and 150 ms (0.95 ± 0.34) ($p = 0.007$, 95% CI [− 0.398, − 0.046], Hedges' $g = -0.27$) and 90 ms (0.75 ± 0.35) and 150 ms ($p = 0.004$, 95% CI [− 0.352, − 0.050], Hedges' $g = -0.25$) during 25% of MVC (Fig. 4).

Unconditioned vs conditioned LEP

Unconditioned LEP was compared to the conditioned LEP at each time delay at the three contraction intensities. During 25% of MVC, conditioned LEP amplitude was statistically lower than unconditioned LEP at 60 ms ($t_{(14)} = -3.128$, $p = 0.007$, 95% CI [− 0.464, − 0.087], Hedges' $g = -0.62$), but not at 90 ms ($t_{(14)} = -2.397$, $p = 0.075$, 95% CI [− 0.505, − 0.028], Hedges' $g = -0.58$), 120 ms ($t_{(14)} = -1.285$, $p = 0.220$, 95% CI [− 0.292, 0.073], Hedges' $g = -0.18$), nor 150 ms ($t_{(14)} = 0.722$, $p = 0.482$, 95% CI [− 0.248, 0.123], Hedges' $g = -0.13$).

During 50% of MVC, statistical differences were found at 60, 90 and 150 ms ($t_{(14)} = -3.052$, $p = 0.009$, 95% CI [− 0.634, − 0.111], Hedges' $g = -0.76$, $t_{(14)} = -2.843$, $p = 0.013$, 95% CI [− 0.446, − 0.062], Hedges' $g = -0.44$ and $t_{(14)} = -3.099$, $p = 0.008$, 95% CI [− 0.502, − 0.091], Hedges' $g = -0.52$, respectively), where the conditioned LEP was lower than the unconditioned LEP. There were no statistically significant differences in conditioned versus unconditioned LEP amplitude at 120 ms ($t_{(14)} = -2.073$, $p = 0.057$, 95% CI [− 0.451, 0.008], Hedges' $g = -0.36$).

During 75% of MVC, the conditioned LEP amplitude was significantly lower than unconditioned LEP (Fig. 4) at 60 ms and 150 ms ($t_{(14)} = -3.348$, $p = 0.005$, 95% CI [− 0.602, − 0.132], Hedges' $g = -0.78$, and $t_{(14)} = -3.377$, $p = 0.005$, 95% CI [− 0.610, − 0.136], Hedges' $g = -0.70$, respectively). But no statistically significant differences were observed at 90 ms nor 120 ms ($t_{(14)} = -2.511$, $p = 0.067$, 95% CI [− 0.429, − 0.034], Hedges' $g = -0.51$ and $t_{(14)} = -2.626$, $p = 0.083$ (corrected), 95% CI [− 0.394, − 0.040], Hedges' $g = -0.52$, respectively).

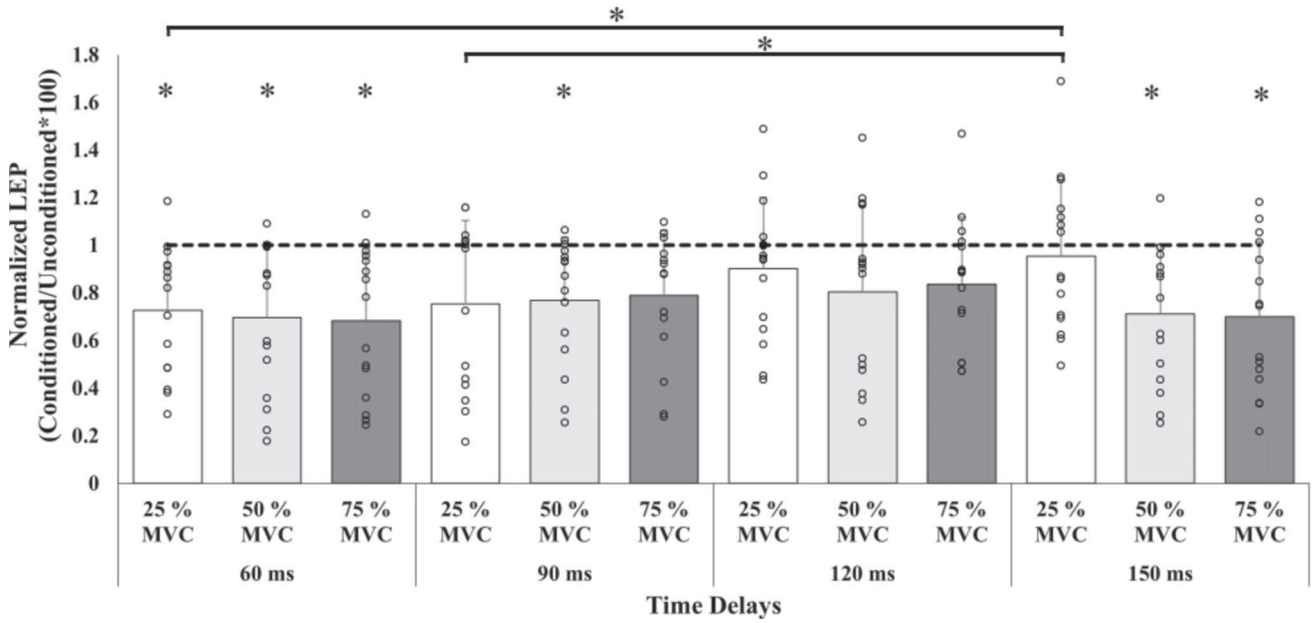

Fig. 4 Mean (± SD) and individual values of conditioned LEP normalized to the unconditioned LEP. The dashed line represents the unconditioned LEP amplitude. Any data point or bar below the dashed line represents inhibition and any data or bar above the dashed line represents facilitation of the conditioned LEP. Bars represent the mean values at each contraction intensity and time delay. The circles represent each participant's data at each contraction intensity and time delay. *$p < 0.05$ vs unconditioned LEP amplitude

Discussion

This is the first study to directly test spinal excitability at different time delays during TMS-evoked SP, and during different contraction intensities, in the lower-limbs (specifically RF). Our results showed reduced spinal excitability during the first 60 ms in RF during all contraction intensities, extending to 90 ms at 50% of MVC and further reductions were observed at 150 ms during 50 and 75% of MVC.

These results conflict with a previous study that used CMEPs during a 25% of MVC contraction in upper limb (Yacyshyn et al. 2016); the conditioned CMEP showed differences from the unconditioned response also at 120 and 150 ms after TMS. However, our results agree with early studies conducted using H-reflex methodology in both upper- and lower-limbs (Fuhr et al. 1991; Ziemann et al. 1996) despite that H-reflex data could be influenced by changes in presynaptic inhibition, which is absent in our methods. The results suggest that reduced spinal excitability is present but largely limited to ≤ 90 ms after TMS in lower-limb muscles, at low contraction intensities (i.e., < 25%of MVC). Nevertheless, differences between upper- and lower-limbs have previously been presented by Giesebrecht et al. (2010). They reported a facilitatory response to spinal stimulation in tibialis anterior after 10 s MVC, in contrast of spinal inhibition observed by Gandevia et al. (1999) in biceps brachii after 5–10 s MVC contraction, discussing different physiological mechanisms in upper- and lower-limbs muscles.

Compiling the existing literature provides indirect support for the present study's finding in that contraction intensity influenced the duration of reduced spinal excitability during SP. First, Finn et al. (2018) did not observe reduced spinal excitability at 100 ms (TMS induced a 200 ms SP), given that the conditioned TMEP was similar to the amplitude of the unconditioned TMEP when standardized to 50% of the M-max (as in the current study). Conversely Brownstein et al. (2021) did observe reduced spinal excitability since both conditioned TMEP and LEP amplitude at 100 ms (TMS included 200 ms SP) were lower than their respective unconditioned amplitudes, again when spinal stimulation was standardized at 50% of the M-max. As Finn et al. (2018) employed contraction intensities of 25% of MVC, whereas Brownstein et al. (2021) employed 50% of MVC, this suggests that contraction intensity influences the duration of reduced spinal excitability. In directly assessing this hypothesis, spinal excitability was reduced at 60 ms but no longer at 90 ms after TMS contracting to 25% of MVC, matching the findings of Finn et al. (2018). However, reductions in conditioned LEP were observed at 90 ms during 50% of MVC and at 150 ms during 50% and 75% of MVC, providing support for and extending the findings of Brownstein et al. (2021). Thus, we suggest that increased contraction intensity modulates spinal excitability distinctly in that reduced stimulation-induced responses are apparent at longer time delays when contracting at a higher intensity.

The suggested mechanisms for the decrease in spinal excitability during TMS-evoked SP are: afterhyperpolarization (AHP), recurrent inhibition via Renshaw cells, Ia interneuron unloading through reciprocal inhibition, and/or GTO inhibition (Mills 1988; Fuhr et al. 1991; Ziemann et al. 1993; Yacyshyn et al. 2016). Although AHP, RI and GTO inhibition are dependent on the preceding motor-neuron activity (Hultborn & Pierrot-Deseilligny 1979; Ziemann et al. 1993) and the size of the conditioned test stimuli (Hultborn & Pierrot-Deseilligny 1979), AHP may not account for more than ~ 56 ms, since discharge rate at 50% of MVC is ~ 18 pps in the VL (Kamen & Knight 2004). There is evidence that AHP could impact excitability up to approx. 100 ms, depending on motor-neuron firing rate (Piotrkiewicz et al. 2007), as observed in upper-limb muscles. Thus, the exact duration of the influence of AHP is still unresolved in different muscles. However, converging evidence suggests that this may not be the case in explaining the difference between conditioned LEP amplitude during 25% versus 50% of MVC at 90 ms in the present study.

Among the TMS-evoked SP studies, Ziemann et al. (1993) found that the conditioned/unconditioned H-reflex amplitude progressively decreased with increasing contraction intensity in the soleus muscle (SOL). The authors argued that Renshaw cells might have a stronger influence on TMS-evoked SP inhibition, rather than GTOs or muscle spindles, since the decrease in spinal excitability was ~ 50 ms, and those monosynaptic feedback mechanisms start to exert an influence after ~ 40 ms in SOL. Although RI may only account for ~ 40 ms (Pierrot-Deseilligny & Burke 2005), it could influence discharging rate (Granit et al. 1960). Since stimulator output was not statistically different in 25% and 50% of MVC conditions, a plausible mechanism to explain the prolonged decrease from 60 to 90 ms in spinal excitability at higher contraction intensities could be recurrent inhibition via Renshaw cells.

In the present study, the interstimulus intervals of 60 and 90 ms could also be affected by modified muscle spindle or GTO activity to the cortico-spinal tract. The spindles provide muscle length feedback and GTOs provide tensile feedback (Enoka 2008; Nichols 2018). When there is an increase in contraction intensity, GTOs increase their discharge rate, increasing Ib inhibition (Houk et al. 1970). Further, the TMS-induced muscle twitch has been suggested to also engage GTOs increasing Ib inhibition (Yacyshyn et al. 2016). It is conceivable that the combination of higher intensity contractions and muscle twitch-induced Ib inhibition could be enhanced in the present study's 50% of MVC trials.

Therefore, GTOs may be one candidate for the continued decrease of spinal excitability with increasing contraction intensity.

One interesting finding in the present study was the observed return of conditioned/unconditioned LEP to baseline during 25% and 75% of MVC at 90 ms and at 120 ms for all conditions, but then a second reduction in spinal excitability at 150 ms during 50% and 75% of MVC (Figs. 4 and 5). An involuntary EMG activity burst (80–150 ms) has been previously observed in upper- (Calancie et al. 1987; Holmgren et al. 1990; Butler et al. 2012) and lower-limbs (Dimitrijević et al. 1992), categorized as "low level EMG" (Butler et al. 2012) or "breakthrough EMG" (Hupfeld et al. 2020), and its origin is not known. But this involuntary EMG activity has been postulated to arise from cortical pathways (Holmgren et al. 1990; Dimitrijević et al. 1992), spinal reflex (Dimitrijević et al. 1992; Butler et al. 2012) and/or agonist and antagonist muscle activity, through polysynaptic excitatory and inhibitory potentials to the motor-neuron (Calancie et al. 1987). This involuntary activity was also observed in 11 of our 15 participants (Fig. 5), with onset latencies between 83 and 130 ms and lengths of 28–91 ms. Additionally, the size of the response increased at 75% vs 25% of MVC (Table 1). Muscle spindles have been considered as a mechanism for the involuntary EMG activity. After the TMS-evoked twitch, there is a period of relaxation, where sarcomeres lengthen and the muscle spindles could induce a monosynaptic reflex (Hupfeld et al. 2020; Škarabot et al. 2019b). Since increases in voluntary contraction increased the relaxation ratio and reduced the time to peak relaxation in knee extensor (Vernillo et al. 2022) muscle spindles could be responsible for the involuntary EMG activity. However, latencies of the patellar tendon reflex in RF were 16–22 ms (Frijns et al. 1997), and time to peak relaxation in knee extensors were ~ 140 ms and ~ 160 ms during contractions of 75% and 50% of MVC, respectively (Vernillo et al 2022). Thus, muscle spindles could provide feedback but not as early as the involuntary EMG activity observed in the present study. Consequently, one possible explanation for the return to baseline in spinal excitability at 90 ms during 75% of MVC and 120 ms during contractions > 50% of MVC could be afferent feedback provided by synergist and/or antagonist muscles from the same limb and contralateral limb (i.e., heteronymous feedback) (Houk et al. 1970; Calancie et al. 1987; Zehr et al. 2001; Wilmink & Nichols 2003; Manning & Bawa 2011). Wilmink & Nichols (2003) found that there were both excitatory and inhibitory effects from the vastii muscles on RF following stretches in cat forelimb. Furthermore, Zehr et al. (2001) showed a long-latency reflex in various muscles of the contralateral limb at 90 ms after peroneal nerve stimulation. Thus, at higher contraction intensities, heteronymous afferent signalling could be responsible for the return of spinal excitability at 90–120 ms, via an excitatory reflex that alters motor-neuron excitability at such time delays. Thus, we speculate that heteronymous feedback specifically affected the 120 ms time delay (and to a certain extent also the 90 ms delay) no longer influences conditioned LEP amplitude at 150 ms, allowing reduced spinal excitability to be observed with the lumbar stimulation method at higher contraction intensities. Nevertheless, this proposal should be specifically investigated in future.

Strength and limitations

A strength of the study is the use of LS methodology to assess spinal excitability of the lower-limbs, which targets the cortico-spinal tract directly, and the positioning of the electrodes has been verified via response tests. These procedures are in-line with those of Škarabot et al. (2019a) who showed that LS can activate the cortico-spinal tract without activating dorsal and ventral roots.

Nevertheless, limitations need to be considered in the present study. TMS during different trials were not employed, in addition to spinal electrical stimulation, to compare cortico-spinal and spinal excitability at the same time delays (60, 90, 120 and 150 ms). This could have provided information regarding ongoing cortical inhibition along with spinal level inhibition (as employed by Fuhr et al. (1991) and Inghilleri et al. (1993). However, the number of trials needed would have compromised the present study's ability to restrict neuromuscular fatigue during the testing session and tripled the number of transcranial stimulations. Second, we acknowledge that employing voluntary contractions in the present study's methodology does not allow controlling for the background EMG activity/torque (Škarabot et al. 2019b) when unconditioned and conditioned LEP were elicited, since the unconditioned LEP was elicited during a period of voluntary muscle activity as opposed to during the SP. Third, sample size estimation suggested that 18 participants were needed to obtain medium effect sizes for torque × time delay interaction. We observed a significant interaction in normalized LEP but post-hoc comparisons have likely been underpowered to detect pairwise comparisons as only 15 participants were available for the final analysis.

Conclusion

The present study confirmed that spinal excitability decreases up to 60 ms during the TMS-evoked SP in the lower-limbs when assessed through LS regardless of contraction intensity. Contraction intensity appeared to affect the duration of decreased spinal excitability, with evidence of reduced excitability at 150 ms during 50% and 75% of MVC and also reduced spinal excitability at 90 ms during 50% of MVC. Thus, interpretation of (changes in) SP

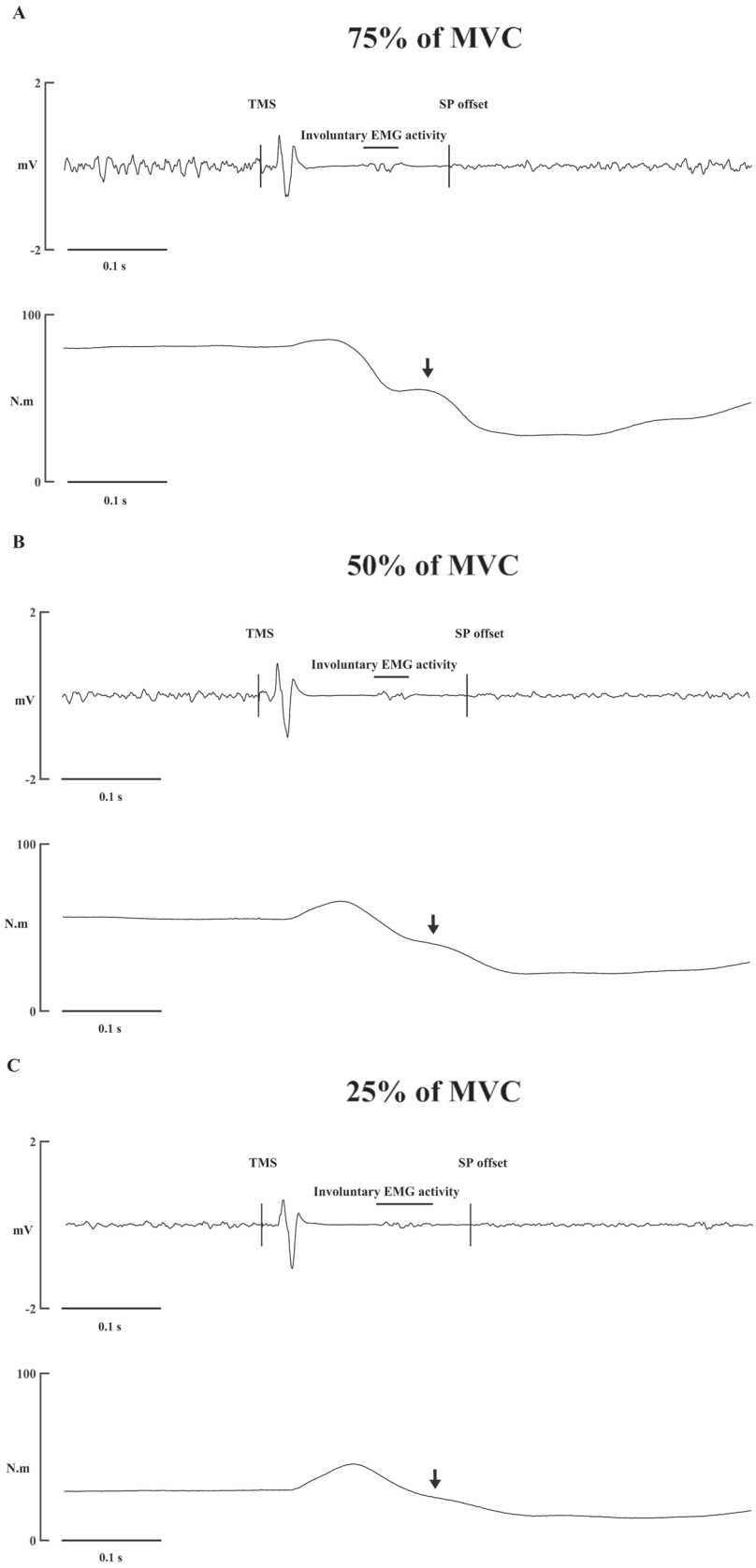

Fig. 5 Involuntary EMG activity during the SP of a participant during different trials at **A** 75% of MVC, **B** 50% of MVC and **C** 25% of MVC. Upper traces represent the EMG signal and lower traces represent torque signal. The arrow points to the possible effect of the involuntary EMG in the torque trace. This phenomenon was observed in 11/15 participants. *TMS* transcranial magnetic stimulation, *SP* silent period

duration being attributable to intracortical inhibition should be made with caution in future studies, particularly during higher contraction intensities. The present study demonstrates that paired TMS-LS could be a potential method to understand changes in spinal excitability (during SP at different contraction intensities) when testing various neurophysiological phenomena; e.g., examining acute fatigue or long-term adaptation.

Author contributions Conceptualization: GGG, JA, SW; Piloting and lab set up: GGG, PA, GH; Data collection and data analysis: GGG, PA, SW; writing original draft: GG, SW; writing-reviewing-editing: GGG, PA, GH, JA, SW; Final approval of the manuscript: GGG, PA, GH, JA, SW.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors do not have any conflicts of interest to report relevant to this manuscript. The authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed. All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or material discussed in this manuscript.

References

Barker AT, Jalinous R, Freeston IL (1985) Non-invasive magnetic stimulation of human motor cortex. The Lancet 325(8437):1106–1107

Brownstein CG, Souron R, Royer N, Singh B, Lapole T, Millet GY (2020) Disparate kinetics of change in responses to electrical stimulation at the thoracic and lumbar level during fatiguing isometric knee extension. J Appl Physiol 128(1):159–167

Brownstein CG, Espeit L, Royer N, Ansdell P, Škarabot J, Souron R, Lapole T, Millet GY (2021) Reductions in motoneuron excitability during sustained isometric contractions are dependent on stimulus and contraction intensity. J Neurophysiol 125(5):1636–1646

Butler JE, Petersen NC, Herbert RD, Gandevia SC, Taylor JL (2012) Origin of the low-level EMG during the silent period following transcranial magnetic stimulation. Clin Neurophysiol 123(7):1409–1414

Calancie B, Nordin M, Wallin U, Hagabarth KE (1987) Motor-unit responses in human wrist flexor and extensor muscles to transcranial cortical stimuli. J Neurophysiol 58(5):1168–1185

Damron LA, Dearth DJ, Hoffman RL, Clark BC (2008) Quantification of the corticospinal silent period evoked via transcranial magnetic stimulation. J Neurosci Methods 173(1):121–128

Day BL, Dressler D, Maertens de Noordhout A, Marsden CD, Nakashima K, Rothwell JC, Thompson PD (1989a) Electric and magnetic stimulation of human motor cortex: surface EMG and single motor unit responses. J Physiol 412(1):449–473

Day BL, Rothwell JC, Thompson PD, Maertens de Noordhout A, Nakashima K, Shannon K, Marsden CD (1989b) Delay in the execution of voluntary movement by eletrical or magnetic brain stimulation in intact man. Brain 112:649–663

Dimitrijević MR, Kofler M, McKay WB, Sherwood AM, Van der Linden C, Lissens MA (1992) Early and late lower limb motor evoked potentials elicited by transcranial magnetic motor cortex stimulation. Electroencephalogr Clin Neurophysiol 85(6):365–373

Enoka RM (2008) Neuromechanics of human movement, 4th edn. Human Kinetics, pp 251–255

Finn HT, Rouffet DM, Kennedy DS, Green S, Taylor JL (2018) Motoneuron excitability of the quadriceps decreases during a fatiguing submaximal isometric contraction. J Appl Physiol 124(4):970–979

Frijns CJM, Laman DM, Van Duijn MAJ, Van Duijn H (1997) Normal values of patellar and ankle tendon reflex latencies. Clin Neurol Neurosurg 99(1):31–36

Fuhr P, Agostino R, Hallett M (1991) Spinal motor neuron excitability during the silent period after cortical stimulation. Electroencephalogr Clin Neurophysiol 81(4):257–262

Gandevia SC, Petersen N, Butler JE, Taylor JL (1999) Impaired response of human motoneurones to corticospinal stimulation after voluntary exercise. J Physiol 521(3):749–759

Giesebrecht S, Martin PG, Gandevia SC, Taylor JL (2010) Facilitation and inhibition of tibialis anterior responses to corticospinal stimulation after maximal voluntary contractions. J Neurophysiol 103(3):1350–1356

Granit R, Haase J, Rutledget LT (1960) Recurrent inhibition in relation to frequency of firing and limitation of discharge rate of extensor motorneurons. J Physiol 154:308

Hermens HJ, Freriks B, Disselhorst-Klug C, Rau G (2000) Develompent of recommendations for SEMG sensors and sensor placement procedures. J Electromyogr Kinesiol 10:361–374

Hofstoetter US, Freundl B, Binder H, Minassian K (2018) Common neural structures activated by epidural and transcutaneous lumbar spinal cord stimulation: elicitation of posterior root-muscle reflexes. PLoS ONE 13(1):1–22

Holmgren H, Larsson LE, Pedersen S (1990) Late muscular responses to transcranial cortical stimulation in man. Electroencephalogr Clin Neurophysiol 75(3):161–172

Houk JC, Singer JJ, Goldman MR (1970) An evaluation of length and force feedback to soleus muscles of decerebrate cats. J Neurophysiol 33(6):784–811

Hultborn H, Pierrot-Deseilligny E (1979) Changes in Recurrent Inhibition during voluntary soleus contractions in man studied by an H-reflex technique. J Physiol 297:229–251

Hupfeld KE, Swanson CW, Fling BW, Seidler RD (2020) TMS-induced silent periods: a review of methods and call for consistency. J Neurosci Methods 346:108950

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

Kamen G, Knight CA (2004) Training-related adaptations in motor unit discharge rate in young and older adults. J Gerontol—Series A Biol Sci Med Sci 59(12):1334–1338

Kidgell DJ, Goodwill AM, Frazer AK, Daly RM (2013) Induction of cortical plasticity and improved motor performance following unilateral and bilateral transcranial direct current stimulation of the primary motor cortex. BMC Neurosci 14:64

Latella C, Teo W-P, Harris D, Major B, VanderWesthuizen D, Hendy A (2017) Effects of acute resistance training modality on corticospinal excitability, intra-cortical and neuromuscular responses. Eur J Appl Physiol 117(11):2211–2224

Manca A, Ginatempo F, Cabboi MP, Mercante B, Ortu E, Dragone D, De Natale ER, Dvir Z, Rothwell JC, Deriu F (2016) No evidence of neural adaptations following chronic unilateral isometric training of the intrinsic muscles of the hand: a randomized controlled study. Eur J Appl Physiol 116(10):1993–2005

Manning CD, Bawa P (2011) Heteronymous reflex connections in human upper limb muscles in response to stretch of forearm muscles. J Neurophysiol 106(3):1489–1499

Martin PG, Butler JE, Gandevia SC, Taylor JL (2008) Noninvasive stimulation of human corticospinal axons innervating leg muscles. J Neurophysiol 100(2):1080–1086

McDonnell MN, Orekhov Y, Ziemann U (2006) The role of GABAB receptors in intracortical inhibition in the human motor cortex. Exp Brain Res 173(1):86–93

McNeil CJ, Martin PG, Gandevia SC, Taylor JL (2009) The response to paired motor cortical stimuli is abolished at a spinal level during human muscle fatigue. J Physiol 587(23):5601–5612

McNeil CJ, Butler JE, Taylor JL, Gandevia SC (2013) Testing the excitability of human motoneurons. Front Hum Neurosci 7:152

Mills KR (1988) Excitatory and inhibitory effects on human spinal motoneurones from magnetic brain stimulation. Neurosci Lett 94(3):297–302

Nichols TR (2018) Distributed force feedback in the spinal cord and the regulation of limb mechanics. J Neurophysiol 119(3):1186–1200

Petersen NT, Taylor JL, Gandevia SC (2002) The effect of electrical stimulation of the corticospinal tract on motor units of the human biceps brachii. J Physiol 544(1):277–284

Pierrot-Deseilligny E, Burke D (2005) The circuitry of the human spinal cord. Cambridge University Press

Piotrkiewicz M, Kudina L, Mierzejewska J, Jakubiec M, Hausmanowa-petrusewicz I (2007) Age-related change in duration of afterhyperpolarization of human motoneurones. J Physiol 585(2):483–490

Rossi S, Hallett M, Rossini PM, Pascual-Leone A, Avanzini G, Bestmann S, Berardelli A, Brewer C, Canli T, Cantello R, Chen R, Classen J, Demitrack M, Di Lazzaro V, Epstein CM, George MS, Fregni F, Ilmoniemi R, Jalinous R, Ziemann U (2009) Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clin Neurophysiol 120(12):2008–2039

Ruotsalainen I, Ahtiainen JP, Kidgell DJ, Avela J (2014) Changes in corticospinal excitability during an acute bout of resistance exercise in the elbow flexors. Eur J Appl Physiol 114(7):1545–1553

Sidhu SK, Cresswell AG, Carroll TJ (2013) Corticospinal responses to sustained locomotor exercises: moving beyond single-joint studies of central fatigue. Sports Med 43(6):437–449

Škarabot J, Ansdell P, Brownstein CG, Thomas K, Howatson G, Goodall S, Durbaba R (2019a) Electrical stimulation of human corticospinal axons at the level of the lumbar spinal segments. Eur J Neurosci 49(10):1254–1267

Škarabot J, Mesquita RNO, Brownstein CG, Ansdell P (2019b) Myths and methodologies: how loud is the story told by the transcranial magnetic stimulation-evoked silent period? Exp Physiol 104(5):635–642

Taylor JL (2006) Stimulation at the cervicomedullary junction in human subjects. J Electromyogr Kinesiol 16(3):215–223

Taylor JL, Butler JE, Allen GM, Gandevia SC (1996) Changes in motor cortical excitability during human muscle fatigue. J Physiol 490(2):519–528

Taylor JL, Butler JE, Gandevia SC (1999) Altered responses of human elbow flexors to peripheral-nerve and cortical stimulation during a sustained maximal voluntary contraction. Exp Brain Res 127(1):108–115

Taylor JL, Petersen NT, Butler B, Gandevia SC (2002) Interaction of transcranial magnetic stimulation and electrical transmastoid stimulation in human subjects. J Physiol 541(3):949–958

Taylor JL, Todd G, Gandevia SC (2006) Evidence for a supraspinal contribution to human muscle fatigue. Clin Exp Pharmacol Physiol 33(4):400–405

Triggs WJ, Cros D, Macdonell RAL, Chiappa KH, Fang J, Day BJ (1993) Cortical and spinal motor excitability during the transcranial magnetic stimulation silent period in humans. Brain Res 628(1–2):39–48

van Melick N, Meddeler BM, Hoogeboom TJ, Nijhuis-van der Sanden MWG, van Cingel REH (2017) How to determine leg dominance: the agreement between self-reported and observed performance in healthy adults. PLoS ONE 12(12):e0189876

Vernillo G, Barbi C, Temesi J, Giuriato G, Giuseppe F, Martignon C, Schena F, Venturelli M (2022) Reliability of relaxation properties of knee-extensor muscles induced by transcranial magnetic stimulation. Neurosci Lett 782:136694

Walker S, Blazevich AJ, Haff GG, Tufano JJ, Newton RU, Häkkinen K (2016) Greater strength gains after training with accentuated eccentric than traditional isoinertial loads in already strength-trained men. Front Physiol 7:1–12

Wilmink RJH, Nichols TR (2003) Distribution of heterogenic reflexes among the quadriceps and triceps surae muscles of the cat hind limb. J Neurophysiol 90(4):2310–2324

Yacyshyn AF, Woo EJ, Price MC, McNeil CJ (2016) Motoneuron responsiveness to corticospinal tract stimulation during the silent period induced by transcranial magnetic stimulation. Exp Brain Res 234(12):3457–3463

Zehr EP, Collins DF, Chua R (2001) Human interlimb reflexes evoked by electrical stimulation of cutaneous nerves innervating the hand and foot. Exp Brain Res 140(4):495–504

Ziemann U, Netz J, Szelényi A, Hömberg V (1993) Spinal and supraspinal mechanisms contribute to the silent period in the contracting soleus muscle after transcranial magnetic stimulation of human motor cortex. Neurosci Lett 156(1–2):167–171

III

CORTICAL AND SPINAL RESPONSES TO SHORT-TERM RESISTANCE TRAINING AND DETRAINING IN YOUNG AND OLDER ADULTS IN RECTUS FEMORIS MUSCLE

by

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ORIGINAL ARTICLE

Cortical and spinal responses to short-term strength training and detraining in young and older adults in rectus femoris muscle

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Abstract

Introduction Strength training mitigates the age-related decline in strength and muscle activation but limited evidence exists on specific motor pathway adaptations.

Methods Eleven young (22–34 years) and ten older (66–80 years) adults underwent five testing sessions where lumbar-evoked potentials (LEPs) and motor-evoked potentials (MEPs) were measured during 20 and 60% of maximum voluntary contraction (MVC). Ten stimulations, randomly delivered, targeted 25% of maximum compound action potential for LEPs and 120, 140, and 160% of active motor threshold (aMT) for MEPs. The 7-week whole-body resistance training intervention included five exercises, e.g., knee extension (5 sets) and leg press (3 sets), performed twice weekly and was followed by 4 weeks of detraining.

Results Young had higher MVC (~ 63 N·m, $p = 0.006$), 1-RM (~ 50 kg, $p = 0.002$), and lower aMT (~ 9%, $p = 0.030$) than older adults at baseline. Young increased 1-RM (+ 18 kg, $p < 0.001$), skeletal muscle mass (SMM) (+ 0.9 kg, $p = 0.009$), and LEP amplitude (+ 0.174, $p < 0.001$) during 20% MVC. Older adults increased MVC (+ 13 N·m, $p = 0.014$), however, they experienced decreased LEP amplitude (− 0.241, $p < 0.001$) during 20% MVC and MEP amplitude reductions at 120% (− 0.157, $p = 0.034$), 140% (− 0.196, $p = 0.026$), and 160% (− 0.210, $p = 0.006$) aMT during 60% MVC trials. After detraining, young and older adults decreased 1-RM, while young adults decreased SMM.

Conclusion Higher aMT and MEP amplitude in older adults were concomitant with lower baseline strength. Training increased strength in both groups, but divergent modifications in cortico-spinal activity occurred. Results suggest that the primary locus of adaptation occurs at the spinal level.

Keywords Aging, resistance training · TMS · Lumbar stimulation · Cortico-spinal excitability · Lower-limbs

Abbreviations

1-RM	One-maximum repetition
aMT	Active motor threshold
ANOVA	Analysis of variance
CI	Coefficients intervals
CV	Coefficient of variance
cSP	Cortical silent period
EMG	Electromyography
ES	Effect size
GABA	Gamma-aminobutyric acid
H-reflex	Hoffmann's reflex
Hz	Hertz
ICC	Intra-class correlation
ICF	Intra-cortical facilitation
LEP	Lumbar-evoked potential
LICI	Long intracortical inhibition
LS	Lumbar stimulation
MEP	Motor-evoked potential
M-max	Maximum compound action potential
MVC	Maximal voluntary contraction
RF	Rectus femoris
SICI	Short intracortical inhibition

Communicated by William J. Kraemer.

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SMM Skeletal muscle mass
TMS Transcranial Magnetic Stimulation

Introduction

Aging is a complex process causing functional declines at both the cortical (Baudry et al. 2015; Clark and Taylor 2011) and spinal levels (Baudry et al. 2015; Geertsen et al. 2017; Hortobágyi et al. 2018; Kido et al. 2004). Neuronal atrophy, particularly within the motor cortex, can affect axonal regeneration potentially reducing motor cortex excitability (Fathi et al. 2010; Oliviero et al. 2006) and decreasing cortical inhibition (Christie and Kamen, 2014; Oliviero et al. 2006). Spinal motor-neurons, the last executors of neural commands from the cortex, are also susceptible to age-related changes such as a decline in population (Cruz-Sánchez et al. 1998; Tomlinson and Irving, 1977) and synaptic input reorganization. These changes can lead to a decrease in maximal force production, power, and physical function (Clark and Taylor 2011; Hunter et al. 2016).

Cortico-spinal excitability is evaluated using transcranial magnetic stimulation (TMS) to induce action potentials, producing a motor-evoked potential (MEP) (Barker and Jalinous 1985). Changes in MEP indicate the cortico-spinal tract's integrity (Day et al. 1989; Kobayashi and Pascual-Leone 2003). During voluntary contraction, TMS causes a pause in electromyography (EMG), known as the cortical silent period (cSP) (Mills 1988). The duration of the cSP provides insights into intracortical inhibition (Inghilleri et al. 1993; Taylor et al. 1996), which varies depending on the target muscle (Yacyshyn et al. 2016; Gomez-Guerrero et al. 2023a) and contraction intensity (Gomez-Guerrero et al. 2023a).

Cortical and spinal excitability are inseparable from MEP responses (Taylor 2006), thus, electrical stimulation at the spinal level is needed for specific insight into spinal motor-neurons. Given the importance of lower-limb function for ambulation (Landin et al. 2016), which predicts disability and mortality (Guralnik et al. 1995; Millington et al. 1992), methodologies targeting lower-limb muscles in aging individuals are needed. Traditional peripheral-nerve stimulation has been questioned (McNeil et al. 2013), and direct spinal-cord stimulation at corticomedullary and thoracic levels can cause discomfort. In contrast, lumbar stimulation (LS), validated in healthy young adults (Škarabot et al. 2019a), has shown reliability during 20 and 60% muscular voluntary contraction (MVC) in active healthy adults (18–75 years old) (Gomez-Guerrero et al. 2023b) and is well-tolerated by young males (Brownstein et al. 2020), inducing an action potential in spinal motor-neurons and eliciting a lumbar-evoked potential (LEP) in the anterior thigh muscles' EMG.

Strength training interventions are a safe and robust method to decelerate the aging process by enhancing functional capacity in untrained older adults (Siddique et al. 2022). In healthy young adults, strength training induces neural adaptations, during the first 3–4 weeks, by inducing plastic changes at the cortical (Weier et al. 2012; Goodwill et al. 2012) and spinal level (Aagaard et al. 2002; Holtermann et al. 2007). Specifically, increased MEP amplitude at 110–140% aMT was observed in m.rectus femoris (RF) within a recruitment curve (90–140% active motor threshold (aMT)) following twelve sessions of heavy-squat training (4 sets, 6–8 repetitions, at 80% 1-repetition maximum (1-RM)) in healthy young adults (Weier et al. 2012). A meta-analysis (Kidgell et al. 2017) reported that strength training may induce cortico-spinal adaptations in young adults, indicated by both increased MEP amplitude and decreased cSP duration. On the other hand, 6 sessions of strength training over 3 weeks in the ankle dorsiflexors did not change MEP amplitude but decreased cSP length in both untrained young and older adults (Christie and Kamen 2014); currently the only study to use TMS to evaluate a strength-training intervention in older adults.

Similarly, 2 to 3 weeks (6–9 training sessions in total) of strength training in older adults did not show spinal adaptations as measured by Hoffman-reflex (H-reflex) amplitude (Christie and Kamen 2014; Unhjem et al. 2020). Nevertheless, spinal adaptations have been documented following 3 to 14 weeks of strength training when measured during maximal (100% of MVC) (Aagaard et al. 2002) or submaximal (20 and 60% of MVC (Holtermann et al. 2007); and 10% of MVC (Vila-Chã et al. 2012)) contractions in young adults. Therefore, it is not presently clear whether adaptations in inhibitory pathways observed in young adults is due to an age effect per se or whether older adults may simply require more than 3 weeks/nine training session to achieve the same level of adaptation as the young. Consequently, it remains unclear whether neural plasticity resulting from strength training occurs at a cortical or spinal level or perhaps involves both (Siddique et al. 2022), and whether certain neural adaptations are specific to young and older age.

Finally, short- and long-term withdrawal (i.e., detraining) from strength training leads to decreased strength in young and older adults (Häkkinen et al. 2000, 1981). Although there is currently no specific investigation as to how cortical and spinal mechanisms affect the decrease in strength in young and older adults after a detraining period (Hortobágyi et al. 2021), some studies have reported a decrease in EMG activity and strength after a short-term (i.e., 3 to 6 weeks) detraining period in older adults (Häkkinen et al. 2000; Toraman 2005). Consequently, utilizing a training–detraining model would enhance confidence in interpreting causality from accompanying MEP and LEP changes along with strength level**.** Therefore, the aim of this study was to evaluate cortical and spinal adaptations in RF during a 7-week

strength training period that included a 4-week detraining period in both young and older adults.

Material and methods

Participants

Twenty-seven participants volunteered for the study (14 female). The recruitment process and exclusion of participants is shown in Fig. 1. Therefore, the data presented in

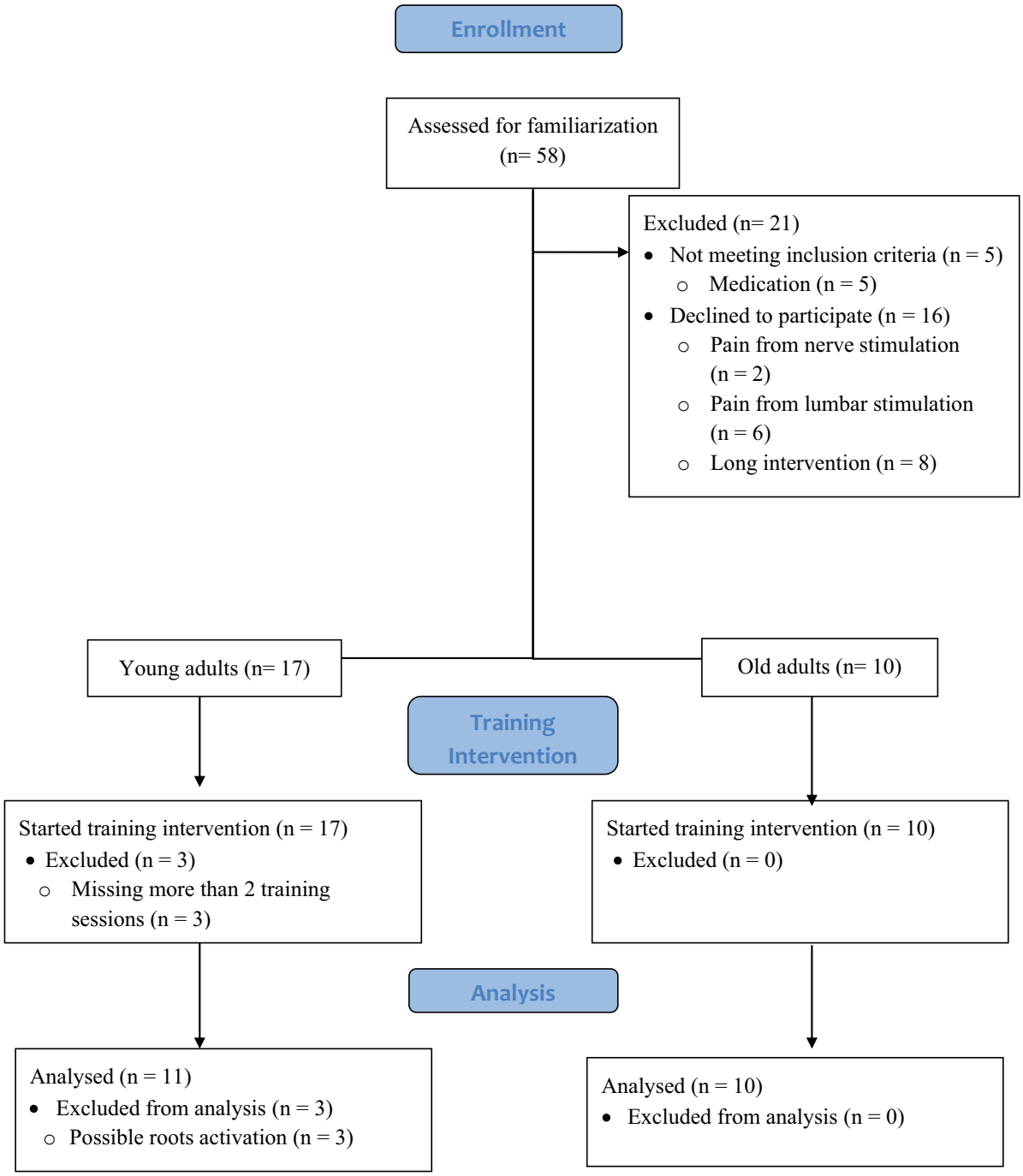

Fig. 1 Flow chart of study enrollment, strength-training intervention, and analysis

Table 1 are representative of the 21 (11 young adults (6 female) and 10 older adults (6 female)) volunteers fulfilling all study requirements. All included participants were free from musculoskeletal injury in the lower-limbs for the last 6 months and neurologic illness, were not taking any medications known to affect the nervous system and had no contraindications to TMS, which was assessed via a health questionnaire (Rossi et al. 2011). Before testing, all participants were fully informed of the procedures and possible risks, and each participant provided written informed consent. The Ethical Committee of the University of Jyväskylä provided a statement for the study (857/13.00.04.00/2021) and the study was conducted in accordance with the ethical standards establish in the *Declaration of Helsinki* (2013).

Experimental set-up

Participants visited the laboratory on five different testing periods and one familiarization session (Fig. 2A). All participants were instructed to maintain their regular dietary habits up to two hours prior to the testing session, consume 500 ml of water immediately before the test, abstain from consuming caffeine within the 12 h leading up to the examination, and refrain from engaging in strenuous physical activities 48 h preceding each testing session. The study's initial phase consisted of a familiarization session in which participants were introduced to all instructions and stimulation parameters pertinent to the subsequent testing sessions. This session also served as a preliminary assessment of the LS placement and the determination of TMS intensity for active motor threshold (aMT). Then, testing periods were defined as control testing (Con), pre-training testing (Pre), mid-training testing (Mid), post-training testing (Post) and detraining testing (De) (Fig. 2A). Every testing period was structured the same: A LS session, a TMS session and a one-maximum repetition session (1-RM) conducted within a 7-day period. Sessions for each participant were consistently scheduled at the same time of the day, and there was a 48- to 72-h interval between LS, TMS and 1-RM (Fig. 2B).

To assess responses in RF, participants sat in a custom-built chair with a calibrated load cell (Faculty of Sport and Health Sciences, University of Jyväskylä, Finland) with the hip and knee at 90° flexion and the shank strapped by a non-elastic restraint ~ 2 cm superior to the ankle malleoli. The voltage signal originating from the load cell was calibrated and converted into torque (N·m). All measures were performed on the right (i.e., dominant) leg assessed by self-report of which foot they primarily kick a ball (van Melick et al. 2017).

Every session followed the same structure. Once the participant was secured to the dynamometer, the maximum compound action potential (M-max) was assessed in a relaxed condition (i.e., M-maxpre). As a warm-up, two contractions at ~ 50 and ~ 80% of estimated MVC were performed. Then, two MVC trials were performed 60 s apart (i.e., MVCpre). Verbal encouragement and visual feedback were provided to motivate participants to produce maximal effort and torque was recorded. The reliability of this method was excellent (CV = 4.6%; ICC = 0.987).

In every testing session, visual feedback was provided to the participants to produce the required submaximal torque and then a single LS or TMS stimulus was delivered manually. Contractions at 20 and 60% of MVC were held for 5–8 s, because RF MEP amplitude seemingly increases until 50–75% of MVC (Martin et al. 2006; Oya et al. 2008; Goodall et al. 2009; Škarabot et al. 2019a). Sets of ten stimulations were given per condition and per contraction level as a single block, giving a total of 40 LS and 60

Table 1 Mean ± standard deviation and statistical comparison of older versus younger adults of measurements during control

	Young adults	Older adults	Between-group *p*-value	95% CI [lower-bound, upper-bound]	Hedges' *g*
Age (years)	27 ± 5	71 ± 4	–	–	–
Height (m)	1.74 ± 0.10	1.66 ± 0.06	–	–	–
Body mass (kg)	83.99 ± 24.23	74.73 ± 9.49	$p = 0.272$	[− 26.41, 7.89]	− 0.47
Body Mass Index (kg/m^2)	26.78 ± 5.83	27.19 ± 3.30	$p = 0.857$	[− 4.58, 3.85]	− 0.08
Skeletal muscle mass (kg)	32.54 ± 7.88	27.59 ± 3.82	$p = 0.088$	[− 11.15, 0.66]	− 0.75
Body fat mass (kg)	26.14 ± 13.13	24.34 ± 9.12	$p = 0.723$	[− 12.23, 8.64]	− 0.15
MVC (N·m)	202 ± 53	139 ± 38	$p = 0.006$	[− 105.54, − 20.84]	− 1.31
1-RM (kg)	127 ± 42	77 ± 16	$p = 0.002$	[− 79.93, − 21.12]	− 1.51
M-max (mV)	2.65 ± 1.25	1.23 ± 0.50	$p = 0.003$	[− 2.32, − 0.53]	− 1.41
LEP stimulation intensity (mA)	262 ± 93	200 ± 77	$p = 0.136$	[− 144.89, 21.29]	− 0.69
aMT (%)	31 ± 6	40 ± 11	$p = 0.030$	[0.91, 16.33]	0.98

CI confidence interval, *m* meter, *kg* kilogram, *MVC* maximal voluntary contraction, *N* Newton, *1-RM* one-repetition maximum, *M-max* maximal compound action potential, *mV* millivolt, *LEP* Lumbar-evoked potential, *mA* milliampere, *aMT* active motor threshold

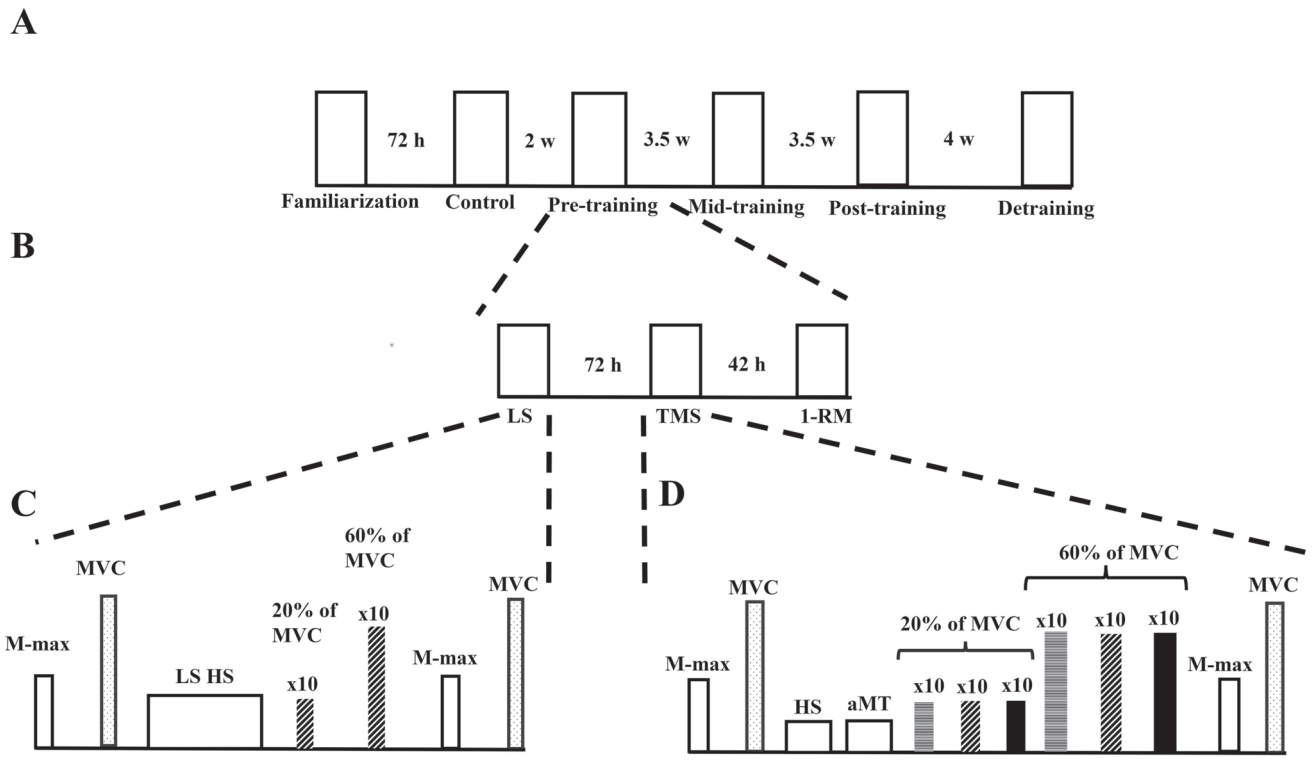

Fig. 2 Description of the experimental timeline. **A** order of the six different testing periods. The time between testing sessions refers to the total time between one test period to the next. **B** Example of the testing sessions set up within a testing period. The time in-between the sessions is the minimum amount of time between test. **C** lumbar stimulation session set-up. **D** TMS stimulation sessions set-up. *H* hours, *W* weeks, *LS* lumbar stimulation, *TMS* transcranial magnetic stimulation, *1-RM* one-repetition maximum, *M-max* maximum compound action potential, *MVC* maximal voluntary contraction, *HS* hotspot, *aMT* active motor threshold

TMS stimulations. To avoid fatigue, 30 s and 45 s rest was given between contractions during 20 and 60% of MVC, respectively, and 60 s and 180 s rest was given between the sets of 10 contractions. At the end of the protocol, M-max (M-maxpost) and MVC (MVCpost) were re-assessed (Fig. 2 C and D).

Bipolar surface electromyography and torque

Muscle activity was recorded using adhesive Ag/AgCl electrodes (30 × 20 mm, BlueSensor N, Ambu, Penang, Malaysia) from RF according to SENIAM guidelines (Hermens et al. 2000). Skin was shaved, abraded with sandpaper, and wiped with alcohol before positioning the electrodes in a bipolar arrangement with a 20 mm center-to-center distance. Impedance was set < 2 kΩ, and the reference electrode was positioned on the patella. EMG electrode positions were marked with a permanent marker over the skin, photographs were taken and the distance from the iliac crest to the middle of the electrode pair was recorded. In addition, during the training period, the marks were redrawn by the research assistant after every training session. EMG data were sampled online at 3000 Hz, amplified (1000 ×) and bandpass filtered (16–1000 Hz; Neurolog System, Digitimer Ltd, UK) using CED Power1401-3 (Cambridge Electronic Design Ltd, Cambridge, UK).

Torque was sampled at 1000 Hz, amplified by a custom-built amplifier (ForAmps 1 v1.2, University of Jyväskylä, Finland) and converted by a 16-bit A/D board (CED Power1401-3, Cambridge Electronics Design, Cambridge, UK) in combination with Spike2 software (version 6.10, Cambridge Electronic Design, Cambridge, UK).

Peripheral nerve stimulation

Transcutaneous electrical stimulation of the femoral nerve (32 mm cathode/anode arrangement; Polar Neurostimulation Electrodes, Espoo, Finland) was performed to elicit M-max in RF (1 ms squared pulse duration; Digitimer DS7AH, Hertfordshire, UK). Electrodes were placed 2 cm apart and positioned at each side of the femoral nerve, located by palpation and identification of the femoral artery (Walker et al. 2016). M-max was elicited by gradually increasing stimulator output intensity until the EMG response plateaued. To ensure a supramaximal response was elicited, this intensity

was further increased by 50% and two individual simulations were given (Table 1).

Lumbar stimulation

Transcutaneous electrical LS was used to elicit LEPs with a constant-current stimulator (1 ms square pulse duration; Digitimer DS7AH, Hertfordshire, UK) via self-adhesive electrodes (Polar Neurostimulation Electrodes, Espoo, Finland). Originally, the cathode (5 × 9 cm) was centered over the first lumbar vertebra and the anode (circular shape; 3.2 cm diameter) was placed on the midline of the vertebral column ~ 5 cm above the top edge of the cathode as described by Škarabot et al. (2019a).

Potential activation of ventral roots was examined from the onset latency of the LEP of an increasing stimulator intensity (Petersen et al. 2002) up to 25% of the M-max and also tracking LEP amplitude during increasing voluntary contraction while maintaining stimulator output intensity to that which produced a LEP amplitude of 25% of the M-max (Taylor et al. 2002). Should the ventral roots be activated by the stimulation procedures, onset latency would have shortened with an increase in stimulator intensity and LEP amplitude would have been the same during increased voluntary contraction (Petersen et al. 2002; Taylor et al. 2002). Three participants demonstrated no change in LEP amplitude with an increase in voluntary torque during offline analyses, and they were, therefore, removed from further analyses.

Dorsal root activation was assessed via paired LS with a 50 ms time delay, where the second LEP amplitude was compared to the first. Paired stimulation was conducted at rest, with the stimulator output intensity set to produce a LEP equivalent to 25% of the M-max. Evidence of dorsal-root activation would manifest as a decrease in the second LEP compared to the first, attributed to post-activation depression at the motor-neuron pool between the two stimuli (Hofstoetter et al. 2018). If the participant failed any of the tests (i.e., dorsal or ventral stimulation protocols), the electrodes were relocated 1 cm higher, until the participant passed all tests, or the anode was place between the third and fourth thoracic vertebrae. To ensure the placement was the same in all sessions, the distance from the 7th cervical vertebra to the anode (21.7 ± 4.1 cm) and from the bottom of the anode to the top of the cathode (3.7 ± 1.1 cm) (i.e., inter-electrode distance) were taken. All remaining participants showed no sign of the responses described and reported that they found LS to be tolerable. Once the placement was confirmed, stimulator intensity was kept to that which produced a LEP of 25% of the M-max at rest, and this stimulation intensity was used throughout the session (Table 1, Fig. 2C). The reliability of this method is reported in Gomez-Guerrero et al. (2023b) and considered moderate-to-good (ICC: 20% of MVC = 0.632; 60% of MVC = 0.520).

Transcranial magnetic stimulation

Single TMS pulses were delivered using a MagStim 200^2 magnetic stimulator (MagStim Co., Ltd., Whitland, UK) connected to a concave double-cone coil positioned over the left cortical hemisphere for RF with a posterior-to-anterior current orientation. The hotspot was defined at rest as the position eliciting the largest visible MEP recorded by EMG using the same intensity (approx. 50–70% stimulator output). Once the hotspot was found, the coil position was marked with a permanent marker on the scalp to maintain the same position throughout the protocol. Active motor threshold (aMT) was determined by increasing stimulator intensity in 5% steps, starting at 30% of the stimulator output. Thereafter, stimulator intensity was decreased in steps of 1% until clear MEPs (> 100 µV) were elicited in three out of five stimulations during unilateral isometric contractions of the right limb at 10% of MVC. Sets of ten single TMS stimulations were delivered in a random order for each of the assigned conditions (i.e., 120, 140 and 160% aMT) during unilateral isometric contractions at 20% and 60% of MVC (Fig. 2D). The reliability of these methods is reported in Gomez-Guerrero et al. (2023b) and considered good-to-excellent (ICC: 20% of MVC = 0.821–0.861; 60% of MVC = 0.901–0.941).

Knee extension one-repetition maximum

All participants performed a bilateral concentric knee extension (David 200, David Health Solutions Ltd, Helsinki, Finland) one-repetition maximum (1-RM) test during the 5 test periods (Fig. 2A). First, each participant went through anthropometric analysis (Inbody 770, Inbody Co. Ltd, Seoul, Korea). Then, a 5 min cycling (1 kg load at 70 rpm) warm-up was performed followed by a series of submaximal warm-up sets (6 repetitions at an estimated 10-RM load, 3 repetitions at an estimated 6-RM load, 1-repetition at an estimated 3-RM load). Thereafter, single repetitions were performed until the participant could no longer lift the load from the beginning knee angle of ~ 85° to the required knee angle (≥ 170° knee angle), by visual inspection. The last successfully lifted load was recorded as the participant's 1-RM and used to prescribe the load for the first and 4th week of training. Four-to-eight attempts where needed to calculate 1-RM with 1.25 kg precision. Verbal encouragement was provided to motivate participants to produce a maximal effort. 3 minutes rest were provided between attempts. The reliability of this method was excellent (CV = 8.4%; ICC = 0.991).

Strength training sessions

Over the course of the 7 weeks of strength training, participants engaged in a total of 13 supervised sessions of

conventional strength training. Mid-training testing was conducted after seven training sessions. Training sessions were conducted twice-a-week, with at least a 48-h break between sessions. The strength-training program was created following the guidelines provided by Fragala et al. (2019). The training program may be considered whole-body, targeting both upper- and lower-limbs, although we acknowledge that there were no dedicated abdominal or lower back exercises. Nevertheless, one or two exercises per muscle group were performed with a total volume of eight sets per muscle group for the lower-limbs and back/biceps and three sets for chest/triceps (Fragala et al. 2019). Each training session consisted of five different exercises for the upper- and lower-limbs: leg press, knee extension, bicep curl, smith-machine bench press and chest-supported seated row, in that order during normal training sessions. During testing sessions (Pre, Mid, Post), the order was: knee extension, leg press, smith-machine bench press, bicep curl. This training program closely resembles the most potent program for older adults identified in a meta-analysis (Borde et al. 2015). During the last set of the last session of the week, participants performed the maximum number of repetitions for each exercise to adjust either the volume or intensity (according to the estimated %RM) for the following week, so they could perform at least 8 repetitions.

All training sessions started with a warm-up, which consisted of 5 min of cycling and dynamic mobility exercises. During the initial training session, knee extension 1-RM testing was conducted. Subsequently, a 3–5 RM test was performed for the remaining exercise to determine and prescribe the training load. The rest of the sessions consisted of five (knee extension and bicep curl) and three sets (leg press, smith-machine bench press and chest-supported seated row) of 8–10 repetitions at 75–80% of 1-RM. The participants were asked to perform a 2 s-controlled eccentric phase, with no isometric phase and fast concentric phase.

A 4-week detraining period followed the strength-training period. Participants were allowed to maintain their normal physical activity (i.e., cycling, walking, running) during the whole intervention, but strength training was terminated during the detraining period.

Data and statistical analyses

Offline analyses were performed with Spike2 software (version 6.10, Cambridge Electronic Design, Cambridge, UK) to manually obtain M-max amplitude and MVC torque. The other outcome measures were analyzed by a customized MATLAB script (version R2020b, The MathWorks, Inc., Natick, USA). Peak-to-peak amplitude of LEPs and MEPs were analyzed automatically between latencies-of-interest following LS or TMS, respectively. SP duration was defined, as the time from the stimulator artifact to the return of voluntary EMG (Damron et al. 2008). Torque was averaged over the 100 ms before the stimulator artifact (Škarabot et al. 2019b). LEP and MEP amplitude is represented as relative to M-max.

SPSS software (version 26.0, SPSS Inc., Chicago, USA) was used for all statistical methods. Means and standard deviation (SD) were calculated and reported throughout. Normality of the data was tested with the Shapiro–Wilk test and confirmed by a z-score with an acceptance of + 2 to − 2 (e.g., skewness score/skewness score$_{SE}$ and kurtosis score/kurtosis score$_{SE}$) and Q-plots for visualization. Data that did not fulfill those requirements were Log_{10} transformed, which then fulfilled the requirements for normality. A two-way repeated measures ANOVA (5 Time × 2 Group) was employed to assess most outcome variables (MVC, 1-RM, skeletal muscle mass, M-max, aMT, and silent periods of LEPs at 25% of the M-max and MEPs at 120, 140, 160% aMT) during contractions at 20 and 60% of MVC. When assumptions of sphericity were violated, Greenhouse–Geisser corrections were used. Post-hoc Bonferroni adjustments were used when significant main effects were found. To investigate the influence of strength training on the TMS- and LS-induced MEP/LEP amplitude, and to accommodate for missing data points and baseline variability, we employed a Linear Mixed Model (LMM) (Wilkinson et al. 2023). This model served as a robust framework for analyzing our data considering both fixed and random effects simultaneously. Cortico-spinal (MEPs at 120, 140, 160% aMT) and spinal (LEPs at 25% of the M-max) excitability at 20 and 60% of MVC were assessed using the LMM. The model included time (Con, Pre, Mid, Post, and De) and age group (young and older) as main effects and an interaction between age group (young and older) and time with participants as the random effect within the model. Bonferroni adjustments were used when significant main effects were found. Reliability, based on ICCs was categorized as poor (ICC < 0.5), moderate (ICC: > 0.5–< 0.75), good (ICC: > 0.75–< 0.9) and excellent (ICC: > 0.9) (Koo and Li 2016).Data are presented in the Tables by mean and SD, and in the results section by mean difference (MD), effect sizes are represented as partial eta-squared values (η_p^2 = small: 0.01, medium: 0.06, large: 0.14) for the factors of the ANOVA and post-hoc effect sizes reported as Hedge's g (g = small: < 0.3, medium: 0.3–0.8, large: > 0.8). Alpha was set at 0.05.

Results

Baseline between-group comparisons

Main effects for Group were observed for 1-RM ($F_{(1,19)} = 15.94$, $p = 0.001$, $\eta_p^2 = 0.46$), MVC ($F_{(1,19)} = 9.60$, $p = 0.006$, $\eta_p^2 = 0.34$), M-max ($F_{(1,19)} = 20.86$, $p < 0.001$,

$\eta_p^2 = 0.53$), aMT ($F_{(1,19)} = 11.75$, $p = 0.038$, $\eta_p^2 = 0.21$), MEP amplitude during 60% of MVC with 120% aMT ($F_{(1,19)} = 4.65$ $p = 0.044$) and 140% aMT ($F_{(1,19)} = 4.62$ $p = 0.045$), MEP silent period during 20% of MVC with 120% aMT ($F_{(1,19)} = 13.96$, $p = 0.001$, $\eta_p^2 = 0.42$), LEP silent period duration during 20% of MVC ($F_{(1,19)} = 5.60$, $p = 0.029$, $\eta_p^2 = 0.229$), MEP silent period during 60% of MVC with 120% aMT ($F_{(1,19)} = 23.39$, $p < 0.001$, $\eta_p^2 = 0.650$), and LEP silent period duration during 60% of MVC ($F_{(1,19)} = 23.39$, $p < 0.001$, $\eta_p^2 = 0.552$).

During the first measurement session (i.e., control), young adults were stronger than older adults, and had a higher M-max and lower aMT (Table 1 and Fig. 3). Further, during control, MEP amplitude at 120% and 140% aMT was greater in the older group during 60% of MVC (Fig. 4D and E). Silent period duration was longer in older adults during both 20% of MVC (99 ± 15 ms versus 117 ± 18 ms,

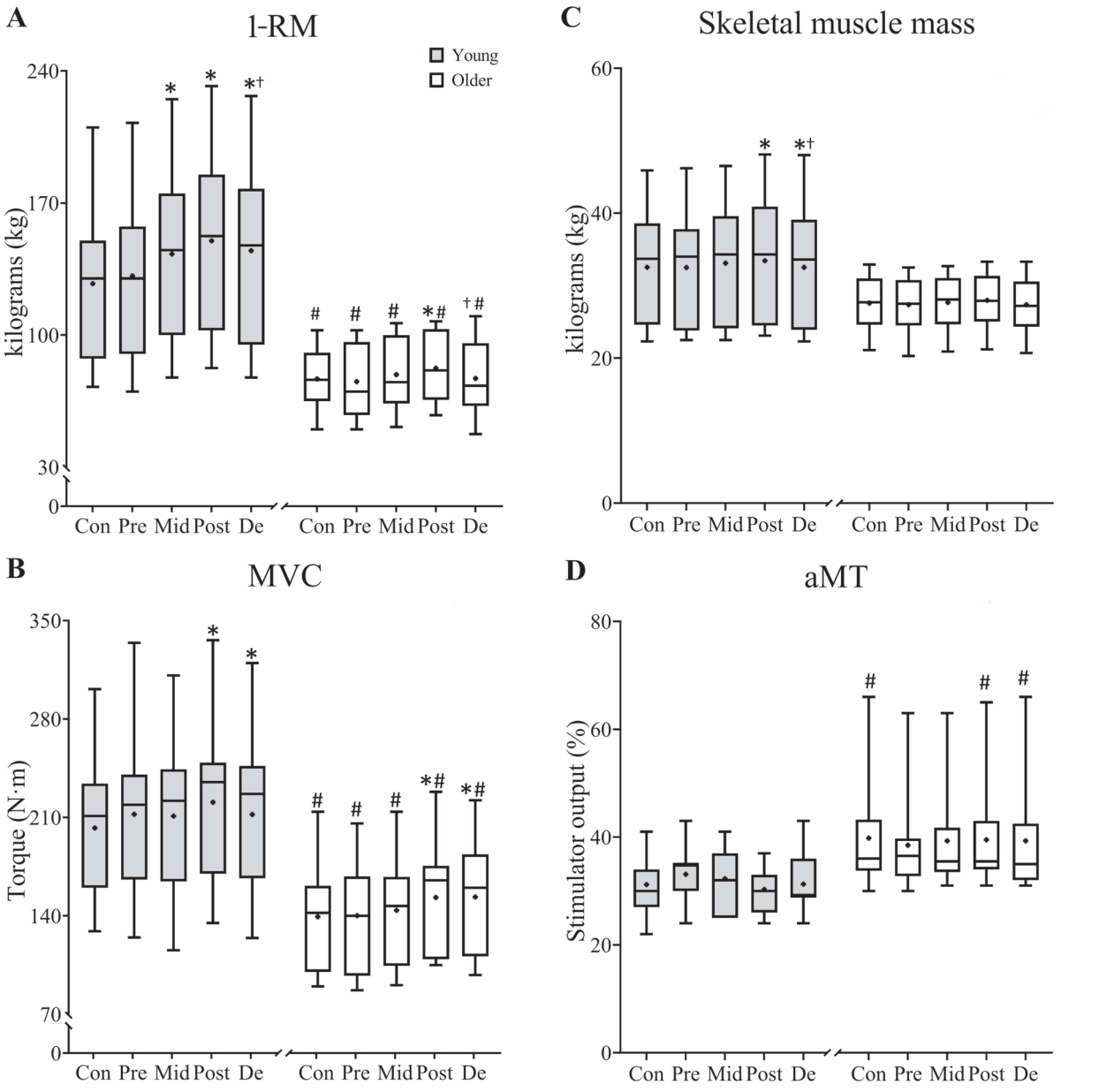

Fig. 3 Box and whiskers plots showing the comparisons of group and time effect in young and older adults for **A** 1-RM, **B** MVC, **C** skeletal muscle mass and **D** aMT. Each figure shows quartiles and whiskers (minimum and maximum), the median (line in the box), mean (+ in the box) for each group (young: filled box and older: blank box) and session. *$p < 0.05$ post hoc within-group analysis compared to pre-training. $p < 0.05$ post hoc within-group analysis compared to post-training. #$p < 0.05$ post hoc between-group analysis compared to the older group

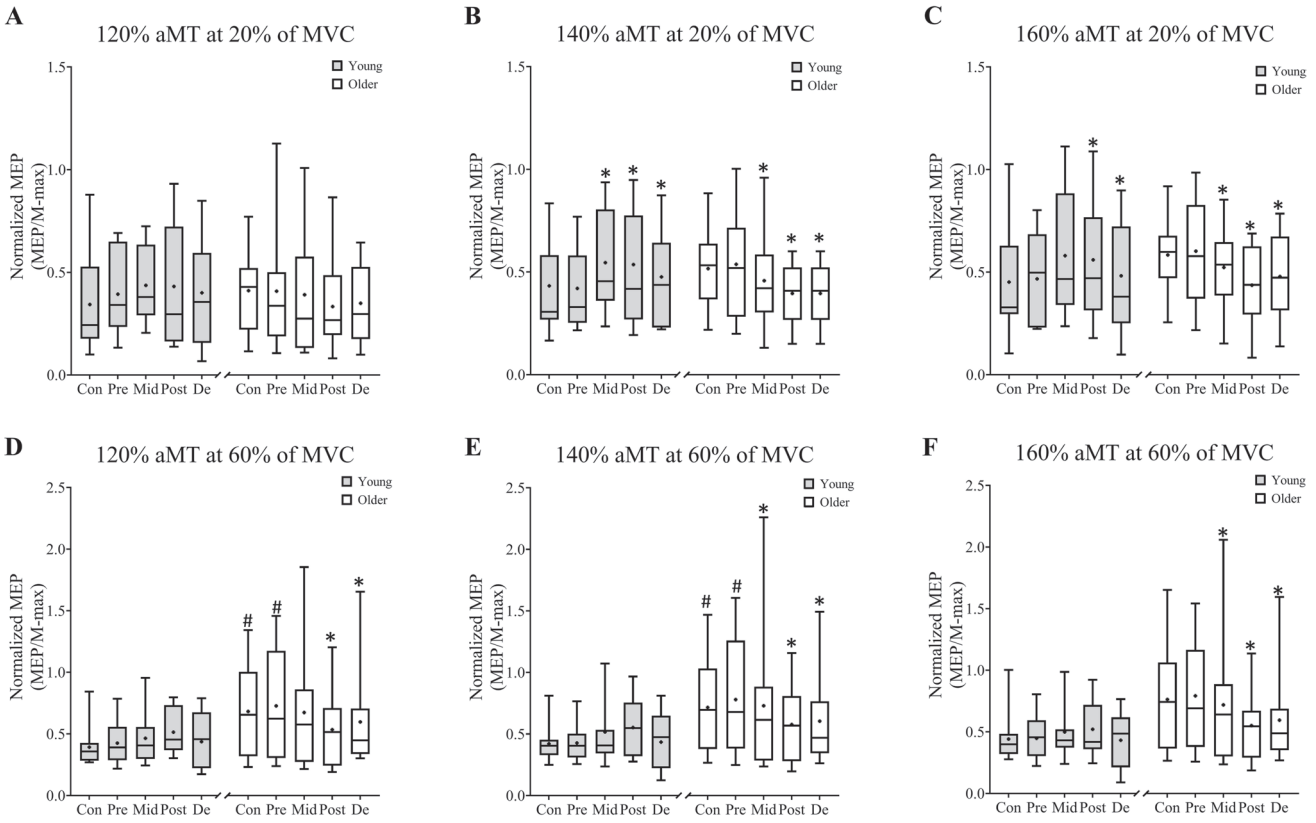

Fig. 4 Box and whiskers plots showing the comparisons of group and time effect in young and older adults for different aMT intensities at 20% of MVC (120% aMT: **A**; 140% aMT: **B**; 160% aMT: **C** and 60% of MVC (120% aMT: **D**; 140% aMT: **E**; 160% aMT: **F**).Each figure shows quartiles and whiskers (minimum and maximum), the median (line in the box), mean (+in the box) for each group (young: filled box and older: blank box) and session. *$p<0.05$ post hoc within-group analysis compared to pre-training. #$p<0.05$ post hoc between-group analysis compared to the older group

p = 0.027) and 60% of MVC (94 ± 12 ms versus 121 ± 20 ms, $p = 0.001$) when stimulated at 120% aMT, and during 60% of MVC from LS (62 ± 7 ms versus 82 ± 20 ms, $p = 0.006$) (see supplementary material).

Training-induced adaptations

For 1-RM, main effects for Time ($F_{(2.3,42.9)} = 28.29$, $p < 0.001$, $\eta_p^2 = 0.60$) and Time*Group interaction ($F_{(2.3,42.9)} = 11.06$, $p < 0.001$, $\eta_p^2 = 0.38$) were observed. Post-hoc comparisons showed that young adults increased from Pre to Post ($p < 0.001$) and then decreased from Post to De ($p = 0.011$, Fig. 3A). Older adults did not increase statistically Pre to Post but did Mid to Post ($p = 0.027$) and they also decreased Post to De ($p = 0.012$).

MVC demonstrated a significant main effect for Time ($F_{(4,76)} = 10.13$, $p < 0.001$, $\eta_p^2 = 0.35$). Post-hoc analysis showed that young adults increased significantly Mid to Post ($p = 0.024$) and older increased significantly Pre to Post ($p = 0.014$, Fig. 3B).

Skeletal muscle mass demonstrated a significant main effect for Time ($F_{(2.5,47.8)} = 3.16$, $p < 0.001$, $\eta_p^2 = 0.323$). Here, only young adults increased Pre to Post ($p = 0.009$) and then decreased Post to De ($p < 0.001$, Fig. 3C).

Significant main effects for Time and Time*Group interaction were observed for MEP amplitude during 20% of MVC at 120% aMT (Time: $F_{(4,1021)} = 3.09$, $p = 0.015$; Time*Group: $F_{(4,1021)} = 4.10$, $p = 0.003$), 140% aMT (Time: $F_{(4,1021)} = 4.89$, $p = 0.001$; Time*Group: $F_{(4,1021)} = 14.44$, $p < 0.001$), 160% aMT (Time: $F_{(4,1021)} = 8.12$, $p < 0.001$; Time*Group: $F_{(4,1021)} = 4.10$, $p = 0.003$). In the young adults, significant increases occurred Pre to Post with 140% aMT ($p = 0.023$) and Pre to Mid at 160% aMT ($p = 0.005$). In older adults, significant decreases were observed Pre to Post at 140% ($p < 0.001$) and 160% ($p < 0.001$) aMT (Figure, Fig. 4B and C).

Significant main effects for Time and Time*Group interaction were observed for MEP amplitude during 60% of MVC at 120% aMT (Time: $F_{(4,1021)} = 4.24$, $p = 0.002$; Time*Group: $F_{(4,1021)} = 10.53$, $p < 0.001$), 140% aMT (Time: $F_{(4,1021)} = 7.97$, $p < 0.001$; Time*Group: $F_{(4,1021)} = 13.69$,

$p < 0.001$), 160% aMT (Time: $F_{(4,1021)} = 13.50$, $p = 0.002$; Time*Group: $F_{(4,1021)} = 14.08$, $p < 0.001$). Post-hoc comparisons showed that only older adults decreased Pre to Post with all stimulation intensities ($p < 0.001$, Fig. 4D–F).

Significant main effects for Time ($F_{(4,1021)} = 3.09$, $p = 0.015$) and Time*Group interaction ($F_{(4,1021)} = 4.10$, $p = 0.003$) were observed for LEP amplitude during 20% of MVC. Young adults significantly increased Pre to Post ($p < 0.001$) and subsequently decreased Post to De ($p < 0.001$). Also, in the young adults, there was a significant decrease from Con to Pre ($p = 0.022$). In older adults, a significant decrease occurred Pre to Post ($p < 0.001$) (Table 2).

Significant main effects for Time ($F_{(4, 1021)} = 8.45$, $p < 0.001$) and Time*Group interaction ($F_{(4, 1021)} = 6.66$, $p < 0.001$) were LEP amplitude during 60% of MVC. Post hoc showed that young significantly decreased Con to Pre ($p < 0.001$), further decreased Pre to Mid ($p = 0.023$), and then increased Mid to Post ($p < 0.001$) (Table 2).

Discussion

This study addressed the lack of knowledge regarding cortico-spinal and spinal adaptations to short-term strength training and detraining in young and older adults, specifically in the lower-limbs. The results showed an increase in maximum strength for both groups after seven weeks of training and a partial reversal following four weeks of detraining. The main result of interest was that young adults demonstrated increased cortico-spinal and spinal excitability as a consequence of training, but older adults showed the opposite, i.e., decreased cortico-spinal and spinal excitability. Furthermore, the present study revealed that older adults required greater stimulation intensity to elicit an MEP (i.e., aMT), cortico-spinal excitability at higher contraction intensity was greater, and cortical and spinal inhibition was greater in older adults at baseline accompanying the between-group strength differences suggesting an effect of age.

The observed differences in 1-RM and MVC between young and older adults would be expected due to the age-related reduction in maximal strength (Bemben et al. 1991). Further, both young and older adults responded positively to a short-term strength training intervention observed through increases in 1-RM and MVC, again as expected from previous studies (Christie and Kamen 2014; Häkkinen et al. 2000; Walker and Häkkinen 2014). The 1-RM increases in the present study of Δ14% and Δ9% in young and older adults, respectively, are similar to those reported by Walker and Häkkinen (2014) over ten weeks of training. Interestingly, increases in lean leg mass in that study occurred only in the younger group (Walker et al. 2014), and only the young group increased skeletal muscles mass in the present study. These converging results suggest that neural mechanisms, rather than morphologic, may be responsible for increased maximal strength in previously untrained older adults when initiating strength training. Previously untrained young adults, on the other hand, appear to improve maximal strength through a combination of neural and morphologic mechanisms.

Cortico-spinal excitability

An interesting observation was the consistent decrease in MEP excitability in the older group, independent of the contraction intensity. These changes became apparent as early as

Table 2 Mean ± standard deviation and statistical results from Linear Mix Models fixed effects of normalized LEP amplitude (LEP/M-max) for young and older groups at different contraction intensities and post-hoc comparison

	Control	Pre-training	Mid-training	Post-training	Detraining	Time *p*-value	Time*Group *p*-value	Group *p*-value
20% MVC								
Young adults	0.36 ± 0.13*	0.30 ± 0.10	0.31 ± 0.23	0.48 ± 0.23*	0.36 ± 0.22+	$p = 0.003$	$p < 0.001$	$p = 0.857$
95% CI	[0.30, 0.43]	[0.21, 0.39]	[0.26, 0.51]	[0.37, 0.59]	[0.30, 0.48]			
Older adults	0.35 ± 0.11	0.40 ± 0.21	0.37 ± 0.22	0.30 ± 0.10*	0.35 ± 0.13			
95% CI	[0.30, 0.43]	[0.30, 0.49]	[0.25, 0.51]	[0.18, 0.42]	[0.25, 0.45]			
60% MVC								
Young adults	0.51 ± 0.22*	0.41 ± 0.21	0.34 ± 0.21*	0.47 ± 0.20	0.41 ± 0.20	$p < 0.001$	$p < 0.001$	$p = 0.313$
[95% CI]	[0.39, 0.62]	[0.29, 0.54]	[0.25, 0.54]	[0.33, 0.60]	[0.31, 0.55]			
Older adults	0.50 ± 0.21	0.51 ± 0.23	0.48 ± 0.30	0.48 ± 0.25	0.51 ± 0.24			
[95% CI]	[0.39, 0.63]	[0.37, 0.63]	[0.34, 0.64]	[0.34, 0.62]	[0.39, 0.64]			

MVC maximal voluntary contraction, *LEP* lumbar-evoked potential, *M-max* maximal compound action potential, *CI* confidence intervals

*$p < 0.05$ post hoc within-group analysis compared to pre-training

+$p < 0.05$ post hoc within-group analysis compared to post-training

three weeks into the training. Our results differ from those reported by Christie and Kamen (2014) who reported that two weeks of training (six training sessions) did not induce significant changes in MEP amplitude in the m.tibialis anterior. The authors noted decreases of 4–6% (n.s) in MEP amplitude in the older adults. The magnitude of those results was similar to our results (− 7 to 8%) after 3 weeks/6 sessions of strength training but ours further decreased (to − 12 to 21%) after 7 weeks/ 13 sessions of strength training. Therefore, cortico-spinal adaptation in older adults seems to require more training duration than in young adults.

Furthermore, the interaction, and within-group changes of LEP amplitude parallel those of MEP amplitude; older adults showing a reduction in LEP amplitude at 20% of MVC. In addition, LEP amplitude increased in the young group from pre- to post-training at 20% of MVC and then decreased back to baseline after detraining. No clear or systematic changes were observed in either group during 60% of MVC trials, and the observed fluctuations may be due to the relatively high typical error/reliability values of this method (Gomez-Guerrero et al. 2023b). Nevertheless, one previous study investigating short-term strength training effects (Ansdell et al. 2020) observed no changes in MEP nor LEP amplitude at a group level; where large inter-individual differences apparent with approximately half of the group increasing and half decreasing amplitude after 12 sessions of 4 sets of 6–8 back squat repetitions. In contrast, Lundbye-Jensen et al. (2005) demonstrated decreased cortico-spinal excitability in untrained healthy young adults after thirteen training sessions spread over 4 weeks. This effect was observed at several higher TMS stimulator output intensities (160–220% rMT), similar to our differences observed at 140 and 160% aMT. The authors discussed that those changes could potentially be at subcortical levels through changes in spinal motor-neuron firing rate and/or intrinsic firing properties, although this was not specifically tested. In support, Vila-Chã et al. (2012) and Aagaard et al. (2002) observed spinal adaptations, through better modulation of inhibitory pathways, after 3 weeks and 14 weeks of strength training in younger adults. Thus, in the present study, the older group adapted to the training by reducing their MEP amplitude down to the level of the young and these adaptations could be at a spinal level.

Conversely, small magnitude but statistically significant increases in MEP excitability occurred in the young group after strength training, as has been previously reported (Goodwill et al. 2012; Kidgell et al. 2017; Weier et al. 2012). Goodwill et al. (2012) and Weier et al. (2012) found that a short-term training intervention, twelve sessions, produced an increase in MEP amplitude of RF when measured at 10% of MVC. Those results are in line with our results at 20% of MVC. However, and importantly for our interpretation, MEP excitability assessed at 60% of MVC did not show significant changes in the young. Strength training and maximal strength has been proposed as a specific skill (Buckner et al. 2017), and 12 sessions of arm flexion–extension visuomotor tracking skill training (Lundbye-Jensen et al. 2005) along with 12 sessions of 3 s concentric and 4 s eccentric tempo-controlled bicep curl strength training (Leung et al. 2017) has been shown to increase MEP amplitude after four weeks. Since the participants were required to hold the force level constant prior to stimulation (~ 2 s), it may be that lower force levels challenge the sensorimotor system to a greater extent than higher contraction levels, as previously evident in force steadiness tasks (Laidlaw et al. 2000). Therefore, we propose that the statistically significant but small magnitude changes in excitability in the young observed only during 20% of MVC trials reflect the sensorimotor integration needed for force steadiness, a so-called 'skill element' of strength training.

Our results showed higher aMT in older adults compared to younger adults, which is an indicator of cortico-spinal excitability (Pascual-Leone et al. 1995; Wassermann 2002). Should this reflect a decline in cortico-spinal excitability with age, as interpreted in previous studies (Bashir et al. 2014; Cirillo et al. 2011), this would directly conflict the MEP amplitude data of the present study. The aging process may lead to reduced activation of cortico-spinal neurons or disrupted synchronization among these neurons leading to a cancelation phase (Pitcher et al. 2003; Magistris et al. 1998). Notably, despite the impact of strength training and subsequent detraining on MEP and LEP amplitudes, aMT remained unchanged across interventions and age groups suggesting a discrepancy between the measures as an indicator of excitability. Previous studies have discussed (Wassermann 2002; Hassanlouei et al. 2017) that caution is advised in interpreting aMT due to factors such as a reduction in motor cortex size (Marner et al. 2003; Salat et al. 2004) and increase in skull thickness (Lillie et al. 2016) with age that potentially increases the coil-to-cortex distance, meaning a requirement for higher intensities for action potential generation. It may be that the between-group differences in aMT of the present study is due to cortex size or skull thickness rather than cortico-spinal excitability per se. While our study did not directly address these factors, our results underscore the need for further investigation to identify the precise mechanisms.

In addition, at higher contraction intensities in the present study, the older group showed greater MEP amplitude than the younger group at baseline. Further, Hassanlouei et al. (2017) showed that individuals engaged in higher physical activity (> 10,000 steps/day) demonstrated lower MEP amplitude in m.vastus lateralis than the ones with low physical activity (< 10,000 steps/day), independent of age. Moreover, cast immobilization has been shown to increase cortico-spinal excitability, when measured at 120% rMT

(Roberts et al. 2007). Both studies discuss that modulation of different inhibitory pathways at the cortical level could modify cortico-spinal excitability due to the lack of exercise. These data suggest that better trained muscles for gross force production, remembering that older adults are generally less physically active than young (Martin et al. 2014), are characterized by lower cortico-spinal excitability responses to TMS.

Cortical and spinal inhibition

Our results showed that neither strength training nor detraining affected MEP or LEP cSP duration. This is somewhat unexpected as meta-analyses have shown reductions in cSP duration following strength training (Kidgell et al. 2017; Mason et al. 2019), at least in young adults. Nevertheless, within these meta-analyses there have been studies showing no changes in cSP, thus, our data is not without precedent. For example, 12 strength training sessions of 4 sets of 6–8 repetitions with 80% 1-RM using 3 s concentric and 4 s eccentric tempo-controlled contractions led to no changes in biceps brachii cSP in healthy young adults (Kidgell et al. 2011).

At baseline, our results showed that MEP SP at 120% aMT and LEP SP were significantly longer for the older group independently of the contraction intensity used. cSP is an indication of intracortical inhibition (Inghilleri et al. 1993) mediated by Gamma-aminobutyric acid (GABA) inhibitors, particularly involving the activity of $GABA_B$ receptors (Siebner et al. 1998). Consequently, prolonged cSP indicates greater $GABA_B$ activity and longer intracortical inhibition in the older group. These results contradict previous findings, where SP durations were reported shorter (Christie and Kamen 2014; Sale and Semmler 2005) or not different (Fujiyama et al. 2012) comparing younger and older adults at baseline. However, it should be noted that MEPs were either of similar amplitude (Christie and Kamen 2014) or smaller (Sale and Semmler 2005) than the younger adults in those previous studies, which contrasts the higher MEP and LEP amplitudes for the older adults here. Given the correlation between cSP and MEP amplitude (Orth and Rothwell 2004), it is plausible that normalization of cSP to MEP amplitude in the older group might have led to an interpretation of increased inhibition in older adults, due to the decreased MEP size and no changes in cSP in the older adults.

Moreover, the present study showed decreased MEP amplitude following strength training while the SP duration from cortical and spinal stimulation remained unchanged. Therefore, normalizing the SP to MEP or LEP amplitude, would modify the interpretation of excitatory and inhibitory processes influencing the observed outcomes. Thus, the observed decrease in MEP/LEP amplitude and the conserved SP may indicate greater contribution of cortical and/or spinal inhibition in older adults after training, which may improve movement efficiency and result in increased strength.

Strengths and limitations

This study is the first to provide evidence of cortical and spinal excitability and inhibition adaptations to a 7-week strength training intervention in young and older adults. In addition, it also provides information from a detraining period, which strengthens inferences that can be drawn from the causality of the intervention. Furthermore, cortico-spinal responses were recorded during different contraction intensities. Clearer between-group differences (at baseline) were observable at 60% of MVC compared to 20% of MVC, and this finding could direct future studies comparing differences between groups. In addition, the detraining period provides support that the intervention caused the observed alterations in the outcome measures and helps to identify the mechanisms of improved strength. The young increased and decreased both strength and muscle mass concomitantly, suggesting that morphologic adaptations were a large factor in the strength increase. Conversely, the older adults maintained both strength and the altered MEP/LEP amplitude after detraining suggesting that neural adaptations predominantly underpinned the strength gain.

As a limitation, the strength-training program was performed dynamically and mainly bilaterally. Thereby, the unilateral isometric test was non-specific and could have influenced the ability to identify neural adaptations. TMS paired-pulse paradigms (i.e., SICI, LICI, ICF), peripheral stimulation paradigms (H-reflex) and/or paired H-reflex -TMS (cortical recurrent inhibition) were not measured in this study because an increased number of contractions per session would have increased the risk of fatigue. This could have provided more specific information about how strength training modulates cortical and spinal inhibitory process in young and older adults alongside cortico-spinal and spinal excitability.

Conclusions

The present study has shown maximal strength, cortico-spinal excitability and cortical and spinal differences between young and older group at baseline, that are believed to be related to the aging process. Furthermore, the short-term strength-training intervention showed improved strength in both groups and that early cortico-spinal adaptations might be age-dependent as well as specific to contraction level. The decrease in MEP amplitude at 60% of MVC indicates cortico-spinal adaptations in the older adults. In addition, LEP amplitude changes in young and older could suggest

spinal adaptation as the primary site after strength training in young and older adults, proving strength training as a beneficial tool to decelerate aging.

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Data availability The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors do not have any conflicts of interest to report relevant to this manuscript. The authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed. All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or material discussed in this manuscript.

References

Aagaard P, Simonsen EB, Andersen JL, Magnusson P, Dyhre-poulsen P, Dyhre-Poulsen P (2002) Neural adaptation to resistance training: changes in evoked V-wave and H-reflex responses. J Appl Physiol 92:2309–2318

Ansdell P, Brownstein CG, Škarabot J, Angius L, Kidgell D, Frazer A, Hicks KM, Durbaba R, Howatson G, Goodall S, Thomas K (2020) Task-specific strength increases after lower-limb compound resistance training occurred in the absence of corticospinal changes in vastus lateralis. Exp Physiol 105(7):1132–1150

Barker AT, Jalinous R (1985) Non-invasive magnetic stimulation of human motor cortex. Lancet 325(8437):1106–1107

Bashir S, Perez JM, Horvath JC, Pena-Gomez C, Vernet M, Capia A, Alonso-Alonso M, Pascual-Leone A (2014) Differential effects of motor cortical excitability and plasticity in young and old individuals: a Transcranial Magnetic Stimulation (TMS) study. Front Aging Neurosci 10(6):111

Baudry S, Collignon S, Duchateau J (2015) Influence of age and posture on spinal and corticospinal excitability. Exp Gerontol 69:62–69

Bemben MG, Massey BH, Bemben DA, Misner JE, Boileau RA (1991) Isometric muscle force production as a function of age in healthy 20 to 74-yr-old men. Med Sci Sports Exerc 23(11):1302–1310

Borde R, Hortobágyi T, Granacher U (2015) Dose-response relationships of resistance training in healthy old adults: a systematic review and meta-analysis. Sports Med 45(12):1693–1720

Brownstein CG, Souron R, Royer N, Singh B, Lapole T, Millet GY (2020) Disparate kinetics of change in responses to electrical stimulation at the thoracic and lumbar level during fatiguing isometric knee extension. J Appl Physiol (1985) 128(1):159–167. https://doi.org/10.1152/japplphysiol.00635.2019

Buckner SL, Jessee MB, Mattocks KT, Mouser JG, Counts BR, Dankel SJ, Loenneke JP (2017) Determining strength: a case for multiple methods of measurement. Sports Med 47(2):193–195

Christie A, Kamen G (2014) Cortical inhibition is reduced following short-term training in young and older adults. Age 36(2):749–758

Cirillo J, Todd G, Semmler JG (2011) Corticomotor excitability and plasticity following complex visuomotor training in young and old adults. Eur J Neurosci 34(11):1847–1856

Clark B, Taylor J (2011) Age-related changes in motor cortical properties and voluntary activation of skeletal muscle. Curr Aging Sci 4(3):192–199

Cruz-Sánchez FF, Moral A, Tolosa E, De Belleroche J, Rossi ML (1998) Evaluation of neuronal loss, astrocytosis and abnormalities of cytoskeletal components of large motor neurons in the human anterior horn in aging. J Neural Transm 105(6–7):689–701

Damron LA, Dearth DJ, Hoffman RL, Clark BC (2008) Quantification of the corticospinal silent period evoked via transcranial magnetic stimulation. J Neurosci Methods 173(1):121–128

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

Fathi D, Ueki Y, Mima T, Koganemaru S, Nagamine T, Tawfik A, Fukuyama H (2010) Effects of aging on the human motor cortical plasticity studied by paired associative stimulation. Clin Neurophysiol 121(1):90–93

Fragala MS, Cadore EL, Dorgo S, Izquierdo M, Kraemer WJ, Peterson MD, Ryan ED (2019) Resistance training for older adults: position statement from the national strength and conditioning association. J Strength Cond Res 33(8):2019–2052

Fujiyama H, Hinder MR, Schmidt MW, Garry MI, Summers JJ (2012) Age-related differences in corticospinal excitability and inhibition during coordination of upper and lower limbs. Neurobiol Aging 33(7):1484.e1–14

Geertsen SS, Willerslev-Olsen M, Lorentzen J, Nielsen JB (2017) Development and aging of human spinal cord circuitries. J Neurophysiol 118(2):1133–1140

Gomez-Guerrero G, Ansdell P, Howatson G, Avela J, Walker S (2023a) Contraction intensity modulates spinal excitability during transcranial magnetic stimulation-evoked silent period in rectus femoris muscle. Eur J Appl Physiol. https://doi.org/10.1007/s00421-023-05367-1

Gomez-Guerrero G, Avela J, Enroth M, Häkkinen E, Ansdell P, Howatson G, Walker S (2023b) Test–retest reliability of cortico-spinal measurements in the rectus femoris at different contraction levels. Front Neurosci 17:1239982

Goodall S, Romer LM, Ross EZ (2009) Voluntary activation of human knee extensors measured using transcranial magnetic stimulation. Exp Physiol 94:995–1004

Goodwill AM, Pearce AJ, Kidgell DJ (2012) Corticomotor plasticity following unilateral strength training. Muscle Nerve 46(3):384–393

Guralnik JM, Ferrucci L, Simonsick EM, Salive ME, Wallace RB (1995) Lower-extremity function in persons over the age of 70 years as a predictor of subsequent disability. N Engl J Med 332(9):556–561

Häkkinen K, Komi P, Tesch P (1981) Effect of combined concentric and eccentric strength training and detraining on force-time, muscle fiber and metabolic characteristics of leg extensor muscles. Scand J Med Sci Sports 3:50–58

Häkkinen K, Alen M, Kallinen M, Newton RU, Kraemer WJ (2000) Neuromuscular adaptation during prolonged strength training, detraining and re-strength-training in middle-aged and elderly people. Eur J Appl Physiol 83:51–62

Hassanlouei H, Sundberg CW, Smith AE, Kuplic A, Hunter SK (2017) Physical activity modulates corticospinal excitability of the lower limb in young and old adults. J Appl Physiol 123(2):364–374

Hermens HJ, Freriks B, Disselhorst-Klug C, Rau G (2000) Developent of recommendations for SEMG sensors and sensor placement procedures. J Electromyogr Kinesiol 10:361–374

Hofstoetter US, Freundl B, Binder H, Minassian K (2018) Common neural structures activated by epidural and transcutaneous lumbar spinal cord stimulation: Elicitation of posterior root-muscle reflexes. PLoS ONE 13(1):1–22

Holtermann A, Roeleveld K, Engstrøm M, Sand T (2007) Enhanced H-reflex with resistance training is related to increased rate of force development. Eur J Appl Physiol 101(3):301–312

Hortobágyi T, van de Waardt LE, Tokuno CD, Taube W, Papegaaij S (2018) Age-related reversal of spinal excitability during anticipatory postural control. Eur J Appl Physiol 118(12):2577–2585

Hortobágyi T, Granacher U, Fernandez-del-Olmo M, Howatson G, Manca A, Deriu F, Taube W, Gruber M, Márquez G, Lundbye-Jensen J, Colomer-Poveda D (2021) Functional relevance of resistance training-induced neuroplasticity in health and disease. Neurosci Biobehav Rev 122:79–91

Hunter SK, Pereira HM, Keenan KG (2016) The aging neuromuscular system and motor performance. J Appl Physiol 121(4):982–995

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

Jensen JL, Marstrand PC, Nielsen JB (2005) Motor skill training and strength training are associated with different plastic changes in the central nervous system. J Appl Physiol 99(4):1558–1568

Kidgell DJ, Stokes MA, Pearce AJ (2011) Strength training of one limb increases corticomotor excitability projecting to the contralateral homologous limb. Mot Control 15(2):247–266

Kidgell DJ, Bonnano DR, Frazer AK, Howatson G, Pearce AJ (2017) Corticospinal response following strength training: a systematic review and meta-analysis. Eur J Neurosci 46(11):2648–2661

Kido A, Tanaka N, Stein RB (2004) Spinal excitation and inhibition decrease as humans age. Can J Physiol Pharmacol 82(4):238–248

Kobayashi M, Pascual-Leone A (2003) Basic principles of magnetic stimulation. Lancet 2:145–156

Koo TK, Li MY (2016) A guideline of selecting and reporting Intraclass correlation coefficients for reliability research. J Chiropr Med 15:155–163

Laidlaw DH, Bilodeau M, Enoka RM (2000) Steadiness is reduced and motor unit discharge is more variable in old adults. Muscle Nerve 23(4):600–612

Landin D, Thompson M, Reid M (2016) Actions of two Bi-Articular muscles of the lower extremity: a review article. J Clin Med Res 8(7):489–494

Leung M, Rantalainen T, Teo WP, Kidgell D (2017) The corticospinal responses of metronome-paced, but not self-paced strength training are similar to motor skill training. Eur J Appl Physiol 117(12):2479–2492

Lillie EM, Urban JE, Lynch SK, Weaver AA, Stitzel JD (2016) Evaluation of skull cortical thickness changes with age and sex from computed tomography scans. J Bone Miner Res 31(2):299–307

Magistris MR, Rösler KM, Truffert A, Myers JP (1998) Transcranial stimulation excites virtually all motor neurons supplying the target muscle. A demonstration and a method improving the study of motor evoked potentials. Brain 121:437–450

Marner L, Nyengaard JR, Tang Y, Pakkenberg B (2003) Marked loss of myelinated nerve fibers in the human brain with age. J Comp Neurol 462(2):144–152. https://doi.org/10.1002/cne.10714

Martin PG, Gandevia SC, Taylor JL (2006) Output of human Motoneuron pools to corticospinal inputs during voluntary contractions. J Neurophysiol 95:3512–3518

Martin KR, Koster A, Murphy RA, Van Domelen DR, Hung MY, Brychta RJ, Chen KY, Harris TB (2014) Changes in daily activity patterns with age in U.S. men and women: National health and nutrition examination survey 2003–04 and 2005–06. J Am Geriatr Soc 62:1263–1271

Mason J, Frazer AK, Pearce AJ, Goodwill AM, Howatson G, Jaberzadeh S, Kidgell DJ (2019) Determining the early corticospinal-motoneuronal responses to strength training: a systematic review and meta-analysis. Rev Neurosci 30(5):463–476

McNeil CJ, Butler JE, Taylor JL, Gandevia SC (2013) Testing the excitability of human motoneurons. Front Hum Neurosci 7:152

Millington PJ, Myklebust BM, Shambes GM (1992) Biomechanical analysis of the sit-to-stand motion in elderly persons. Arch Phys Med Rehabil 73(7):609–617

Mills KR (1988) Excitatory and inhibitory effects on human spinal motoneurones from magnetic brain stimulation. Neurosci Lett 94(3):297–302

Oliviero A, Profice P, Tonali PA, Pilato F, Saturno E, Dileone M, Ranieri F, Di Lazzaro V (2006) Effects of aging on motor cortex excitability. Neurosci Res 55(1):74–77

Orth M, Rothwell JC (2004) The cortical silent period: intrinsic variability and relation to the waveform of the transcranial magnetic stimulation pulse. Clin Neurophysiol 115(5):1076–1082

Oya T, Hoffman BW, Cresswell AG (2008) Corticospinal-evoked responses in lower limb muscles during voluntary contractions at varying strengths. J Appl Physiol 105:1527–1532

Pascual-Leone A, Nguyet D, Cohen LG, Brasil-Neto JP, Cammarota A, Hallett M (1995) Modulation of muscle responses evoked by transcranial magnetic stimulation during the acquisition of new fine motor skills. J Neurophysiol 74(3):1037–1045

Petersen NT, Taylor JL, Gandevia SC (2002) The effect of electrical stimulation of the corticospinal tract on motor units of the human biceps brachii. J Physiol 544(1):277–284

Pitcher JB, Ogston KM, Miles TS (2003) Age and sex differences in human motor cortex input-output characteristics. J Physiol 546(Pt 2):605–613

Roberts DR, Ricci R, Funke FW, Ramsey P, Kelley W, Carroll JS, Ramsey D, Borckardt JJ, Johnson K, George MS (2007) Lower limb immobilization is associated with increased corticospinal excitability. Exp Brain Res 181(2):213–220

Rossi S, Hallett M, Rossini PM, Pascual-Leone A (2011) Screening questionnaire before TMS: An update. Clin Neurophysiol 122(8):1686

Salat DH, Buckner RL, Snyder AZ, Greve DN, Desikan RS, Busa E, Morris JC, Dale AM, Fischl B (2004) Thinning of the cerebral

cortex in aging. Cereb Cortex 14(7):721–730. https://doi.org/10.1093/cercor/bhh032
Sale MV, Semmler JG (2005) Age-related differences in corticospinal control during functional isometric contractions in left and right hands. J Appl Physiol 99(4):1483–1493
Siddique U, Frazer AK, Avela J, Walker S, Ahtiainen JP, Howatson G, Tallent J, Kidgell DJ (2022) Determining the cortical, spinal and muscular adaptations to strength-training in older adults: a systematic review and meta-analysis. Ageing Res Rev 82:101746
Siebner HR, Dressnandt J, Auer C, Conrad B (1998) Continuous intrathecal baclofen infusions induced a marked increase of the transcranially evoked silent period in a patient with generalized dystonia. Muscle Nerve 21(9):1209–1212
Škarabot J, Ansdell P, Brownstein CG, Thomas K, Howatson G, Goodall S, Durbaba R (2019a) Electrical stimulation of human corticospinal axons at the level of the lumbar spinal segments. Eur J Neurosci 49(10):1254–1267
Škarabot J, Tallent J, Goodall S, Durbaba R, Howatson G (2019b) Corticospinal excitability during shortening and lengthening actions with incremental torque output. Exp Physiol 103(12):1586–1592
Taylor JL (2006) Stimulation at the cervicomedullary junction in human subjects. J Electromyogr Kinesiol 16(3):215–223
Taylor JL, Butler JE, Allen GM, Gandevia SC (1996) Changes in motor cortical excitability during human muscle fatigue. J Physiol 490(2):519–528
Taylor JL, Petersen NT, Butler B, Gandevia SC (2002) Interaction of transcranial magnetic stimulation and electrical transmastoid stimulation in human subjects. J Physiol 541(3):949–958
Tomlinson BE, Irving D (1977) The numbers of limb motor neurons in the human lumbosacral cord throughout life. J Neurol Sci 34(2):213–219
Toraman NF (2005) Short term and long term detraining: Is there any difference between young-old and old people? Br J Sports Med 39(8):561–564
Unhjem R, Tøien T, Charlotte A, Kvellestad G, Øren TS, Wang E (2020) External resistance is imperative for training-induced efferent neural drive enhancement in older adults. J Gerontol A Biol Sci Med Sci 76(2):224–232
van Melick N, Meddeler BM, Hoogeboom TJ, Nijhuis-van der Sanden MWG, van Cingel REH (2017) How to determine leg dominance: The agreement between self-reported and observed performance in healthy adults. PLoS One 12(12):e0189876
Vila-Chã C, Falla D, Correia MV, Farina D (2012) Changes in H reflex and V wave following short-term endurance and strength training. J Appl Physiol 112:54–63
Walker S, Häkkinen K (2014) Similar increase in strength training after a short-term resistance training due to different neuromsucular adaptations in young and older men. J Strength Cond Res 28(11):3041–3048
Walker S, Blazevich AJ, Haff GG, Tufano JJ, Newton RU, Häkkinen K (2016) Greater strength gains after training with accentuated eccentric than traditional isoinertial loads in already strength-trained men. Front Physiol 7:1–12
Wassermann EM (2002) Variation in the response to transcranial magnetic brain stimulation in the general population. Clin Neurophysiol 113(7):1165–1171
Weier AT, Pearce AJ, Kidgell DJ (2012) Strength training reduces intracortical inhibition. Acta Physiol (oxf) 206(2):109–119
Wilkinson RD, Mazzo MR, Feeney DF (2023) Rethinking the statistical analysis of neuromechanical data. Exerc Sport Sci Rev 51(1):43–50
Yacyshyn AF, Woo EJ, Price MC, McNeil CJ (2016) Motoneuron responsiveness to cortico-spinal tract stimulation during the silent period induced by transcranial magnetic stimulation. Exp Brain Res 234(12):3457–3463