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Shaping exercise performance and neuromuscular fatigue through neuromodulation: unleashing the power of brain-muscle connections

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PhD Thesis

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Sommario

La fatica neuromuscolare rappresenta una ridotta abilità di esprimere forza da parte del muscolo durante un compito motorio. La letteratura propone protocolli atti a limitare lo sviluppo di questo evento fisiologico modulando le componenti periferiche (es., muscolo). D'altro canto, la stimolazione magnetica transcranica ripetitiva è uno strumento in diffusione per regolare i network neuronali e le funzioni cerebrali in ambito clinico e di ricerca. Tuttavia, i protocolli di stimolazione theta burst (TBS), più veloci e di pari efficacia, rimangono confinati alla sfera clinica per il trattamento di disturbi neuropsicologici. Ad oggi, infatti, i meccanismi fisiologici ancora poco chiari e l'alta variabilità di risposta alla TBS ne limitano l'utilizzo e l'applicazione a largo spettro. Invece, grazie all'induzione di effetti a lungo termine eccitatori (TBS intermittente) ed inibitori (TBS continua), la TBS può risultare un approccio valido per la modulazione delle componenti centrali anche nel campo della fatica neuromuscolare. Quindi, il proposito di questa tesi è quello di verificare l'efficacia della TBS eccitatoria ed inibitoria sulle componenti della fatica neuromuscolare, e i suoi aggiustamenti durante l'esercizio fisico in soggetti giovani e sani. Nel Capitolo 1 è fornita una breve introduzione e una revisione della letteratura con le informazioni necessarie a capire il fenomeno della fatica neuromuscolare, gli strumenti per la sua verifica, e i meccanismi fisiologici correlati. In seguito, nel Capitolo 2 sono presentati gli obiettivi ed ipotesi perseguiti in questa tesi. Nei Capitoli 3, 4, e 5, si possono trovare i risultati degli studi originali sull'argomento in oggetto. In fine, il Capitolo 6 fornisce un sommario ed una visione d'insieme dei risultati, sottolineando punti di forza e limitazioni del lavoro, e fornendo idee per investigazioni future.

Abstract

Neuromuscular fatigue represents a reduced ability of the muscle to express force during a motor task. The literature proposes protocols to limit the development of this physiological event by modulating the peripheral components (e.g., muscle). On the other hand, repetitive transcranial magnetic stimulation is a widespread tool for regulating neuronal networks and brain functions in clinical and research settings. However, theta burst stimulation (TBS) protocols, which are faster and equally effective, remain confined to the clinical sphere for treating neuropsychological disorders. To date, the still unclear physiological mechanisms, and the high variability of response to TBS limit its wide-ranging use and application. Instead, thanks to the induction of long-term excitatory (intermittent TBS) and inhibitory (continuous TBS) effects, TBS may be a valid approach for the modulation of the central components also in the field of neuromuscular fatigue. Therefore, this thesis aims to verify the effectiveness of excitatory and inhibitory TBS on the components of neuromuscular fatigue, and its adjustments during physical exercise in young and healthy subjects. Chapter 1 provides a brief introduction and a review of the literature with the necessary information to understand the phenomenon of neuromuscular fatigue, the tools for its verification, and the related physiological mechanisms. Subsequently, in Chapter 2 the objectives and hypotheses pursued in this thesis are presented. In Chapters 3, 4, and 5, you can find the results of original studies on the topic in question. Finally, Chapter 6 provides a summary and overview of the results, highlighting the strengths and limitations of the work, and providing ideas for future investigations.

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I remember the first time I crossed the threshold of this university to look for Prof. Massimo Venturelli's office, still unaware that he would be my supervisor for the next years. I want to acknowledge him, for his mentorship and direction in my doctoral studies. Thank him for allowing me to work in one of the most intriguing and challenging research fields. Even more thank him for always trusting me and pushing me further, especially in the difficult moments.

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Many thanks to all my colleagues, Alice, Samuel, Fabio, Massimo, Anna, Chiara, Gianluca, Luca, Alberto, and Matteo. They have always been essential, the first reason to come to work, to find encouragement, advice and to have fun.
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List of scientific papers and communications

The results of this dissertation produced the following papers and communications.

Papers

1. **Martignon C**, Barbi C, Vernillo G, Sidhu S, Andani ME, Schena F, Venturelli M. Theta burst stimulation modulates performance fatigability by shaping central fatigue and corticospinal excitability. *Submitted / Under review.*

2. **Martignon C**, Barbi C, Vernillo G, Andani ME, Schena F, Venturelli M. Gender differences in performance fatigability do not emerge after theta burst stimulation. *In progress.*

3. **Martignon C**, Barbi C, Vernillo G, Sidhu S, Andani ME, Schena F, Venturelli M. The cross-over effect of theta-burst stimulation: another strategy to modulate performance fatigability. *In progress.*

Posters and oral communications

1. **Martignon C**, Barbi C, Laginestra FG, Giuriato G, Pedrinolla A, Vernillo G, Schena F, Venturelli M. Neuromodulatory effects of theta burst on performance and fatigue: from the cortex to the muscle. *13th Congresso Nazionale SISMES. Milano,* 2022.

2. **Martignon C**, Barbi C, Vernillo G, Emadi Andani M, Schena F, Venturelli M. Do gender differences in performance fatigability emerge after neuromodulation with theta burst stimulation? *28th Annual ECSS Congress. Paris,* 2023.

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evoked potential; SP, silent period; CMEP, cervicomedullary motor evoked potential; sTBS, sham theta burst stimulation; iTBS, intermittent theta burst stimulation; cTBS, continuous theta burst stimulation. * among protocols $P \leq .05$; # from PRE $P \leq .05$; § from POST_1 $P \leq .05$; ° from POST_2 $P \leq .05$; + from POST_3 $P \leq .05$ (n = 10).

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List of abbreviations

AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid
AMT	Active motor threshold
ATP	Adenosine triphosphate
Ca^{2+}	Calcium ions
MEP	Cervico medullary motor evoked potential
cTBS	Continuous theta burst stimulation
EMG	Electromyography
GABA	γ-aminobutyric acid
H^+	Hydrogen ions
iTBS	Intermittent theta burst stimulation
K^+	Potassium ions
LTD	Long-term depression
LTP	Long-term potentiation
M1	Primary motor cortex
MEP	Motor evoked potential
MET	Task metabolic equivalent
MDF	Median power frequency
M_{max}	Maximal compound muscle action potential
MVIC	Maximal voluntary isometric contraction
MVC	Maximal voluntary contraction
Na^+	Sodium ions
NMDA	N-methyl-D-aspartic acid
O_2	Oxygen
Pi	Inorganic phosphate
$Q_{tw,pot}$	Twitch force potentiated at rest
RMS	Root mean square
rTMS	Repetitive transcranial magnetic stimulation
SP	Silent period
TBS	Theta burst stimulation
tDCS	Transcranial direct current stimulation
TMS	Transcranial magnetic stimulation
TTF	Time-to-task failure
VA	Voluntary activation

Introduction

Neuromuscular fatigue

The field of fatigue has traditionally been one of the main and debated topics in exercise physiology research. The reason that approached many researchers to this comes from the impact that fatigue has on most of our daily activities, involving healthy young people, the elderly, and patients too. Nevertheless, however widespread and studied this phenomenon is, it remains a topic full of questions. Already in the 19th century, the study of fatigue and its mechanisms aroused the right interest to demonstrate that multiple factors contributed to the reduction of the muscle's ability to generate force. In this context, Angelo Mosso was a pioneer in stating that in addition to the intrinsic failure of the muscle, other mental tasks could influence the outcome of the muscle and, therefore, the level of fatigue (Mosso, 1891). The dual nature of fatigue suggested by Mosso is still valid today, albeit revisited (Enoka & Duchateau, 2016). Indeed, Enoka and Duchateau (2016) suggested that fatigue can be divided into two components: perceived fatigability (i.e., changes in the feelings of exhaustion and energy) and performance fatigability (i.e., the decline in an objective measure of performance over a discrete period). The neuromuscular system appears to play a key role in the performance decline during a given task, so much so that it gives fatigue the name "neuromuscular fatigue". Neuromuscular fatigue can be defined as "a reduction in the force-generating capacity of a muscle or muscle group" (Bigland-Ritchie & Woods, 1984; Gandevia, 2001) but a clear and unique definition of this phenomenon remains elusive. What is certain is the set of events that arise along the motor descending pathway that affect the expression of force (Gandevia, 2001) (Figure 1.1). Firstly, upstream from the motor cortex but still in the higher brain areas the movement is planned. Subsequently, descending neural drive is transmitted from the motor cortex to the spinal motor neurons for the recruitment of the motor units needed to initiate the task. Once the action potentials travel through the motor axons, they reach the neuromuscular junction, where they are transmitted to the muscle fibers. Finally, the stimulus spreads along the muscle membrane and the resulting calcium ions (Ca^{2+}) released from the sarcoplasmic reticulum induce cross-bridges formation in the actomyosin complex and the production of muscle force. However, damage to any part of the described neuromuscular system may lead to an

impairment of the level of force. Indeed, when failure happens at sites proximal to the neuromuscular junction, the phenomenon is termed central fatigue, while at or distal to the neuromuscular junction it refers to peripheral fatigue (Gandevia, 2001; Allen *et al.*, 2008). Despite the choice of the task (Bilodeau, 2006), the intensity and duration of the exercise (Behm & St-Pierre, 1997; Place *et al.*, 2009), as well as the demographical characteristics of the participants (Hunter *et al.*, 2009; Sundberg *et al.*, 2019) can differently influence the appearance of neuromuscular fatigue, this model is now accepted and recognized throughout the scientific world.

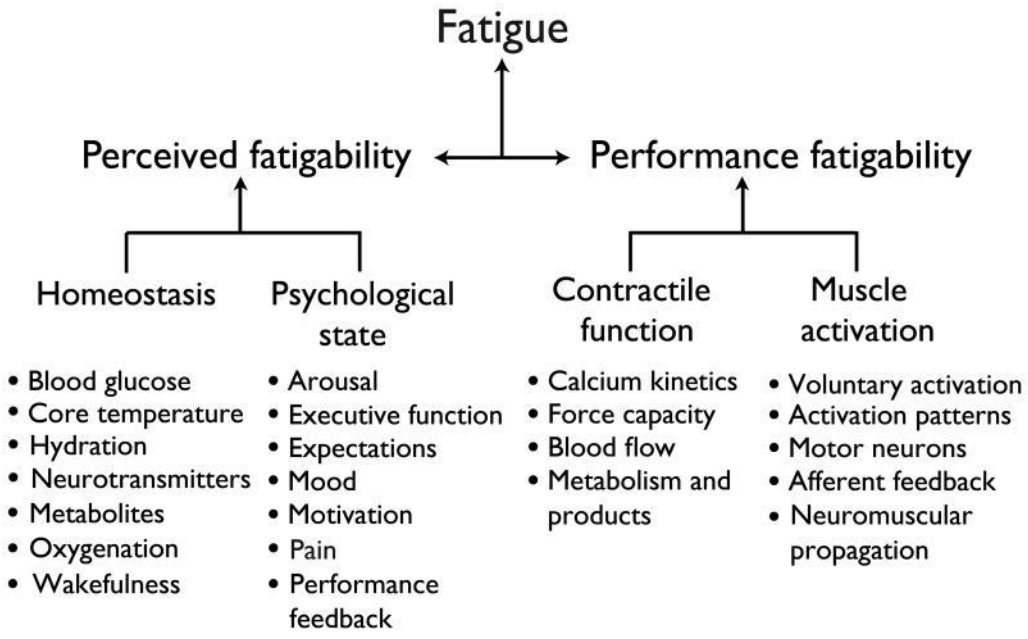

Figure 1.1. *Taxonomy proposed by Enoka and Duchateau (2016).*

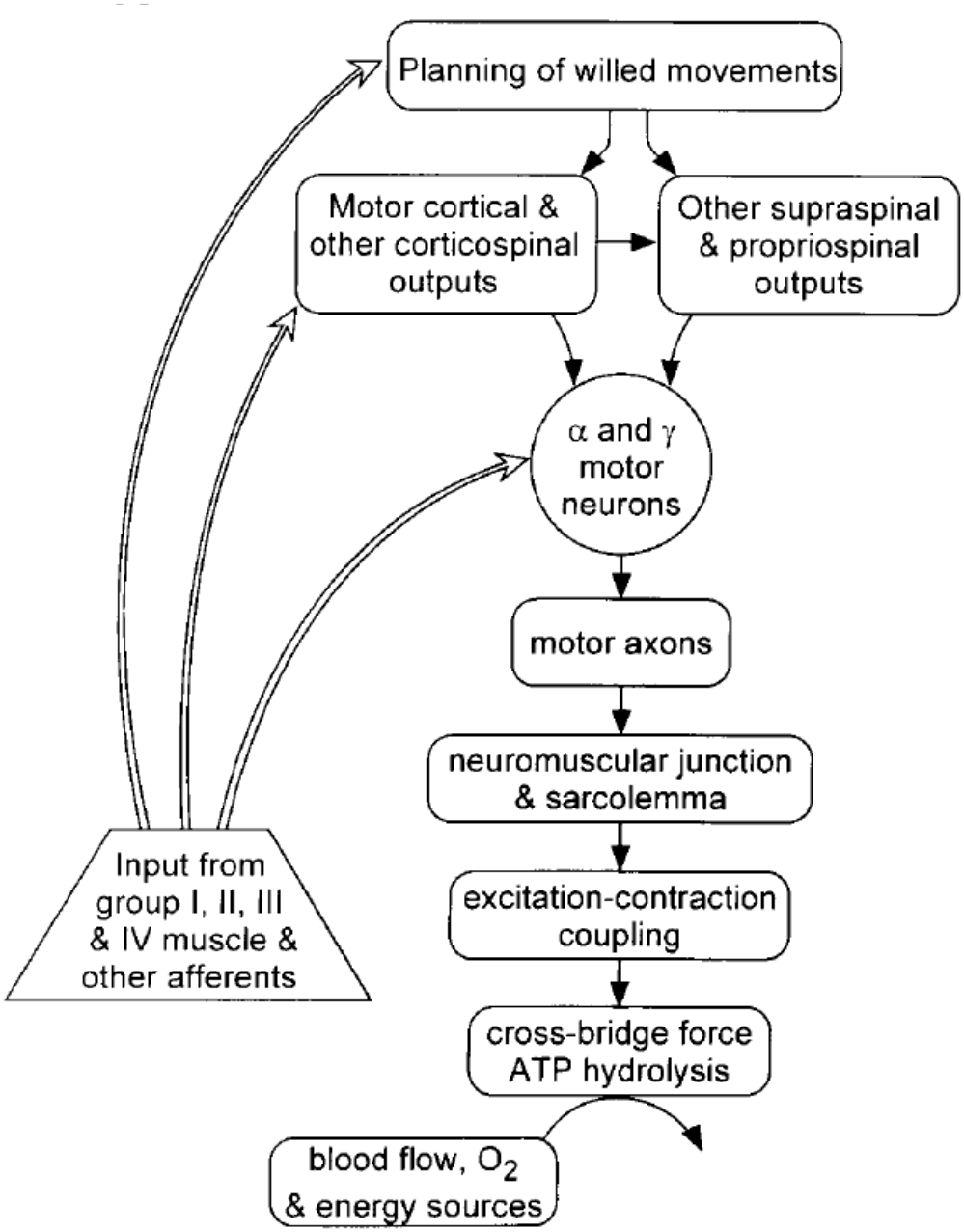

Figure 1.2. *Processes involved in the production of voluntary force. Taken from Gandevia, 2001.*

Central determinants of fatigue

Central fatigue can be explained as the progressive exercise-induced reduction of voluntary activation of the muscle (Gandevia, 2001; Taylor & Gandevia, 2008) and it is usually assessed using the twitch interpolation technique (Merton, 1954). This technique, developed by Merton in 1954 and considered the gold standard today in investigating central fatigue, consists of delivering a supramaximal muscle or nerve stimulation while the subject is performing a maximal voluntary contraction. This stimulation (electrical or magnetic), can activate all the motor units that are not recruited voluntarily, provoking an extra twitch in the force output. This extra twitch is called superimposed twitch and when it appears during contraction, it means that voluntary activation of the exercising muscle is less than 100% and that there may be a central deficit that does not allow an adequate expression of force. Changes in voluntary activation can be induced by previous fatiguing exercises performed by the subject or by the presence of pathologies. However, to obtain the correct percentage value of voluntary activation it is necessary to subtract the ratio between the superimposed twitch and a twitch evoked at rest from the maximal obtainable voluntary activation (i.e., 100%). This technique relies upon the fact that a linear, inverse relationship exists between the superimposed twitch amplitude and the voluntary force at the time of stimulation (Taylor, 2009). This technique has often been the subject of debate due to its high variability and low sensitivity, however, remaining a valid tool for determining central fatigue (Dotan *et al.*, 2021; Place, 2021). To assess the presence of central fatigue it is necessary to use maximal contractions. In the case of a submaximal exercise, brief maximal voluntary contractions are often utilized at regular intervals (Bigland-Ritchie & Woods, 1984). Another approach to inducing central fatigue by avoiding maximal voluntary contractions is performing low-intensity contractions (<30% or 15% maximal voluntary contraction) which, if prolonged over time, also lead to a decrease in voluntary activation (Søgaard *et al.*, 2006*b*). Despite the wide range of fatiguing tasks to induce central rather peripheral fatigue, the most effective is represented by the low-intensity sustained contraction compared to the intermittent, high-intensity, or maximal ones (Bigland-Ritchie *et al.*, 1983; Taylor & Gandevia, 2008).

The development of central fatigue can result from several adaptations that the central nervous system makes. Some of them may be increased or decreased motor unit recruitment, change in the firing rate of the active motor units (Heckman & Enoka, 2012), modulation of intrinsic properties of the motor neuron, altered release of neuromodulators or increased inhibitory inputs (Taylor & Gandevia, 2008).

Mechanisms of central fatigue

Central fatigue begins when the neural drive cannot fully activate the working muscle anymore (Gandevia, 2001). A lowering in motor neuron firing rates seems to play a crucial role in the development of central fatigue during both maximal and submaximal fatiguing contractions (Bigland-Ritchie *et al.*, 1983; Garland *et al.*, 1994). One of the most accredited hypotheses is explained by the muscle wisdom. This theory suggested that this decrease was a mechanism activated by the muscle to preserve its ability to generate force (Marsden *et al.*, 1983). Contrarily, a more recent study proposed that slowing of motor neuron firing rates does not "protect" against fatigue, but rather enhances it (Fuglevand & Keen, 2003). However, three mechanisms are known to potentially contribute to this phenomenon: a decrease in the excitatory input, modulation of intrinsic properties of the motor neuron, and an increase in inhibitory inputs (Taylor & Gandevia, 2008).

Descending excitatory drive and corticospinal excitability

Performing a fatiguing exercise leads to a decrease in the descending drive from the motor cortex after some time, making the task less than optimal. This happens because the drive from the cortex decreases during fatiguing exercise, or it does not work properly to drive the spinal motor neurons, which may have become less sensitive to the synaptic input (Taylor & Gandevia, 2008). Evidence that investigated the functionality of the corticospinal pathway during exercise argued that the level of excitability of this trait plays a fundamental role. Corticospinal excitability can be defined as the efficacy of the corticospinal pathway to relay neural signals from the superior brain areas to the exercising muscle (Weavil & Amann, 2018). In this respect, transcranial magnetic stimulation is a non-invasive

procedure that has been traditionally employed to understand the role of cortical and spinal excitability in fatigue. Transcranial magnetic stimulation is generally applied to the contralateral motor cortex to generate a synchronous, short-latency electromyographic response called motor-evoked potential from the target muscle. This response is typically normalized by the maximum compound muscle action potential (M_{max}) to account for changes in peripheral transmission. Increases in motor-evoked potential represent an augmented overall corticospinal excitability while conversely, smaller motor-evoked potentials represent a decrease. However, this observed response does not allow to distinguish the contribution of cortical vs spinal mechanisms. For this reason, another magnetic or electrical stimulation can be delivered to the cervicomedullary junction, near the pyramidal decussation to bypass the motor cortex. The observed response is termed cervicomedullary motor-evoked potential and it corresponds to the activation of the corticospinal axons immediately after the stimulation. Motor-evoked potential and cervicomedullary motor-evoked potential can be highly affected during exercise (Søgaard *et al.*, 2006*a*). If transcranial magnetic stimulation is administered during the task, an increase in motor-evoked potential is observed (Taylor *et al.*, 1996) due to an increased excitatory drive which recruits fresh motor units, counteracting the loss in spinal excitability and contractile failure at the muscle level (Taylor & Gandevia, 2008). However, during a sustained maximal voluntary contraction, a twitch-like response from transcranial magnetic stimulation is detected indicating that motor cortical output is suboptimal (Taylor *et al.*, 1996). Therefore, as suggested by *in vivo* and *in vitro* studies, supraspinal fatigue develops even though the excitability of the motor cortex increases (Brownstone, 2006). This is because motor neurons become less responsive to synaptic input and motor neuron excitability decreases (Søgaard *et al.*, 2006*c*).

Intracortical inhibitory mechanisms

The silent period is the main phenomenon underlying inhibitory intracortical mechanisms. This event appears when a transcranial magnetic stimulation pulse is delivered during a contraction and the motor-evoked potential is followed by a period of silence in the electromyographic signal lasting ~200-300ms. These mechanisms representing both spinal and cortical components (Weavil & Amann,

2018) are regulated by γ-aminobutyric acid concentration (GABA), which is an inhibitory neurotransmitter present in the central nervous system. Its role is to hyperpolarize the post-synaptic neuron making it less likely to fire in response to a given input (Rosenthal *et al.*, 1967). The lengthening of the silent period is strictly dependent on the task the performer is doing. On one hand, during a fatiguing contraction, the silent period increases its duration (Søgaard *et al.*, 2006*c*; Taylor & Gandevia, 2008; Vernillo *et al.*, 2018) or does not change (Goodall *et al.*, 2012; Sidhu *et al.*, 2017). On the other hand, a previous study found that intermittent contractions with attenuated feedback from group III/IV afferents could shorten the silent period, demonstrating a role of these afferents in increasing intracortical inhibition (Hilty *et al.*, 2011).

Exercise-induced changes in neurotransmitters

Neurotransmitters are physiological molecules with a fundamental role in transmitting information among neurons and other cells within the central nervous system. Among all the neurotransmitters circulating in the human organism, two are particularly associated with changes in central fatigue: dopamine, and noradrenaline. However, serotonin could have an important role in fatigue development especially if interacts with other neural substances (Roelands & Meeusen, 2010). Studies on animals and humans suggest that changes in brain serotonin concentration can impair or improve performance (Bailey *et al.*, 1993). Accordingly, it seems that after the administration of serotonin reuptake inhibitor (i.e., paroxetine) subjects performed worse than the control test on a cycling task (Wilson & Maughan, 1992). Even in animal models the increase in serotonin can lead to imbalances which, however, concern motor neuron excitability (Perrier & Cotel, 2008). On the other hand, several studies denied the effect of serotonin on exercise capacity (Piacentini *et al.*, 2002; Roelands *et al.*, 2009), while suggesting a collaboration among different neurotransmitter systems for the appearance of fatigue (Roelands & Meeusen, 2010).

Peripheral determinants of fatigue

When biochemical and metabolic alterations emerge distally to the neuromuscular junction, the organism experiences peripheral fatigue. The reason why it is also

called contractile fatigue is explained during high-intensity exercise by the failure of the excitation-contraction coupling that is the communication between electrical events in the plasma membrane of skeletal muscle fibers and Ca^{2+} release from the sarcoplasmic reticulum, which leads to contraction (Allen *et al.*, 2008; Taylor & Gandevia, 2008). This phenomenon is strictly associated with homeostatic changes related to the level of lactate, ions, phosphates, and hydrogen in the bloodstream. Several studies showed how the rate of development of fatigue is tightly related to oxygen (O_2) delivery (Amann & Calbet, 2008). Muscle perfusion during muscle contractions plays a crucial role in the development of peripheral fatigue due to the pressure exerted by the muscle on the vasculature (Barcroft & Millen, 1939). Indeed, higher contraction forces cause a higher hindrance to blood flow and O_2 towards the muscle (Barcroft & Dornhorst, 1949) and out of the muscle for the washout of metabolic by-products (Amann *et al.*, 2006; Broxterman *et al.*, 2014). The first studies on the appearance of peripheral fatigue used protocols on isolated fibers (Allen *et al.*, 2008), while peripheral fatigue can also be measured *in vivo* by observing the exercise-induced changes in the force produced by an electrical single, doublet, or tetanic supramaximal stimulation (Behm *et al.*, 1996). A single nerve/muscle stimulation results in a mechanical force response and an electrical muscle action potential (e.g., M-wave). The latter is given by the spatial summation of individual action potential arising from the depolarization of the motor axons and it is thought to reflect the propagation of action potentials across the sarcolemma after a supramaximal intensity stimulus (Rodriguez-Falces & Place, 2018). The choice of a supramaximal stimulation compared to a maximal one (usually between 120% and 200% of the intensity evoking a plateau) depends on the threshold for axonal excitation which may increase during the development of fatigue (Kernell & Monster, 1982). In this way, the maximal stimulation intensity obtained in the non-fatigued state may not be enough to recruit the same motor units in the fatigued state, resulting in a wrong estimation of exercise-induced peripheral fatigue during the interpolated twitch technique, used also to verify the presence of central fatigue. Moreover, during this technique, another important factor to influence the muscle response is potentiation (Rassier & Macintosh, 2000). The potentiation phenomenon derives from previous activation of the muscle, thus, phosphorylation

of the myosin light chain and increases the Ca^{2+} sensitivity of the actomyosin complex (Blazevich & Babault, 2019).

Excitation-contraction coupling

The phenomenon of excitation-contraction coupling refers to all the steps from the depolarization of the muscle membrane (i.e., sarcolemma) to the beginning of cross-bridges formation (Dulhunty, 2006). Firstly, the alpha motor neuron releases acetylcholine in the synaptic cleft at the level of the neuromuscular junction, where it binds to receptors on the sarcolemma (Allen *et al.*, 2008). This allows the opening of ion channels for the passing of sodium (Na^+) and potassium (K^+) through the muscle membranes. At this moment depolarization takes place with the generation of an action potential that travels through the transverse tubular system. Here dihydropyridine receptors, activated by the spreading action potential, interact with the sarcoplasmic reticulum Ca^{2+} release channels (i.e., ryanodine receptors) and lead to the release of Ca^{2+} into the cytosol (Dulhunty, 2006). In the cytosol Ca^{2+} binds to troponin, causing a change in the structure of the tropomyosin, which makes the myosin binding sites available on the actin filament (Williams & Ratel, 2009). Hence, the myosin attaches to the actin filament creating a cross-bridge and producing force (Williams & Ratel, 2009). However, when the discharge of motor neurons ends, the sarcolemma repolarizes, and all these processes stop. Indeed, Ca^{2+} is reuptake into the sarcoplasmic reticulum to restore the basal level in the myoplasm and promote relaxation (Williams & Ratel, 2009). Disruptions in any of these processes lead to excitation-contraction coupling failure and peripheral fatigue development.

Mechanisms of peripheral fatigue

It has been suggested that the peripheral fatigued state can interest every step in the chain from the motor neuron, neuromuscular junction, sarcolemmal membrane, excitation-contraction coupling, accumulation of metabolites, or depletion of fuels. Especially, two phenomena may generally but not always lead to a lower muscle force production: a failure in the neuromuscular transmission of the action potentials at the level of the sarcolemma or the impairment of the contractile intrinsic processes (Place *et al.*, 2010). In this regard, Ca^{2+} is the main responsible

for the alteration downstream of the sarcolemma in three ways: a decrease in the myofibrillar force-generating capacity, an impaired myofibrillar Ca^{2+} sensitivity, and an impaired Ca^{2+} release from the sarcoplasmic reticulum (Allen *et al.*, 2008; Olsson *et al.*, 2020). Intracellular Ca^{2+} is the signaling molecule that governs the execution of a muscle contraction, and it determines the number of cross bridges that can be formed. However, the progressive accumulation of other several metabolites and glycogen depletion may cause excitation-contraction coupling failure and inhibition of the contractile machinery by any of the three Ca^{2+}-based mechanisms (Allen *et al.*, 2008).

Acidosis and inorganic phosphate

Evidence for the relation between peripheral fatigue and accumulation of hydrogen ions (H^+) considered acidosis one of the main causes. The increase in H+ induced by high-intensity exercise comes from the accumulation of lactic acid that dissociates in lactate and H^+ leading to a higher acidity and a decrease in pH. This phenomenon, in addition to affecting the correct functionality of several ATP-related enzymes, influences the contractile machinery due to a competition between H^+ and Ca^{2+} for the binding sites on troponin C, lowering the sensitivity for Ca^{2+} and reducing the expression of force (Fabiato & Fabiato, 1978). However, studies carried out at physiological and non-physiological temperatures made clear that acidosis and the decrease in pH are not always relevant for the development of fatigue (Pate *et al.*, 1995; Westerblad *et al.*, 2002). On the other hand, the accumulation of inorganic phosphate (Pi) during high-intensity exercise was observed to inhibit cross-bridge force production and myofibrillar Ca^{2+} sensitivity (Allen *et al.*, 2008) at physiological temperatures. Indeed, only when the cytosolic Pi exceeds a 'critical' threshold, Pi enters the sarcoplasmic reticulum and precipitates with Ca^{2+}, which reduces Ca^{2+} release during excitation-contraction coupling (calcium-phosphate precipitation phenomenon) and decreases force production (Korzeniewski & Rossiter, 2020; Hureau *et al.*, 2022).

Accumulation of extracellular K+

The transmission of an action potential across the muscle membrane to the t-tubules of a muscle fiber is dependent upon Na^+ and K^+ concentrations within the cell. An

imbalance of Na^+ or K^+ prevents correct muscle contraction (McKenna *et al.*, 2007; Kuo & Ehrlich, 2015). During repeated muscle activation, a net K^+ efflux from the muscle cell occurs leading to an increase in extracellular K^+ concentrations at the expense of intracellular K^+ (Juel *et al.*, 2000; Clausen, 2003). Increasing extracellular K^+ impairs force generation due to the depolarization of the cell membrane which will inactivate Na^+ channels, reducing the amplitude of the action potential. The accumulation of extracellular K^+ during prolonged membrane depolarization reduces sarcoplasmic reticulum Ca^{2+} release, impairing excitation-contraction coupling (McKenna, 1992).

Glycogen content

Another critical factor for peripheral fatigue is the lack of glycogen stores during exercise. Numerous studies confirmed the link between impaired muscle function and glycogen depletion, which is known to influence the rate at which adenosine 5'- triphosphate (ATP) can be regenerated. However, the role of energy reserves in inducing fatigue is not entirely clear, because the relation between low glycogen and decreased muscle function is still present after recovery when ATP levels should be physiological again (Chin & Allen, 1997; Ørtenblad *et al.*, 2013). One more probable hypothesis which is based on human and *in vitro* studies on muscle fibers suggests an association between low levels of glycogen and Ca^{2+} handling mechanisms. From this perspective, glycogen decreases immediately after exercise, and with it also the release of Ca^{2+} contributing to fatigue. While after adequate recovery the levels of both return to pre-exercise concentration (Nielsen *et al.*, 2009; Ørtenblad *et al.*, 2011). These findings, therefore, support the importance of glycogen for proper muscle function and fatigue development. They also suggest the combined role of energy stores and Ca^{2+} release in the sarcoplasmic reticulum in a fatigued state of the organism.

The role of group III/IV muscle afferent feedback on central and peripheral fatigue

Group III and IV muscle afferents are small-diameter fibers that firstly synapse in the dorsal horn of the gray matter of the spine (Kaufman & Forster, 1996), while their axons project to spinal and supraspinal sites of the central nervous system.

They generally influence the development of neuromuscular fatigue and all its components during both single-joint and whole-body exercises. Specifically, firing of group III/IV afferent fibers can cause the slowing of motor neurons firing rates, thus, impacting central fatigue and exercise performance. This phenomenon has been detected utilizing post-exercise circulatory occlusion after single-limb fatiguing exercise (Gandevia *et al.*, 1996). Specifically, voluntary activation decreased after a 2-min MVC of the elbow flexors (Gandevia *et al.*, 1996). At the end of the contraction, a blood pressure cuff was inflated proximally to the elbow flexors, maintaining the firing of group III/IV muscle afferents due to metabolite accumulation, and not allowing muscle recovery. Surprisingly, circulation and group III/IV muscle afferents recovered to baseline values when the contraction stopped. Differently, voluntary activation and motoneuronal output remained low for as long as the muscle was kept ischemic. This result implies that even though the neural pathway appears to have recovered, the output from the motor cortex is not able to drive the muscle fully. Therefore, it is plausible that group III/IV afferent feedback-mediated impairments of the voluntary descending drive may happen upstream of the motor cortex. Further evidence on the inhibitory role of group III/IV muscle afferents on motoneuronal output during whole-body exercise comes from studies in which the participants performed high-intensity cycling to exhaustion under control conditions or pharmacological blockade of afferent feedback (Amann *et al.*, 2009). From these studies emerged that the electromyographic signal during exercise (related to central motor drive) was higher when feedback of group III/IV from the legs was blocked. Interestingly, a similar result with an increase in central fatigue was obtained from a remote muscle group at rest (Sidhu et al., 2014).

The firing of group III/IV afferents due to various intramuscular metabolites and metabolic changes within the contracting muscle can adjust ventilation, to ensure an adequate supply of O_2, and slow the onset of peripheral fatigue too (Dempsey *et al.*, 1996). On the contrary, a lower blood flow and O_2 delivery to the muscle affect exercise performance and accelerate the appearance of peripheral fatigue in a few minutes (Amann & Calbet, 2008). This has been demonstrated using a pharmacological approach to block muscle afferents during exercise, leading to an earlier development of fatigue, because the non-working feedback caused

hypoventilation and hypoperfusion of the exercising muscle (Amann et al., 2008 2009). These findings represent interesting evidence that this neural feedback mechanism represents an important determinant of exercise performance to optimize fatigue.

Effect of neuromuscular fatigue on performance

Several studies suggest that the development of neuromuscular fatigue integrates multiple aspects to stop the exercise. One of the most recognized models explains task failure with a critical peripheral fatigue threshold. Close to this threshold, feedback from sensory afferents stops the task or reduces its intensity, so that peripheral fatigue does not exceed (Vanhatalo *et al.*, 2010; Hureau *et al.*, 2014, 2016). The role of muscle afferents in this process seems to work and to be supported by studies that utilized pharmacological blockade of group III/IV afferent feedback. Indeed, when afferent feedback is attenuated, individuals cross the critical threshold, finishing the trial with higher intramuscular metabolites concentration correlated to higher peripheral fatigue (Blain *et al.*, 2016). The concept of critical threshold emerged from other observations based on reproducible peak twitch force reductions immediately following various cycling bouts to task failure (Amann *et al.*, n.d.; Blain *et al.*, 2016)

The sensory tolerance limit is an expanded model that suggests a more global feedback and feedforward loop acting to terminate the task and ensuring tolerable voluntary activity (Todd *et al.*, 2003). Especially, the sum of neural feedback from locomotor and respiratory muscles, organs, and muscles not involved in the exercise are integrated within the brain to regulate the magnitude of the central motor drive and the intensity of exercise. Previous studies utilized a voluntary fatiguing exercise to induce pre-fatigue in a remote muscle causing the sensory tolerant limit to be reached sooner. Indeed, this approach led to impaired endurance performance due to the crucial activation of afferent feedback from both the exercising and the recovering limb combined with the effort from respiratory muscles (Amann *et al.*, 2013; Johnson *et al.*, 2015).

The literature also provides other studies that agreed with the central governor model theory. The model proposes that exercise performance does not exceed the limits and does not damage the homeostasis of the system (Noakes *et al.*, 2004).

This subconscious regulation works by integrating afferent information from the body and brain, which in turn controls muscle recruitment and power output, while the subject can safely complete the task. However, this model has been subjected to much criticism. Indeed, previous studies revealed that the type of task could damage several organs differently and, thus, exercise performance can be limited not just by motor unit recruitment and central governor, but by a variety of factors (e.g., loss of contractile performance of the skeletal muscle, emerging hyperthermia and ischemia) (Weir *et al.*, 2006; Shepard, 2009).

On the other hand, the idea that motivation (e.g., the maximum effort a subject is willing to invest to succeed in a task), and perception of effort (e.g., the result of the central processing of the corollary discharge associated with the central command) regulate endurance performance is part of the psychobiological model (de Morree & Marcora, 2015). Based on this simple theory extended to self-paced exercises, the conscious regulation of pace is primarily determined by the effort perceived by the athlete without any influence by afferent feedback. On the contrary, several authors observed an association between the perception of effort and the feedback system (Amann *et al.*, 2008; Romer & Polkey, 2008). Despite this, the psychobiological model states that perception of effort seems to be generated only by feedforward mechanisms when an efferent copy of the motor and autonomic signal is sent to other brain areas where perception of effort appears. Hence, the shorter time to exhaustion after physiological or psychological alteration is explained by an increased perceived effort for the same workload, leading to maximal effort in less time (Williamson, 2010) which means a higher activation of the central command.

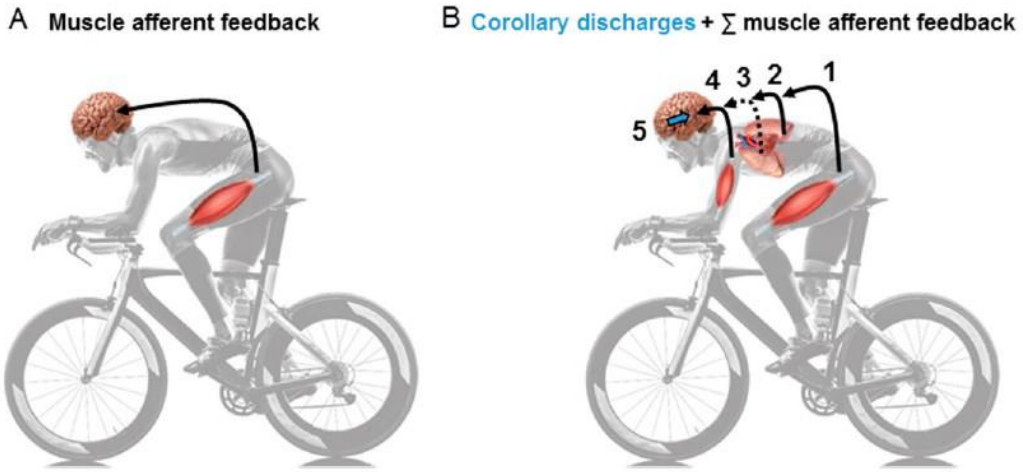

Figure 1.3. *Representation of A) the "critical fatigue threshold" and B) the "sensory tolerance limit" theory. Taken from Hureau et al., 2018.*

Transcranial magnetic stimulation

Transcranial magnetic stimulation is a non-invasive technique that emerged in 1985 by Barker aimed to study the central nervous system through the stimulation of the brain (Barker *et al.*, 1985). Its functionality is based on a short high-intensity current pulse produced in a wire (magnetic coil) which causes a high-intensity magnetic field in the scalp, while an electric field spreads in the targeted cerebral cortex. Hence, ions flow in the brain which ultimately induce brief activation or inhibition of neurons (Di Lazzaro *et al.*, 2008; Rossini *et al.*, 2015) (Fig.1.1). The shape and orientation of the coil influence the pattern and direction of the induced electric field which triggers axons rather than cell bodies of neurons.

Single-pulse transcranial magnetic stimulation has been widely used to assess the conduction of the descending cortico-nuclear and cortico-spinal connections in the primary motor cortex by recording the induced motor-evoked potentials (Rossini *et al.*, 2015). Otherwise, paired-pulse and paired associative protocols offer the possibility to study primary motor cortex excitability through the facilitation and inhibition of motor evoked potentials, for diagnostic and research purposes (Di Lazzaro & Rothwell, 2014).

In the early 90s, it has been introduced repetitive transcranial magnetic stimulation to deliver several pulses of transcranial magnetic stimulation in sequence. This approach has proven to be advantageous to induce long-lasting neuroplastic

changes in the brain and, for this reason, has been mainly applied for therapeutic purposes (Chen *et al.*, 1997; Boutiere *et al.*, 2017). The most studied cortical area with repetitive transcranial magnetic stimulation, thanks to its easy localization, is the motor one where facilitative or inhibitory effects have been observed for a long time. On the other hand, it was more difficult to study other areas of the brain, until the coupling of repetitive transcranial magnetic stimulation with functional brain imaging and electroencephalography helped to assess the neuromodulatory mechanism of action of transcranial magnetic stimulation in different brain regions as well (Burke *et al.*, 2019).

Regardless of the target area, transcranial magnetic stimulation evokes a series of descending waves of corticospinal activity which can be recorded and obtained from the electromyographic signal. The earliest wave is termed D-wave because it is caused by direct activation of the axon of corticospinal neurons in the subcortical white matter after high-intensity stimulation. The later waves are called I-waves because they are due to synaptic activation of the same corticospinal neurons, and they are numbered in order of appearance (Di Lazzaro & Rothwell, 2014). These waves are mainly prompted during repetitive transcranial magnetic stimulation protocols. Pyramidal neurons have a high threshold of activation, so they are not directly stimulated by intensities normally used in repetitive transcranial magnetic stimulation protocols, usually close to the motor threshold. Conversely, they are activated by surrounding interneurons displaying lower thresholds (Di Lazzaro *et al.*, 2008).

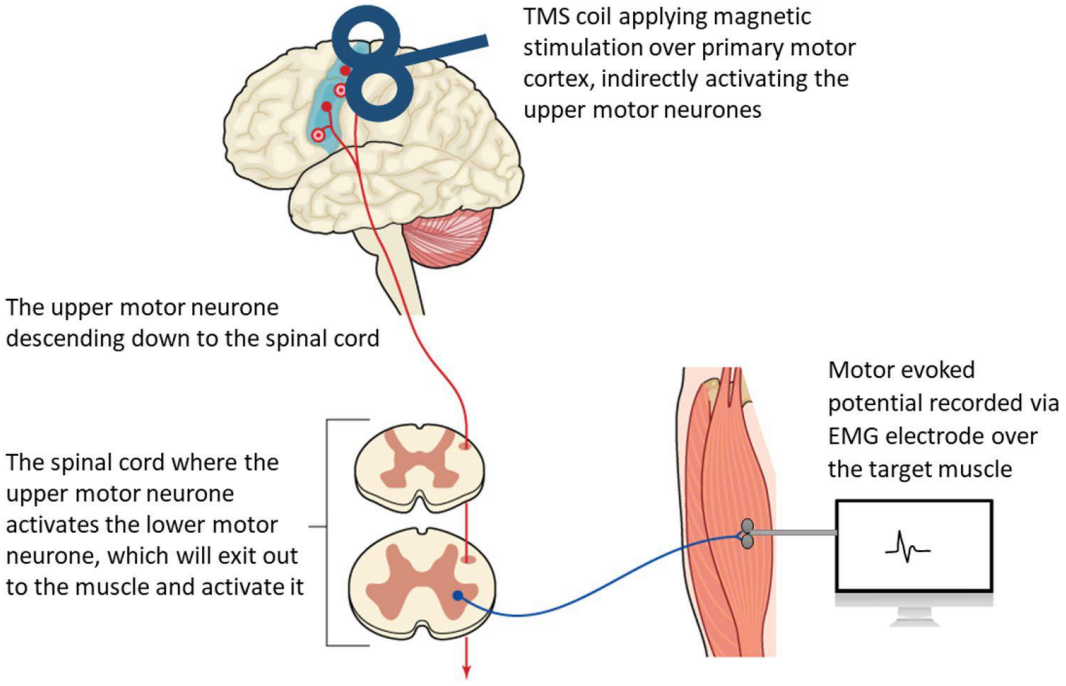

Figure 1.4. *A diagram illustrating how transcranial magnetic stimulation (TMS) over the primary motor cortex (M1) indirectly activates the upper and lower motor neurons of the corticospinal pathway to lead to a muscle contraction recorded as a motor-evoked potential (MEP) using EMG electrodes. Taken from Haavik et al., 2021.*

Conventional and patterned rTMS protocols

The choice of the frequencies and pattern of stimulation determines important neuroscientific effects of excitation or inhibition after repetitive transcranial magnetic stimulation. Conventional repetitive transcranial magnetic stimulation protocols are delivered at high frequencies, with stimulus rates ≥ 5Hz, leading to an excitatory effect; low-frequency protocols, with stimulus rates ≤ 1Hz, produce an inhibitory effect on neuronal excitability. In both cases, the stimulation time is around 20-30 minutes, making experimental and clinical sessions complex. Recently, other protocols used shorter repetitive transcranial magnetic stimulation bursts interleaved by pauses of no stimulation such as theta burst stimulation to mimic brain oscillatory activity (Rossi *et al.*, 2021). Transcranial magnetic stimulation frequencies are derived from the observation of burst discharge (4–7 Hz) of the hippocampus of rats during exploratory behavior and stimuli fire as bursts of 3 pulses at 50-Hz burst applied at 5 Hz every 200 ms). Intermittent theta burst stimulation involves 600 pulses delivered as 20 trains of 2 seconds of

transcranial magnetic stimulation followed by an 8 s rest for about 3 min and increases motor cortex excitability (Huang *et al.*, 2005). Continuous theta burst stimulation is obtained by applying one 40-second transcranial magnetic stimulation train which decreases motor cortex excitability (Huang *et al.*, 2005). The obtained long-lasting effects on cortical excitability are based on long-term potentiation and depression mechanisms (long-term potentiation and long-term depression) and exceed those seen with conventional repetitive transcranial magnetic stimulation protocols (Huang *et al.*, 2005; Suppa *et al.*, 2008). Accordingly, transcranial magnetic stimulation was thought to induce effects that would have lasted more than conventional protocols, but with similar efficacy over time. However, the main advantage of transcranial magnetic stimulation is its short duration, making it more acceptable to participants, and the stability of stimulation parameters, which decreases the variability of parameters among different studies.

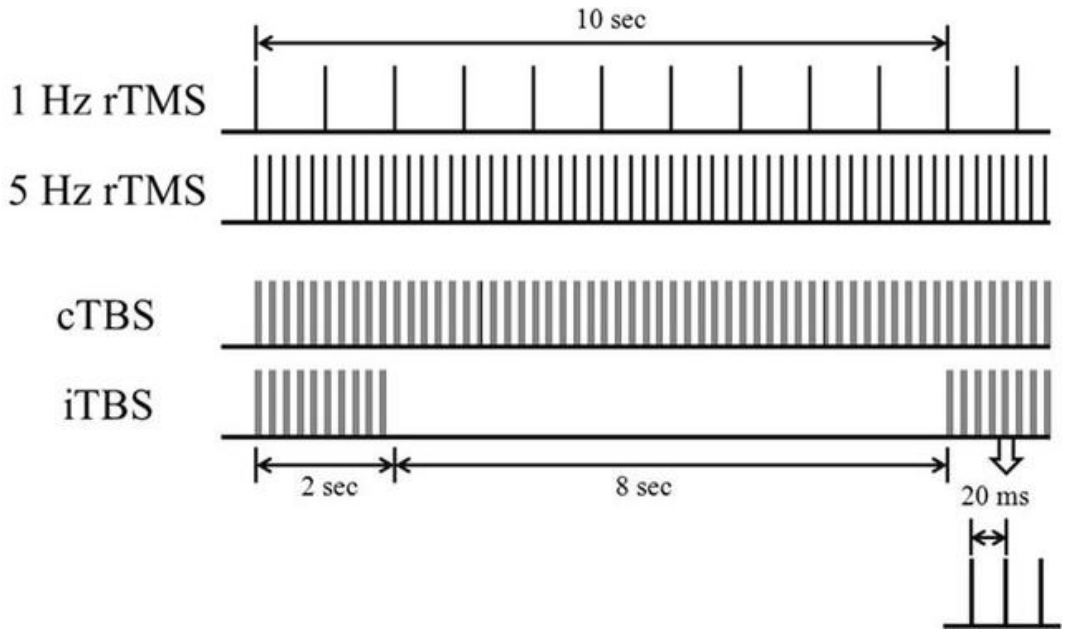

Figure 1.5. *Examples of conventional repetitive transcranial magnetic stimulation (rTMS) at low (1 Hz), high (5 Hz) frequency and patterned rTMS include continuous (cTBS) and intermittent (iTBS) theta burst stimulation. Taken and modified by Fung et al., 2010.*

Cortical plasticity

Neuroplasticity represents the ability of the nervous system to adjust in response to stimuli (e.g., learning, injury) (Bachtiar & Stagg, 2014). Several beneath mechanisms to explain cortical plasticity include synaptic-related processes (Baranyi & Fehér, 1978), changes in excitability of post-synaptic neurons (Woody *et al.*, 1991), the history of pre-existing connections (Jacobs & Donoghue, 1991) and motor cortical reorganization (synaptogenesis and neurogenesis) (Kleim *et al.*, 2004).

Mechanisms of action

The phenomena of long-term potentiation and long-term depression are the basis of synaptic plasticity (Wang & Van Praag, 2012). A potentiation of the synaptic plasticity (i.e., long-term potentiation) is dependent on the summation of excitatory postsynaptic potentials generated by repeated (high frequency) synaptic inputs, or via stimulation delivered with precise timing of inputs from presynaptic to post-synaptic cells. The related mechanisms are thought the be dependent on an initial influx of Na^+ through glutamate-activated alpha-amino-3hydroxy-5-methyl-4-isoxazole propionate (AMPA) channels, or Ca^{2+} through voltage calcium channels (Kew & Kemp, 2005) which lead to both a positively charged intracellular environment and activation of N-methyl-D-aspartate receptors for the transport of further Ca^{2+} and Na^+ into the cell (Collingridge & Bliss, 1995). When enough Ca^{2+} concentration moves into the post-synaptic membrane, a protein kinase pathway triggers to transfer additional AMPA receptors and phosphorylates them. These processes may provoke glutamatergic activation and depolarization of the post-synaptic cell (Ziemann *et al.*, 2004). In contrast to long-term potentiation, long-term depression is due to a reduced Ca^{2+} influx in the post-synaptic cell which promotes a cascade responsible for a long-term depression response (Fung & Robinson, 2014). Contrary to long-term potentiation, this cascade reduces the opening and the number of AMPA receptors from the membrane (Isaac, 2001). With regards to the motor system, Hess and Donoghue (1994) demonstrated that noninvasive brain stimulation induces long-term potentiation-like changes in a rat's primary motor cortex when GABA receptors were blocked with bicuculline.

Moreover, in humans, a lower level of plasticity has been observed through the administration of lorazepam which increases $GABA_A$-mediated inhibition in the primary motor cortex (Ziemann *et al.*, 2001; Teo *et al.*, 2009). Otherwise, further factors known to influence the induction of synaptic plasticity are the dopaminergic, cholinergic, serotonergic, and adrenergic systems and the changes in neuronal circuitries. Indeed, plasticity includes two phenomena: neurogenesis and synaptogenesis which is the generation of new neurons and synapses, respectively. These processes can occur during early development and throughout life in the mammalian brain. In experiments conducted on murine models, instead, both neurogenesis and synaptogenesis have been observed in response to learning tasks in the hippocampus, cerebellum, and motor cortex (Kleim *et al.*, 1996; Gould *et al.*, 1999). Otherwise, in humans, transcranial magnetic stimulation research has demonstrated in trained pianists an altered structural and functional plasticity associated with modified cortical representations of muscles, and interhemispheric inhibition compared with control participants (Chieffo *et al.*, 2016). These findings indicate that continuous activation of neuronal networks can lead to plasticity associated with functional and structural events.

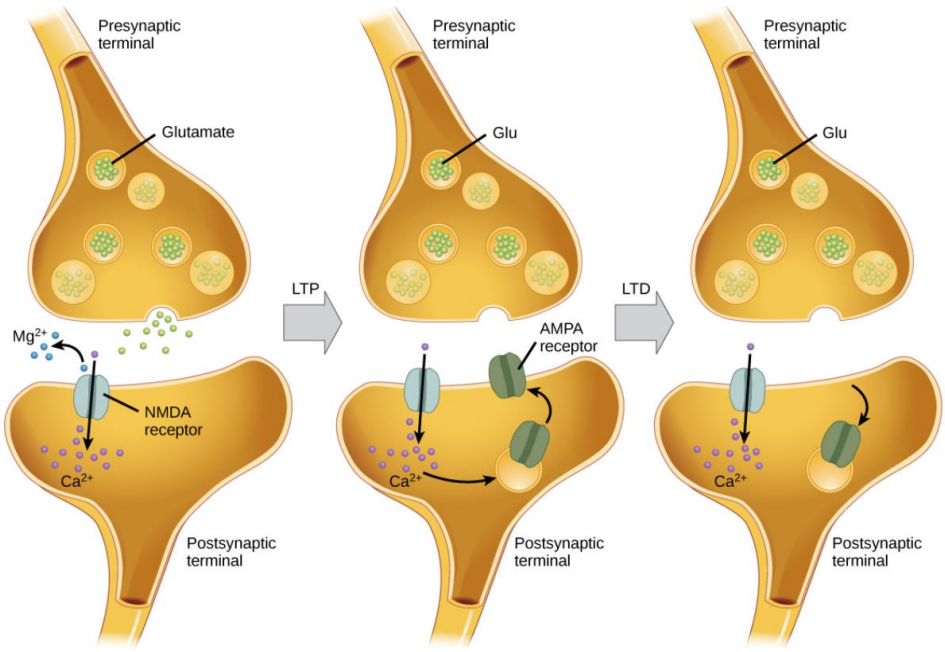

Figure 1.6. *Mechanisms of long-term potentiation (LTP) and long-term depression (LTD). LTP arises after repetitive stimulation of a synapse leading to the activation of calcium-*

(Ca^{2+}) and CaMKII-dependent cellular cascade, which results in the insertion of more AMPA receptors into the postsynaptic membrane. Hence, glutamate (Glu) released from the presynaptic cell can bind to both NMDA and AMPA receptors, depolarizing the membrane more efficiently. LTD occurs when few Glu binds to NMDA (due to the low firing rate of the synaptic neuron). The Ca^{2+} flows through NMDA receptors starting a different calcineurin and protein phosphatase 1-dependent cascade, which results in the endocytosis of AMPA making the postsynaptic neuron less responsive to Glu.

Cortical plasticity behind cortical stimulation

The specific mechanisms of action determining the effects of repetitive transcranial magnetic stimulation and theta burst stimulation protocols are still object to debate. However, both excitatory and inhibitory protocols are known to induce short and long-lasting changes at the primary site of activation and secondary-related areas (Chervyakov *et al.*, 2015). The literature agrees on the involvement of synaptic long-term potentiation and long-term depression events, modulation of neurotransmitter release and receptor expression, growth factors, and activation of neuroprotection pathways (Huang *et al.*, 2017). It has been recently proposed a theoretical model for targeting synaptic strength after theta burst stimulation. It suggests that the variation of the stimulation pattern can change the result of oscillation between facilitation and depression, accounting for plasticity induction from intermittent theta burst stimulation and continuous theta burst stimulation respectively. As mentioned in the previous section, in both the continuous theta burst stimulation and intermittent theta burst stimulation stimulations a chain of events is crucial for plasticity induction. The processes involve mainly glutamatergic and GABAergic pathways, postsynaptic Ca^{2+} influx which activate different kinases that determine the choice between long-term potentiation and long-term depression. Then, synaptic long-term changes occur from the sum of facilitatory and inhibitory oscillations (Rounis & Huang, 2020). The secondary modulation of distant cortical and subcortical areas by repetitive transcranial magnetic stimulation and theta burst stimulation relies mostly on the action of neurotransmitter release, despite the secondary areas may show plastic changes in the opposite direction of modulation compared to the primary target (Cho & Strafella, 2009). Thus, the resulting therapeutic effect is probably due to the modulation of large-scale brain networks involving several areas controlling different physiological pathways (Cirillo *et al.*, 2017). Nowadays conventional and

patterned protocols have been approved by the Food Drug Administration and have important implications for therapeutic applications mainly in the field of neurological, pain, and psychiatric disorders (Lefaucheur *et al.*, 2020).

Modulation of fatigue

Among the several factors that affect neuromuscular fatigue, the central nervous system seems to play a crucial role. As mentioned before, cognitive activity can negatively impact fatigue (Mosso, 1891), and more recent studies detected a lower exercise (Rounis & Huang, 2020) tolerance and a higher initial rating of perceived exertion if heavy cognitive tasks are administered before physical exercise (Marcora *et al.*, 2009). From this perspective, cortical substrates may represent a significant target in the level and development of fatigue. Despite non-invasive techniques such as transcranial magnetic stimulation have been largely used to investigate the impact of fatigue during exercise without finding a clear answer, several pieces of evidence suggest a suboptimal descending output from the motor cortex during fatiguing contraction (Taylor *et al.*, 1996; Gandevia, 2001). In this context, any intervention that limits this alteration by modulating the function of the cerebral cortex could likely improve exercise performance. More in the clinical field than in research, studies on fatigue suggest the use of transcranial direct current stimulation to deliver continuous, low-intensity electrical current, leading to significant changes in cortical excitability and time to exhaustion (Cogiamanian *et al.*, 2007; Williams *et al.*, 2013; Okano *et al.*, 2015) However, despite being now the only approach used in the field of fatigue, its effectiveness still leaves the scientific world skeptical, potentially due to different experimental and methodological setups, and, consequently, contrasting results. On the other hand, alternative approaches to transcranial direct current stimulation to modulate neuromuscular fatigue are scant. Indeed, several studies using theta burst stimulation aim to verify solely the status of the corticospinal pathway after inducing alteration in its excitability without investigating fatigue. For this purpose, Huang et al., 2005 detected modification of the motor evoked potentials after intermittent theta burst stimulation and continuous theta burst stimulation suggesting an increased or decreased excitability, respectively. Other studies using theta burst stimulation are, instead, part of clinical research and are not focused on

treating fatigue. However, they found continuous theta burst stimulation a valid tool in promoting inhibition rather than excitatory mechanisms in epileptic populations (Koc *et al.*, 2017), or intermittent theta burst stimulation an effective approach to improve the resting-state functional connectivity of the motor cortex against multiple sclerosis (Boutiere *et al.*, 2017). Another interesting finding that certified the advantage of continuous theta burst stimulation reduced perception of nociceptive stimuli by acting both on descending pain modulatory systems and some cognitive processes involved in pain perception (Torta *et al.*, 2013).

Experimental aims & hypotheses

Experimental aims

The specific aims of this dissertation were to:

1) Determine whether theta burst stimulation modulates exercise performance by shaping central fatigue and corticospinal excitability.

2) Determine whether the cross-over effect of theta-burst stimulation may represent another strategy to modulate exercise performance.

Hypotheses

To accomplish our aims, we tested the following hypotheses:

1) Alterations induced within brain circuits by intermittent theta burst stimulation or continuous theta burst stimulation (compared to a sham condition) could improve or deteriorate the neuromuscular determinants of force and, after the fatiguing task, further impact performance fatigability and the central neural drive.

2) Alterations induced within the right motor cortex by continuous theta burst stimulation could deteriorate the neuromuscular determinants of force but, after the fatiguing task, performance fatigability and the central neural drive may increase and reduce by intermittent theta burst stimulation and continuous theta burst stimulation, respectively, in the exercising ipsilateral right elbow flexor muscles.

Study I - Theta burst stimulation modulates exercise performance by shaping central fatigue and corticospinal excitability

Based on the article submitted in the Journal of Applied Physiology

Camilla Martignon, Chiara Barbi, Gianluca Vernillo, Simranjit K Sidhu, Mehran Emadi Andani, Federico Schena, Massimo Venturelli

Abstract

Theta-burst stimulation (TBS) over the primary motor cortex modulates activity of the underlying neural tissue, but little is known about its effect on neuromuscular fatigue and its neural correlates. This study assessed the effects of three TBS protocols (facilitatory/intermittent: iTBS; inhibitory/continuous: cTBS, and sham: sTBS) on exercise performance, neuromuscular function, corticospinal excitability and $GABA_B$-mediated intracortical inhibition in twenty young healthy participants with transcranial magnetic and peripheral electrical stimulations at baseline, following TBS (i.e. unfatigued muscle), and after a fatiguing sustained contraction (i.e. fatigued muscle) at 35% of the maximal voluntary isometric contraction (MVIC) of the elbow flexor.

The time-to-task failure was shorter for cTBS (142±51s) and longer for iTBS (214±68s) compared with sTBS (173±65s) ($P<.05$).

In fresh muscle, cTBS reduced MVIC and voluntary activation (VA), increased motor-evoked potential (MEP), and silent period (SP) ($P<0.05$), while iTBS did not cause any change.

In fatigued muscle, MVIC and VA, decreased in all TBS sessions ($P<0.05$). However, the reduction in VA was larger after cTBS (Δ-18±18%) compared with iTBS (Δ-3±5%), and sTBS (Δ-9±9%) ($P<0.001$). Furthermore, in fatigued muscle, the increase in MEP and SP were greater for cTBS ($P<.05$), compared to iTBS and sTBS ($P<.05$).

The outcomes suggest that facilitatory TBS augments exercise performance that is independent of central drive and corticospinal mechanisms whilst inhibitory TBS attenuates exercise performance through an exacerbation in the development of central fatigue and intracortical inhibition.

NEW & NOTEWORTHY

Performance fatigability after theta-burst stimulation (TBS) is of interest to clarify how the neuromuscular system adapts to exogenous stimuli during and after an exercise. In this context, central brain networks received little attention and need to be investigated more fully. Hence, we proposed that exercise performance can be differently affected by divergent TBS protocols localizing and limiting the

appearance of fatigue. From a practical perspective, this finding may help the understanding of the effect of fatigue and modulation on neural processes.

Introduction

Neuromuscular fatigue is manifested in a reduction of the maximal voluntary isometric contraction (MVIC) force (Gandevia, 2001), whilst the endurance of a task is an objective measure of performance over time. Both impairments in MVIC force and endurance can be attributed to one or more sites in the neuromuscular system, including central (i.e., proximal to the neuromuscular junction and encompassing the brain, as well as upper and lower motoneurons) (Gandevia, 2001) and/or peripheral (i.e., within the skeletal muscle) (Allen *et al.*, 2008). Moreover, neuromuscular fatigue and the resulting decrements in the endurance of a task are commonly reported in many clinical populations, including multiple sclerosis, Parkinson's disease, traumatic brain injury, and stroke contributing to the worsening of quality of life and disability (Kluger *et al.*, 2013). However, the understanding of fatigue pathophysiology is limited, and current treatment options rarely lead to meaningful improvements in fatigue.

Theta-burst stimulation (TBS) delivered to the primary motor cortex is a promising alternative to the conventional rTMS protocols as it can produce long-lasting neural changes (i.e., long term potentiation or depression [LTP, LTD] like effects) in less time and with short bursts of stimulation (Hoogendam *et al.*, 2010; Boutiere *et al.*, 2017; Fitzgerald *et al.*, 2020). The frequency of stimulation influences the neural changes (Klomjai *et al.*, 2015). For example, intermittent TBS (iTBS) transiently facilitates the activation of neural networks by influencing the circuitry of the primary motor cortex that generates descending later I-waves (i.e., synchronous activity of corticospinal axons originating from trans-synaptic activation of corticospinal cells) (Di Lazzaro *et al.*, 2008); whereas continuous TBS (cTBS) leads to long-lasting inhibition and suppression of corticospinal excitability by affecting the early I1-wave and leaving the later I-waves unchanged (Di Lazzaro *et al.*, 2008). Since TBS can be used to modulate the neural activity of the brain, it has the potential to influence the development of central fatigue and thus exercise performance during exercise. The application of neuromodulation techniques such as transcranial direct current stimulation has been shown to improve muscular endurance (Imperatori *et al.*, 2018), exercise tolerance (Vitor-Costa *et al.*, 2015), the rate of perceived exertion (Okano *et al.*, 2015; Vitor-Costa *et al.*, 2015; Angius

et al., 2017), heart rate responses (Okano *et al.*, 2015), as well as stress levels and reaction time (Davis, 2013). However, the underlying mechanisms determining this modulation are not clear and the results are highly variable. Given that the use of different TBS protocols can differently modulate the neural activity after a faster administration than compared to conventional protocol of rTMS, it is critical to investigate whether modulation of neural activity via facilitatory (iTBS) and inhibitory (cTBS) TBS of the primary motor cortex would alter central fatigue and exercise performance.

This study aimed to compare the effects of facilitatory versus inhibitory TBS delivered to the primary motor cortex on the neuromuscular fatigue and excitability/inhibition of the corticospinal pathway in a fresh and fatigued muscle. We hypothesised that iTBS and cTBS (compared to sTBS) would improve and attenuate the neuromuscular and corticospinal parameters respectively in both fresh and fatigued muscle.

METHODS

Ethical approval

The study received institutional ethical approval from the University of Verona Research Ethics Committee (IRB #12.R1/2021) and was conducted according to all aspects of the Declaration of Helsinki, apart from registration in a database. Participants provided written, informed consent to volunteer for the study.

Participants

Twenty healthy subjects (five males and fifteen females) volunteered and participated in this study (Table 3.1). The sample size was determined based on the effect of the TBS protocols in three intervals of time (PRE, POST_TBS, POST_TTF) on corticospinal excitability, with an α level of 0.05 and a required power (1-β) of 0.80 (Huang *et al.*, 2005; Bradnam *et al.*, 2010).

Eligibility criteria included the absence of any neurological or cardiovascular disease and contraindications to TMS determined by a questionnaire (Rossi *et al.*, 2021). Participants were instructed to avoid the consumption of caffeine on the day of the experiment and avoid performing any strenuous exercise during the 48 hours

before testing. For the female group, to avoid the influence of different phases of the menstrual cycle on the neuromuscular assessment (Ansdell *et al.*, 2019), the first day of menstruation was considered as day 1 of the cycle and women visited the lab on day 15 ± 3 of their menstrual cycle.

Participant's characteristics	
Age (yr)	24 ± 2
Body weight (Kg)	62 ± 10
Height (cm)	168 ± 0.1
Lever arm (cm)	30 ± 2
MVIC pre (N·m)	82 ± 27
M_{max} *pre (mVs)*	0.14 ± 0.01
MET total	4695 ± 963

Table 3.1. *Participant's characteristics. Data are presented as mean ± SD; n = 20. MVIC, maximal voluntary isometric contraction (mean of the three conditions at pre);* M_{max}*, maximal* M_{wave} *(mean of the three conditions at pre); MET, task metabolic equivalent.*

Study design

The study consisted of four non-consecutive days of examination that were held at the same time of day for each participant to control for within-participant diurnal variation (Ly *et al.*, 2016). On day 1, participants underwent an interview and filled out TMS and physical activity questionnaires (Lee *et al.*, 2011; Rossi *et al.*, 2021). The handedness dominance was verified with the Waterloo Handedness Questionnaire (Steenhuis *et al.*, 1990) which indicated all participants were right-handed. Then participants were familiarized with maximal and submaximal elbow flexor contractions with TMS and electrical nerve stimulation. On days two to four, each participant was asked to carry out a standardized warm-up that consisted of three voluntary contractions at 10%, 30%, 50%, and one at 70% of their MVIC assessed during the familiarization. Each warm-up contraction was 5 s long, with 5 s of rest in between. The warm-up was followed by two 5-s isometric MVICs with 2 min of rest in between. Then participants performed five sets of neuromuscular

evaluations (see "Neuromuscular function evaluation"). The first three neuromuscular evaluations (PRE) were interspersed by at least 1 minute of rest. The fourth neuromuscular evaluation (POST_TBS) was then performed before the fatiguing exercise and soon after the TBS protocol, randomly chosen among iTBS, cTBS, and sTBS to verify the effect of TBS alone without the influence of fatigue. Immediately at the end of the fatiguing sustained contractions at 35% of MVIC, the fifth neuromuscular evaluation was conducted (POST_TTF) (Figure 3.1 B). For each session, a pseudo-randomization was carried out to choose the protocol of TBS to use among iTBS, cTBS, and sTBS. Participants were highly motivated and instructed during all sessions to perform real MVICs, and strongly encouraged during the experiments by the investigators.

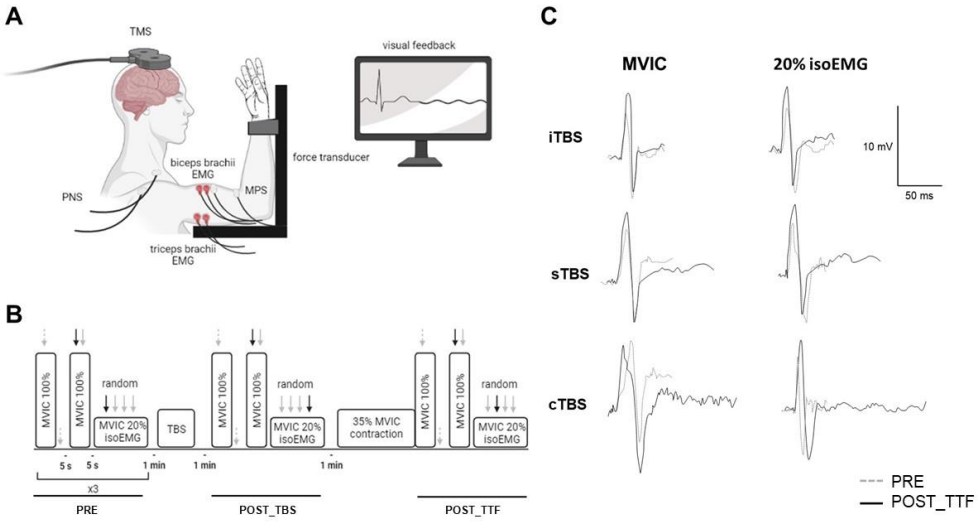

Figure 3.1. *Set up of the experimental session (A), protocol design (B), and raw traces of the motor evoked potentials (MEP) and silent periods from a single male participant (23 years old) during the MVIC and the 20% isoEMG contraction before (PRE) and after (POST_TTF) the submaximal sustained isometric elbow flexors contraction at 35% of the maximal contraction (C). TMS, transcranial magnetic stimulation; PNS, peripheral nerve stimulation; MPS, motor point stimulation; EMG, electromyography; MVIC, maximal voluntary isometric contraction; iTBS, intermittent theta burst stimulation; cTBS, continuous theta burst stimulation; sTBS, sham theta burst stimulation; Black arrows, transcranial magnetic stimulation; Grey dashed arrows, motor point stimulation; Grey arrow, peripheral nerve stimulation.*

Measurements

Force recording. Force was measured by a force transducer (DBBSE-100 kg, A2829; Applied Measurements Limited, Aldermaston Berkshire, UK) previously calibrated and connected to a high-speed acquisition system (PowerLab 16/30; ML880, ADInstruments, Colorado Springs, CO). The signal output was amplified (INT2-L, London Electronics Limited, Sandy, Bedfordshire, United Kingdom) and sampled at 2000 Hz. The participants were seated in an upright position with back support. Both shoulder and elbow joints were flexed at 90° and the wrist of the right arm was attached, via a strap and a rigid steel bar, to the force transducer (Figure 6, Panel A). The force transducer was connected to a fixed bar and located in front of the participants' joints. The participant's dominant arm was placed in a U-shaped hold to ensure that the wrist was connected to the load cell to which force was applied. Visual feedback of the force produced was provided to the participants using a custom-written MATLAB toolbox (The MathWorks, Inc., Natick, MA) (Figure 3.1, Panels A-B).

Surface electromyography. EMG of biceps brachii and triceps brachii was recorded continuously using two pairs of self-adhesive surface electrodes (Ambu Neuroline 715, Ambu A/S, Ballerup, Denmark) in a bipolar configuration with a 20-mm interelectrode distance and the reference on the medial epicondyle of the humerus. Placement of EMG electrodes for biceps brachii was on the line between the medial acromion and the cubital fossa at 1/3 the distance from the cubital fossa and placement for triceps brachii was at 50% of line posterior crista of acromion-olecranon, 2 fingers medial to this line (Hermens *et al.*, 2000). Before the electrode's application, the skin was shaved, prepared by gentle local abrasion using an abrasive paste, and cleaned with an alcohol swab to minimize electrical impedance. The electrodes' location was marked on the skin with indelible ink to maintain the electrode's placement consistent across visits.

Acquisition of the EMG data was done using a Quad Bio Amp (ML135, ADInstruments, Australia), and all signals were integrated and digitalized with a computer-based data acquisition and analysis system (hardware: PowerLab 16/30; ML880, ADInstruments, Bellavista, NSW, Australia, and software: LabChart8, ADInstruments, Bellavista, NSW, CO Australia). The raw EMG signals, acquired at 2 kHz sampling frequency, were band-pass filtered (10-500 Hz).

Transcranial magnetic stimulation. Single TMS pulses were manually delivered to elicit MEP during voluntary contractions of the elbow flexor. The left M1 was stimulated by a magnetic stimulator (Magstim Rapid^2; The Magstim Company Ltd, Whitland, UK) with a 70 mm figure-8 air-cooled coil (Magstim; D70 Air Film Coil; The Magstim Company Ltd, Whitland, UK) to induce a posteroanterior current. To identify the vertex, participants wore a swim cap on which lines were drawn between the preauricular points and from nasion to inion. The coil was held tangentially on the scalp and moved 5 cm from the vertex and rotated 45 degrees from the midline. The exact hotspot for the biceps brachii was identified (and marked on the participants' cap) as the coil location and orientation evoking consistent peak-to-peak amplitude MEP from a biphasic current oriented posterior to anterior across the central sulcus during voluntary contractions at 20% MVIC. The stimulation intensity was defined in relation to the active motor threshold (AMT) as the lowest intensity eliciting MEP amplitudes 200 μV higher than the basal EMG signal for at least 5 out of 10 trials in biceps brachii during brief voluntary contractions at 20% MVIC (Temesi *et al.*, 2014). A stimulus intensity of 120% of the intensity to elicit biceps brachii MEP responses was used throughout the rest of the sessions. Stimulus intensity was determined at the start of each session. Mean stimulus intensities were 58% ± 10%, 59% ± 9%, and 58% ± 11% during iTBS, sTBS, and cTBS, respectively with no difference between sessions ($P > .05$). TMS was always delivered once the participant had contracted to the appropriate force level and the force had stabilized during voluntary contractions. Participants were also instructed to re-contract to the prestimulus force level as quickly as possible after TMS delivery (Mathis *et al.*, 1998).

Theta burst stimulation. For each session, TBS was delivered after the baseline neuromuscular function evaluations on the site drawn on the swim cap, by a magnetic stimulator (Magstim Rapid^2; The Magstim Company Ltd, Whitland, UK) with a 70 mm figure-8 air-cooled coil (Magstim; D70 Air Film Coil; The Magstim Company Ltd, Whitland, UK), used in repetitive mode. Each TBS protocol consisted of bursts containing 3 pulses at 50 Hz and an intensity of 80% AMT repeated at 200 ms intervals. For the iTBS, a 2 s train of TBS was repeated every 10 s for a total of 190 s (600 pulses). For the cTBS, a 40 s train of

uninterrupted TBS was given (600 pulses) (Huang *et al.*, 2005). sTBS was also delivered through the same coil to which a polystyrene panel was attached, and the intensity of stimulation was set to 80% AMT to provide some discharge noise to ensure participants remained blinded to the intervention.

Peripheral nerve stimulation. To evoke maximal M_{wave} (M_{max}) in biceps brachii, single electrical stimuli of 200-µs duration were delivered via a constant-current stimulator (DS7AH, Digitimer; Welwyn Garden City, UK) to the brachial plexus (M_{wave} measurements) and the motor point of biceps brachii (force measurements). For biceps brachii, stimuli were delivered to the brachial plexus trunk at Erb's point with a cathode (32 mm G0451, Globus Corporation, TV, Italy) in the supraclavicular fossa and the anode (32 mm G0451, Globus Corporation, TV, Italy) on the acromion. For motor point stimulation, the cathode (32 mm G0451, Globus Corporation, TV, Italy) was placed on the motor point (on the biceps brachii muscle belly, midway between the anterior edge of the deltoid and the proximal elbow crease with the elbow flexed at 90°), and the anode (32 mm G0451, Globus Corporation, TV, Italy) over the bicipital tendon.

Single stimuli were delivered incrementally in the relaxed muscle until M wave and twitch amplitudes plateaued. A stimulus intensity of 125% of the intensity to elicit M wave peak-to-peak (M_{max}) and maximal twitch responses was used throughout the rest of the experiment. Stimulus intensity was determined at the start of each session. The supramaximal stimulus intensity was 128 ± 72 mA, 135 ± 52, and 137 ± 53 for brachial plexus stimulation during iTBS, cTBS, and sTBS, with no differences between sessions ($P > .05$). The supramaximal stimulus intensity was 120 ± 35 mA, 118 ± 52, and 131 ± 45 for elbow flexor motor point stimulation during iTBS, cTBS, and sTBS, respectively with no difference between sessions ($P > .05$).

Neuromuscular function evaluation. The neuromuscular function evaluation started with three sets of evaluations, separated by 60 s (PRE). Each evaluation consisted of three contractions of the elbow flexor. The first 5 s MVIC was superimposed with a single muscle stimulation delivered at the force plateau (superimposed twitch, SIT) and another stimulation evoked 2 s after the MVIC on the relaxed muscle (i.e. potentiated twitch, $Q_{tw,pot}$). All the peak forces from the

MVIC trials were within 5% of each other. Five seconds after the potentiated twitch, participants performed another 5-s MVIC. Single pulse TMS and PNS were delivered at MVIC, with ~2 s interstimulus intervals. Thereafter, participants performed a 15 s contraction at the EMG corresponding to 20% of the first MVIC force (20% isoEMG). The root mean square (RMS) EMG of the biceps brachii during the 200 ms plateau of baseline MVIC was quantified and then the 20% of this value was calculated and shown on the computer screen by a reference line. Participants were asked to contract at this intensity while they received three TMS and one nerve stimulation every 3 seconds in random order. One neuromuscular function evaluation was then repeated following the TBS protocol (POST_TBS), as well as after the fatiguing task (POST_TTF) (Figure 3.1, Panels A-B).

Fatiguing task. Participants were asked to perform a TTF exercise consisting of a submaximal sustained isometric elbow flexor contraction at 35% of the MVIC, previously recorded during the warm-up. Visual feedback of the force produced, as well as the target force levels, were provided to the participants using a real-time display on a computer screen. Participants contracted to the target force, and once force was attained, they were strongly encouraged by the investigators to maintain that target as long as possible during the session. The task ended when the force dropped by more than 10% compared to the target for more than 5 seconds (Cogiamanian *et al.*, 2007).

Data analysis

Force values were measured for the duration of the entire neuromuscular testing protocol. All data of force expression during the MVIC contractions and at rest were normalized to the lever arm of each participant. Area values for M_{max} and MEPs were measured between cursors marking the initial deflection from the baseline to the second crossing of the horizontal axis (Martin *et al.*, 2006*a*). To account for any changes in the compound muscle action potential, MEPs were normalized to M_{max} values (MEP/M_{max}) recorded during the same contraction. The duration of the SP after TMS was measured by visually inspecting the interval from the stimulus to the return of continuous voluntary EMG (Taylor *et al.*, 1996). During the fatiguing task, the RMS of EMG values were averaged every 10 seconds

and normalized for the M_{max} of the first 10-second interval. The median power frequency (MDF) data were averaged over the first and the last steady-state 5 seconds contraction time of the task (Elfving *et al.*, 2002).

For the calculation of the voluntary elbow flexors activation (VA), SIT and $Q_{tw,pot}$ were used as previously described (Merton, 1954):

$$VA = [1 - (SIT/Q_{tw,pot})] \times 100$$

Statistical analysis

Data in the results section are provided as either individual values or mean ± SD unless otherwise stated. For the non-normally distributed variable (i.e., TTF), the Friedman test was used to compare the TTF across the TBS protocols (iTBS, cTBS, and sTBS). Normal Gaussian distribution of data was confirmed using the Shapiro-Wilk test. If a violation was detected, the data were analysed using non-parametric analysis. For variables among PRE, POST_TBS, and POST_TTF (MVIC, $Q_{tw,pot}$, VA, MEP/M_{max}, and SP) a two-way (3×3) repeated measures ANOVA was used to assess differences over time (PRE, POST_TBS, and POST_TTF) and between the TBS protocols (iTBS, cTBS, and sTBS

For the EMG variable and the MDF assessed during the TTF a two-way (2×2) repeated measures ANOVA was used to assess differences between the TBS protocol (iTBS, cTBS, and sTBS) during the TTF. The compound symmetry, or sphericity, was checked using the Mauchly's test. If significant main or interaction effects were observed, these were followed up by *post-hoc* Bonferroni-corrected pairwise comparisons. To verify the association between the duration of the fatiguing task and the fatiguing task mediated reduction of $Q_{tw,pot}$, Pearson's product moment correlation coefficient (r) was used to examine the bivariate relationships between the percentage difference in $Q_{tw,pot}$ from PRE to POST_TTF and TTF. The precision of estimates is indicated by the 95% confidence intervals (CIs). To verify the effect size and the amount of variance, partial eta-squared (η^2_p) was used. Values closed to 0.01 indicated a small effect, 0.06 a moderate effect, 0.14 a large effect. The significance level was set at $\alpha \leq .05$. Statistical analysis was conducted using IBM™ SPSS™ Statistics (version 26, IBM Corp., Somers, New York).

Results

Effects of theta burst stimulation on the time to task failure

Compared to the sTBS (173 ± 65 s), time-to-task failure (TTF) was longer for the iTBS (214 ± 68 s, +41%, P = .006) and shorter for the cTBS (142 ± 51 s, -31%, P = .025) (Figure 3.2).

There was an effect of time (P < .001; η^2_p = .720), and interaction between time and TBS intervention P = .036; η^2_p = .863) on RMS/M_{max} of the electromyographic (EMG) signal during the TTF (Figure 3.3A). The increase in RMS/M_{max} between the first and the last 10 s intervals was greater for cTBS (+328%) when compared to iTBS (+97%, P = .022), but not when compared to sTBS (+250%, P = .952). The change in RMS/M_{max} was significantly different only between iTBS and cTBS at the end (197 ± 124 *versus* 428 ± 738 %; P = .047) of the TTF.

Similarly, there was an effect of time (P < .001; η^2_p = .118), and an interaction between time and TBS intervention (P < .001; η^2_p = .014) on MDF (Figure 3.3B). The decrease in MDF between the first and the last 5 s intervals of the TTF was greater for cTBS (-30.29%) when compared to iTBS (-24.17%, P = .050), but not when compared to sTBS (-25.95%, P = .264). The change in MDF was significantly different only between iTBS and cTBS at the beginning (68 ± 9 *versus* 67 ± 11 Hz; P = .041) and at the end (52 ± 10 *versus* 46 ± 11 Hz; P = .009) of the TTF (Figure 3.3B).

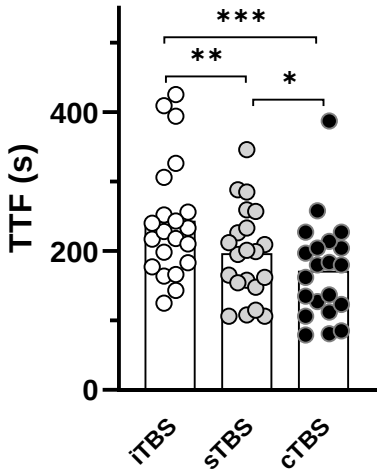

Figure 3.2. *Individual and mean data of the time-to-task failure (TTF) for each protocol. sTBS, sham theta burst stimulation; iTBS, intermittent theta burst stimulation; cTBS, continuous theta burst stimulation. * P ≤ .05, ** P ≤ .01, *** P ≤ .001 (n = 20).*

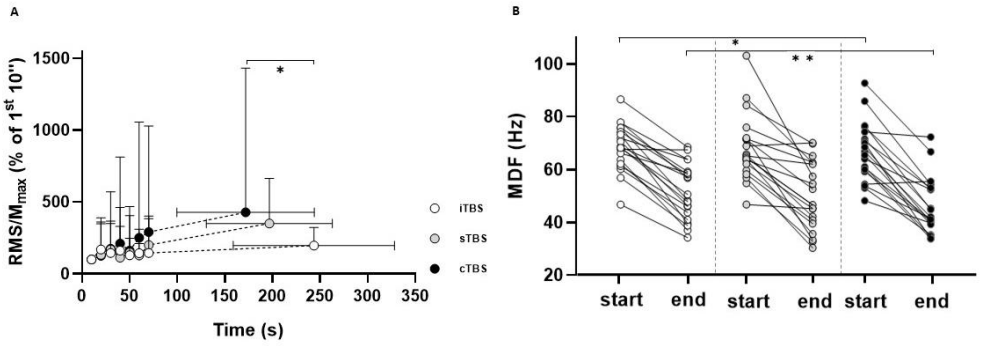

Figure 3.3. *Root mean square (RMS) of the electromyographic data normalised for the maximal M-wave (M_{max}) in the percentage of the first 10 seconds interval where the maximal end interval of iTBS significantly differs from cTBS (A) and individual data of the median power frequency (MDF) (B) for each protocol during the time-to-task failure (TTF) where iTBS and cTBS differ both over the first and last 5 seconds. sTBS, sham theta burst stimulation; iTBS, intermittent theta burst stimulation; cTBS, continuous theta burst stimulation. * P ≤ .05, ** P ≤ .01 (n = 20).*

Effects of theta burst stimulation on force and central fatigue

Maximal Voluntary Isometric Contraction

Figure 3.4A shows the MVIC force before, after each TBS protocol, and immediately after each TTF task. There was an effect of time (P < .001; η^2_p = .719), and an interaction between time and TBS intervention (P = .034; η^2_p = .126). MVIC significantly decreased from PRE to POST_TBS for cTBS only (from 60 ± 27 to 55 ± 26 N·m, P = .002), although MVIC for cTBS at POST_TBS was lower than sTBS (P = .014) but not different from iTBS (P = .143). MVIC decreased for the three TBS paradigms from PRE to POST_TTF (~ -20%, P < .001) and from POST_TBS to POST_TTF (~ -15%, P < .001). However, MVIC at POST_TTF was similar among the three paradigms (P = .977).

Voluntary Activation

Figure 3.4B shows the VA before, after each TBS protocol, and immediately after each TTF task. There was an effect of time (P < .001; η^2_p = .582), and an interaction between time and TBS intervention (P < .001; η^2_p = .292) on VA. VA significantly decreased from PRE to POST_TBS for cTBS only (from 96% ± 3% to 93% ± 4%, P = .004), although VA for cTBS differed from iTBS (P = .002) and not from sTBS (P = .065) in POST_TBS. VA significantly decreased from PRE to POST_TTF for iTBS (from 97% ± 3% to 94% ± 6%, P = .015), sTBS (from 97% ± 3% to 87% ± 10%, P < .001), and for cTBS (from 96% ± 3% to 78% ± 17%, P < .001). The decrease in VA was greater for cTBS (-18% ± 18%) when compared to sTBS (-9% ± 9%, P = .023), and iTBS (-3% ± 5%, P < .001). However, VA in POST_TTF significantly differed for iTBS (P = .015), sTBS, and cTBS paradigms (both P < .001) compared to POST_TBS.

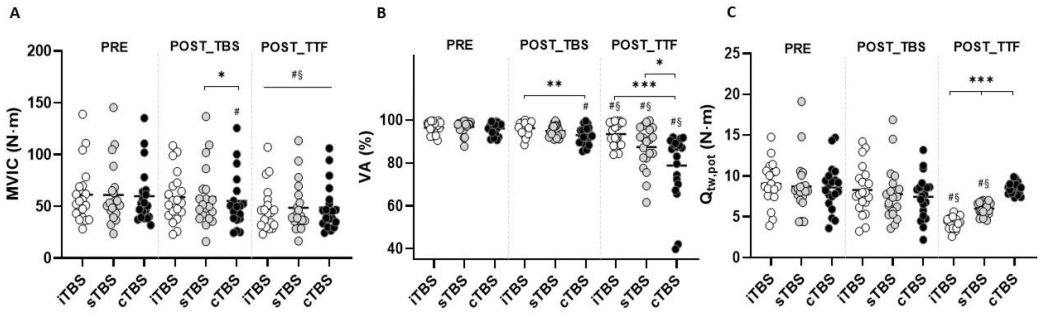

Figure 3.4. *Individual and mean data for MVIC (A), VA (B), and $Q_{tw,pot}$ (C) before (PRE), after TBS (POST_TBS), and after the fatiguing task for each TBS protocol. MVIC, maximal voluntary isometric contraction; $Q_{tw,pot}$, potentiated twitch force at rest; VA, voluntary activation; sTBS, sham theta burst stimulation; iTBS, intermittent theta burst stimulation; cTBS, continuous theta burst stimulation. # from PRE P ≤ .05; § in POST_TTF compared to POST_TBS P ≤ .05; ** P ≤ .01, *** P ≤ .001 (n = 20).*

Effects of theta burst stimulation on the peripheral function

Figure 3.4C shows the $Q_{tw,pot}$ before, after each TBS protocol, and immediately after each TTF task. There was an effect of time (P < .001; η^2_p = .436), and an interaction between time and TBS intervention (P < .001; η^2_p = .666) on

$Q_{tw,pot}$. $Q_{tw,pot}$ showed significant changes between PRE and POST_TBS for iTBS (from 9.0 ± 2.6 to 8.3 ± 3.0 N·m, P = .028), sTBS (from 8.7 ± 3.4 to 7.8 ± 3.3 N·m, P < .001), and cTBS (from 8.6 ± 2.8 to 7.4 ± 2.7 N·m, P < .001), although the drop was not significantly different among the three paradigms. However, after comparing the interquartile ranges, it became evident that these changes were well within the realm of variability between the 25th and 75th percentile. Consequently, although significant, they were not attributed to the TBS as a meaningful result, but rather to the participant's variability (Van De Ruit *et al.*, 2015).

$Q_{tw,pot}$ significantly decreased from PRE to POST_TTF for iTBS (from 9.0 ± 2.6 to 4.3 ± 0.8 N·m, P < .001), and sTBS (from 8.7 ± 3.4 to 5.9 ± 0.8 N·m, P = .001) but not for cTBS (from 8.6 ± 2.8 to 8.4 ± 0.7 N·m, P = .801). However, the reduction in $Q_{tw,pot}$ was greater for iTBS (-47% ± 22%) when compared to sTBS (-23% ± 28%, P < .001) and cTBS (9% ± 40%, P < .001) However, $Q_{tw,pot}$ from POST_TBS to POST_TTF significantly decreased for iTBS, sTBS (P < .001), but not cTBS (P = .093).

Correlational analysis revealed a negative correlation between the percentage difference in $Q_{tw,pot}$ from PRE to POST_TTF and TTF (r = -.397, P = .002) (Figure 3.5).

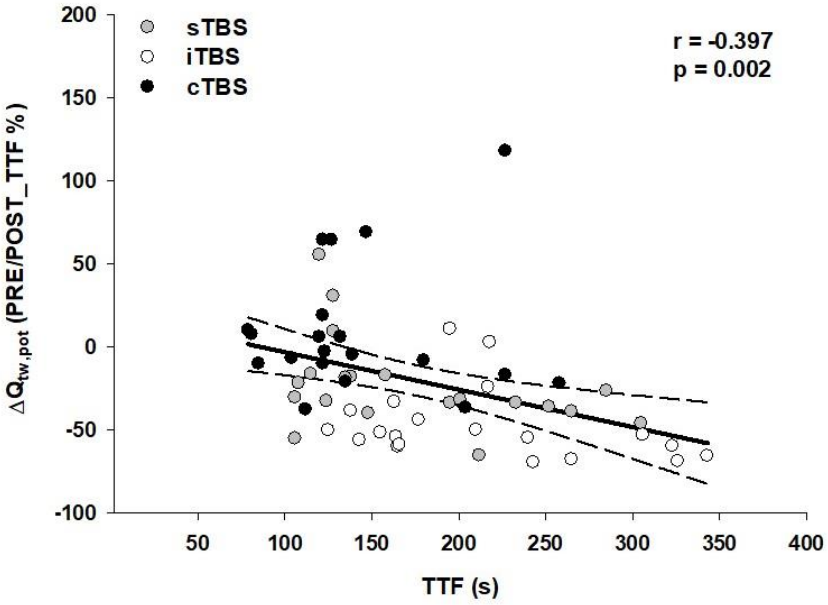

Figure 3.5. *Correlations between the difference from PRE to POST_TTF of potentiated twitch force ($\Delta Q_{tw,pot}$) and the time-to-task failure (TTF) with data of sham theta burst*

stimulation (sTBS), intermittent theta burst stimulation (iTBS), and continuous theta burst stimulation (cTBS) in the POST_TTF.

Effects of theta burst stimulation on corticospinal excitability and inhibition

Corticospinal excitability

Figures 3.6A,B show the MEP/M_{max} during the MVIC and the sustained contraction at 20% isoEMG before, after each TBS protocol, and immediately after each TTF task. During the MVIC, there was an effect of time ($P < .001$; $\eta^2_p = .387$), and an interaction between time and TBS intervention ($P = .011$; $\eta^2_p = .156$) on MEP/M_{max}. MEP/M_{max} significantly increased from PRE to POST_TBS for cTBS only (from 0.49 ± 0.31 to 0.77 ± 0.53, $P = .047$), although the VA for cTBS differed from iTBS ($P = .019$) and not from sTBS condition ($P = .533$) in POST_TBS. MEP/M_{max} significantly increased from PRE to POST_TTF for iTBS (from 0.51 ± 0.32 to 0.69 ± 0.21, $P = .002$), sTBS (from 0.47 ± 0.32 to 0.97 ± 0.51, $P < .001$), and for cTBS (from 0.49 ± 0.31 to 1.2 ± 0.5, $P < .001$). The MEP/M_{max} was lower for iTBS (53% ± 50%) when compared to cTBS (220% ± 226%, $P = .006$), and sTBS (148% ± 142%, $P = .013$) at POST_TTF. However, MEP/M_{max} increased from POST_TBS to POST_TTF for iTBS ($P = .019$) and cTBS ($P = .038$), but not for sTBS ($P = .082$) (Figure 3.6A).

During the sustained contraction at 20% isoEMG, there was an effect of time effect ($P < .001$; $\eta^2_p = .178$), and an interaction between time and TBS intervention ($P = .048$; $\eta^2_p = .057$) on MEP/M_{max}. MEP/M_{max} did not significantly change from PRE to POST_TBS for iTBS (from 0.48 ± 0.21 to 0.39 ± 0.26, $P = .263$), cTBS (from 0.55 ± 0.34 to 0.73 ± 0.88, $P = .276$), and sTBS (from 0.55 ± 0.39 to 0.64 ± 0.60, $P = .456$). Moreover, MEP/M_{max} was not significantly different among the three paradigms in POST_TBS. MEP/M_{max} significantly increased from PRE to POST_TTF for iTBS (from 0.48 ± 0.21 to 0.60 ± 0.31, $P = .009$), for cTBS (from 0.55 ± 0.34 to 0.87 ± 0.36, $P < .001$), but not for sTBS (from 0.55 ± 0.39 to 0.69 ± 0.31, $P = .076$). Furthermore, the increase in MEP/M_{max} from PRE to POST_TTF was greater for cTBS (104% ± 121%), when compared to sTBS (69% ± 109%, $P = .039$), and iTBS (25% ± 26%, $P = .017$). However, MEP/M_{max} from POST_TBS to

POST_TTF significantly differed for iTBS (P = .027), but not cTBS (P = .478), and sTBS (P = .529) (Figure 3.6B).

Corticospinal inhibition

Figures 3.6C,D show the MEP/M_{max} during the MVIC and the sustained contraction at 20% isoEMG before, after each TBS protocol, and immediately after each TTF task. During the MVIC there was an of time (P < .001; η^2_p = .548), and an interaction between time and TBS intervention (P = .013; η^2_p = .112) on SP. SP significantly increased from PRE to POST_TBS for cTBS only (from 120 ± 41 to 130 ± 46 ms, P = .013), although the increase was not significantly different among the three paradigms in POST_TBS. SP significantly increased from PRE to POST_TTF for iTBS (from 121 ± 39 to 130 ± 39 ms, P < .001), sTBS (from 121 ± 36 to 145 ± 48, P < .001), and for cTBS (from 120 ± 41 to 164 ± 44 ms, P < .001). The increase in SP was greater for cTBS (42% ± 37%) when compared to sTBS (20% ± 19%, P < .001), and iTBS (8% ± 9%, P = .019) However, SP from POST_TBS to POST_TTF significantly increased for iTBS (P = .050), cTBS (P = .001), and sTBS (P = .040) (Figure 3.6C).

During the sustained contraction at 20% isoEMG, there was an effect of time (P < .001; η^2_p = .704), and an interaction between time and TBS intervention (P = .001; η^2_p = .225) on SP. SP significantly increased from PRE to POST_TTF for cTBS only (from 109 ± 39 to 126 ± 55 ms, P = .014), although the increase for cTBS differed from iTBS (P = .008) and not from sTBS (P = .185) in POST_TBS. SP significantly increased from PRE to POST_TTF for iTBS (from 110 ± 42 to 130 ± 25 ms, P < .001), sTBS (from 114 ± 45 to 160 ± 42, P < .001), and for cTBS (from 109 ± 39 to 162 ± 27 ms, P < .001). The increase in SP was lower for iTBS (28% ± 33%) when compared to sTBS (56% ± 56%, P = .046, and cTBS (63% ± 55%, P = .001) However, MEP/M_{max} from POST_TBS to POST_TTF significantly increased for iTBS, sTBS, and cTBS (all P < .001) (Figure 3.6D).

Table 3.2	ΔPRE/POST_1 (n = 20)			ΔPRE/POST_2 (n = 20)			ΔPOST_1/POST_2 (n = 20)		
	iTBS	sTBS	cTBS	iTBS	sTBS	cTBS	iTBS	sTBS	cTBS
MVIC (Δ%)	-2.8 ± 11.2	-3.7 ± 8.9	-8.0 ± 11.5	-22.1 ± 6.1	-20.2 ± 8.5	-17.9 ± 8.2	-18.5 ± 14.9	-16.8 ± 9.1	-9.3 ± 16.4
VA (Δ%)	-0.28 ± 3.17	-1.8 ± 3.9	-3.1 ± 4.3	-3.0 ± 5.1	-9.5 ± 9.1	-17.8 ± 18.1	-2.7 ± 4.6	-7.7 ± 10.7	-14.7 ± 15.9
$MEP/M_{max\ MVIC}$ (Δ%)	13.9 ± 83.9	74.5 ± 172.8	118.2 ± 202.2	53.0 ± 50.4	148.3 ± 142.3	219.9 ± 226.1	187.3 ± 470.5	196.5 ± 267.8	119.1 ± 156.3
$MEP/M_{max\ 20\%isoEMG}$ (Δ%)	-3.2 ± 71.1	26.1 ± 78.2	53.5 ± 149.8	25.3 ± 25.9	69.4 ± 108.6	103.7 ± 120.7	74.2 ± 120.4	58.8 ± 96.8	90.1 ± 118.8
SP_{MVIC} (Δ%)	-2.3 ± 30.4	6.6 ± 40.6	8.0 ± 13.2	8.2 ± 9.3	20.4 ± 18.5	42.1 ± 37.3	20.3 ± 39.3	23.2 ± 37.2	34.2 ± 40.3
$SP_{20\%isoEMG}$ (Δ%)	-5.2 ± 23.8	-1.4 ± 23.3	14.7 ± 28.9	27.9 ± 33.2	55.6 ± 55.9	63.5 ± 54.5	39.7 ± 42.3	139.5 ± 404.9	55.6 ± 81.5
$Q_{tw,pot}$ (Δ%)	-9.3 ± 15.0	-11.0 ± 10.6	-13.9 ± 11.0	-47.4 ± 21.9	-23.4 ± 27.9	9.3 ± 39.9	-39.4 ± 30.4	-12.2 ± 35.2	32.2 ± 65.8

Table 3.2. *Differences among time intervals. POST_TBS, post theta burst stimulation; POST_TTF, post time-to-task failure; MVIC, maximal voluntary isometric contraction; VA, voluntary activation; MEP/M_{max}, area under the curve of the motor evoked potential normalised for the maximal M-wave; SP, silent period; $Q_{tw,pot}$, potentiated twitch force; sTBS, sham theta burst stimulation; iTBS, intermittent theta burst stimulation; cTBS, continuous theta burst stimulation.*

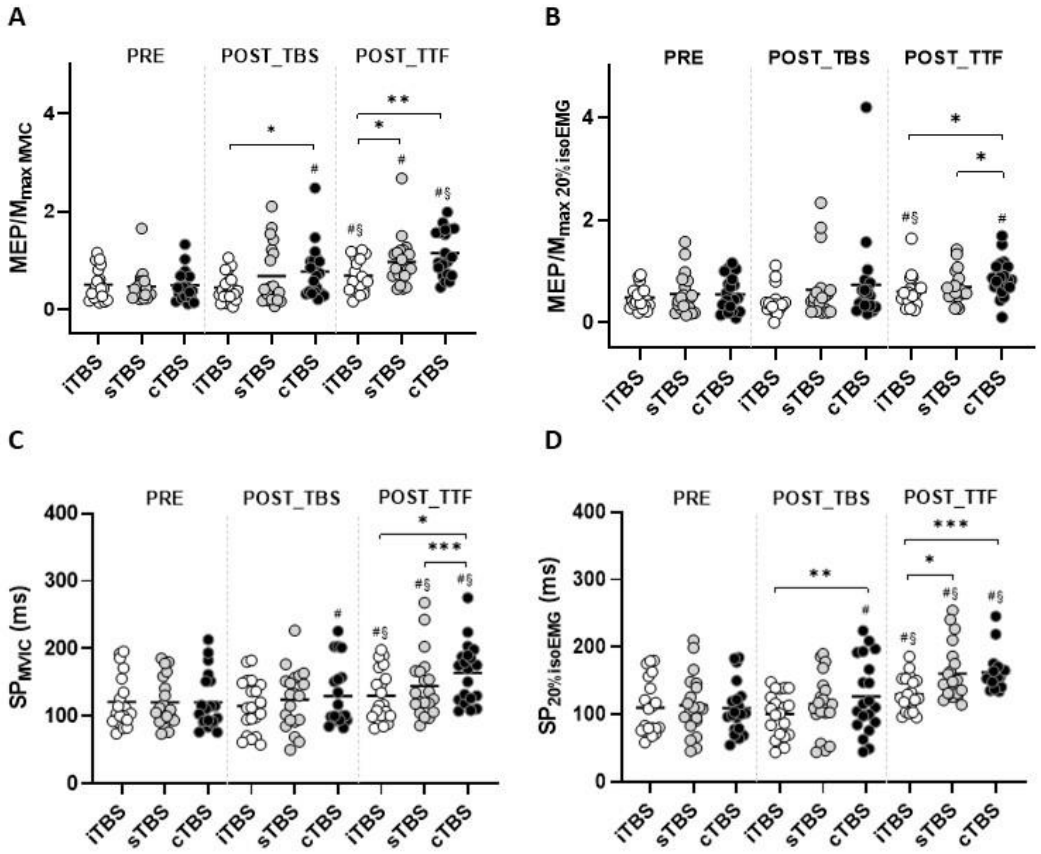

Figure 3.6. *Individual and mean data for the amplitude (A, B) and the silent period (C, D) of the motor evoked potentials before (PRE), after the TBS (POST_TBS), and after (POST_TTF) the fatiguing task for each TBS protocol during MVIC and 20% isoEMG contraction. MEP/M_{max}, area under the curve of the motor evoked potential normalised for the maximal M-wave; SP, silent period; MVIC, maximal voluntary isometric contraction; sTBS, sham theta burst stimulation; iTBS, intermittent theta burst stimulation; cTBS, continuous theta burst stimulation.*

*$^{\#}$ from PRE P ≤ .05; § POST_TTF compared to POST_TBS P ≤ .05; * P ≤ .05, ** P ≤ .01, *** P ≤ .001 (n = 20).*

Discussion

This study aimed to compare the effects of facilitatory iTBS versus inhibitory cTBS delivered to the primary motor cortex on exercise performance, neuromuscular fatigue and excitability/inhibition of the corticospinal pathway in an unfatigued (i.e. fresh) and fatigued muscle. We demonstrate that in an unfatigued muscle, while iTBS had no effect compared to sham, cTBS attenuated maximal muscle force and central drive, increased intracortical inhibition and corticospinal excitability. In addition, while iTBS augmented performance of the fatiguing task, it had no effect on central fatigue, corticospinal excitability and $GABA_B$ mediated intracortical inhibition. In contrast, cTBS reduced performance of the isometric task, increased development of central fatigue, increased corticospinal excitability and $GABA_B$ mediated intracortical inhibition. These outcomes suggest that facilitatory TBS augment exercise performance that appear to be independent of changes in neural drive and corticospinal mechanisms, whilst inhibitory TBS attenuate exercise performance through an exacerbation in the development of central fatigue and intracortical inhibition.

The modulatory effects of TBS in an unfatigued muscle

Both facilitatory and inhibitory TBS protocols are known to induce lasting changes at the site of stimulation and nearby areas (Chervyakov *et al.*, 2015). A theoretical model has been accepted by the current literature for targeting synaptic activity after TBS. This model suggests that the stimulation pattern of iTBS and cTBS can choose between facilitation and depression, respectively, which are constantly oscillating phenomena during the stimulation. In fact, after the application of TBS, both glutamatergic and GABAergic pathways are involved within local excitation–inhibition networks, causing a postsynaptic Ca^{2+} influx in higher or lower concentrations to determine the expression of receptors, growth factors, the choice between LTP or LTD, respectively, and the production of synaptic long-term changes (Huang *et al.*, 2017; Rounis & Huang, 2020).

In the current study looked at the modulatory effects of iTBS and cTBS on the neuromuscular function as well as corticospinal excitability in a relatively unfatigued muscle. Contrarily to our hypothesis, we did not observe any effects on

these parameters induced by facilitatory TBS. This suggests that iTBS did not increase the capacity of corticospinal outputs to appropriately drive motoneurons at maximal voluntary force-generating capacity. However, a part of these results diverged from the study of Huang et al., 2005 (Huang *et al.*, 2005) that obtained an enhancement on the corticospinal excitability on the first dorsal interosseous muscle after iTBS. This difference could be due to differences in target muscle between studies (Martin *et al.*, 2006*b*). In addition to genetic factors and brain state (Cheeran *et al.*, 2008; Suppa *et al.*, 2016), the time interval between the end of TBS and corticospinal measurements is also a consideration. Indeed, our measurements were taken immediately at the end of each TBS protocol. This may not have allowed for sufficient time for TBS induced neuroplastic modulations (Iezzi *et al.*, 2008). The null effect in the early time point after facilitatory TBS was reported in a recent meta-analysis which have in fact described iTBS not as effective in modulating neural circuits as cTBS, unless there is a latency of 20-30 minutes post-stimulation (Chung *et al.*, 2016).

In agreement with our hypothesis, our data showed a decrement in maximal force and central drive and an increased intracortical inhibition after the inhibitory cTBS. It seems reasonable that motor cortical depression through cTBS may have stimulated LTD-like plasticity. This event would have favoured reduction in the efficacy of neuronal synapses and, consequently, in the rate of action potentials fired by the motor neurons, causing a limitation in the amount of force that the motor unit can generate (Huang *et al.*, 2005). In support of this, studies on animal and human models traced the augmented inhibition to an increase in the local concentration of the inhibitory neurotransmitter GABA (Stagg *et al.*, 2009; Romero *et al.*, 2022), and, in addition, evidence from magnetic resonance spectroscopy demonstrated an increase in GABA in the human motor cortex after cTBS to induce inhibition phenomena (Stagg *et al.*, 2009).

However, in contrast to our hypothesis, we also found inhibitory TBS to increase corticospinal excitability. There is evidence to in fact demonstrate that cTBS first induces a small potentiation (Huang *et al.*, 2005; Gamboa *et al.*, 2010*a*), before reversing into depression. The underlying mechanism of this contrary outcome is not clear, although the work of Fung et Robinson, 2014 (Fung &

Robinson, 2014) proposes a temporal dissociation in the protein cascade pathway, where plasticity signalling and expression can occur at distinct time points but influence each other. Our results are also in agreement with the reversal of effects of contractions after the application of neuromodulatory protocols such as TBS (Gentner *et al.*, 2008; Huang *et al.*, 2011). Indeed, the current investigation involved multiple contractions which may have primed the corticospinal pathway prior to application of TBS (Fung & Robinson, 2014).

Central effects of facilitatory TBS in a fatigue muscle

The iTBS protocol resulted in a longer TTF compared to both the sTBS and the cTBS. iTBS can enhance neural drive and the excitation of pyramidal neurons; thereby improving the efficacy of the motor neuronal recruitment, the motor neuronal discharge towards the muscle, and the duration of the task (Di Lazzaro *et al.*, 2008; Giboin *et al.*, 2016). Unexpectedly, the improvement in exercise performance was not paralleled by neuromuscular and corticospinal (excitability/inhibition) changes with iTBS. To the best of our knowledge, no previous studies have investigated the aftereffects of facilitatory TBS on fatigue-related corticospinal and neuromuscular variables. However, multiple blocks of iTBS stimulation have been proposed to bring more improvements in motor cortical excitability than single application (Yu *et al.*, 2020), thus, we are led to think that the number of TBS blocks and the latency between them might influence its effect. A further explanation for the gap between exercise performance and the neuromuscular and corticospinal response could come from a different location of the central networks responsible of the motor drive that cannot be completely assessed with the application of non-invasive stimulation tools (Gandevia, 2001).

Central effect of inhibitory TBS in a fatigued muscle

The cTBS protocol resulted in a shorter TTF compared to both the sTBS and the iTBS. In parallel, the muscle contractile properties were not largely impaired, and the level of peripheral fatigue proved to be low due to the early termination of the task and preservation of the muscle.

Although there is currently no evidence on the effects of cTBS on neuromuscular fatigue, we can speculate that since the continuous repeated bursts act on a different neuronal pool compared to iTBS, it is likely to generate an opposite response. Thus, the inhibitory stimulation should limit Ca^{2+} release and, therefore, in an inhibition of both the cortical circuits and the motor output during the fatiguing task. Indeed, MVIC post cTBS decreased as much as in the other two conditions. This decline, therefore, cannot be related to the TBS type. This was likely an outcome of our experimental design that required participants to perform a sustained submaximal isometric contraction until force reached the same relative value in all conditions. However, we demonstrate that with cTBS, the development of both central fatigue and $GABA_B$ mediated intracortical inhibition increased. As the principal inhibitory neurotransmitter in the brain, GABA has been implicated in cortical plasticity by attenuating the glutamatergic excitatory transmission and inducing LTD-like effect that transpire to the periphery (Cash *et al.*, 2016). Moreover, GABA is also linked to a maximal inhibition of Ca^{2+} current that negatively influences the feedback system leading to weaker cortical activity and a reduced voluntary activation (Klomjai *et al.*, 2015). A longer SP duration can also explain the increase in intracortical inhibition (Taylor *et al.*, 1996; Gandevia, 2001; McNeil *et al.*, 2011). According to Gilio *et al.*, 2007, the lengthening of the SP after cTBS could be elucidated by an inhibition generated through a temporal summation of presynaptic stimuli depending on the time-course delivery of stimulation (Romeo *et al.*, 2000). The sum of many subthreshold high frequency stimuli closed together, as happened for cTBS, coupled with a higher level of central fatigue, could have led to a stronger inhibitory postsynaptic potential and a longer inhibition.

Unexpectedly and in contrast to our hypothesis, corticospinal excitability increased after inhibitory cTBS. Similar findings were obtained after the duration of cTBS was doubled (Gamboa *et al.*, 2010*b*). However, the current study provides first evidence of an increase in corticospinal excitability after a single application. We are also not able to completely exclude the consequence of prior muscle contraction on the subsequent effects of TBS. Indeed, there is some evidence to suggest that the history of the neural network influences the regulation of corticospinal excitability and plasticity. Hence, in the presence of previously

excitatory brain activity then the inhibitory effect of cTBS is attenuated, while without prior voluntary activation the effect of the cTBS could skew the system towards depression (Gentner *et al.*, 2008; Brighina *et al.*, 2010).

Development of peripheral fatigue

Peripheral fatigue manifested after the fatiguing task and the drop from unfatigued to fatigued condition was proportional to the duration of the TTF. For example, $Q_{tw,pot}$ decreased to a greater extent after the fatiguing contraction when iTBS was applied simply because TTF was longer for this session causing the muscle to fatigue to a greater extent. The opposite was seen with cTBS where the magnitude of peripheral fatigue was lower due to the early termination of the task and preservation of the muscle The development of peripheral fatigue was in line with previous studies that showed muscle contractile impairments after sustained submaximal isometric contractions [e.g., (Hunter *et al.*, 2006; Yang *et al.*, 2009)]. During prolonged submaximal exercises, peripheral fatigue is mediated by a lower efficacy of the contractile properties and changes in metabolic and biochemical processes within the muscle (Tornero-Aguilera *et al.*, 2022). It is likely that during the sustained submaximal contractions, intramuscular P_i accumulation reduced the free Ca^{2+} available for release from the sarcoplasmic reticulum (Allen & Westerblad, 2001). This, coupled with increasing recruitment of muscle fibres to counteract the considerable development of fatigue, lead to disrupted skeletal muscle contractile processes (Allen *et al.*, 2008).

Limitations of the study

Although our findings on the application of TBS in the context of fatiguing exercise are promising, there are some limitations to consider. First, the time interval between the end of TBS and the neuromuscular assessment where our study design included the evaluation within one minute from the end of the stimulation. This may not have allowed the desired effects at the neuromuscular and corticospinal level to be recorded. Second, fatigue-related changes in brain activity and corticospinal excitability response also include non-motor cortical regions that

can only be monitored using imaging techniques. Thus, the measurements we applied may not have fully targeted some system behaviors in response to TBS.

Conclusion

Our study showed that facilitatory iTBS increases exercise performance independently of changes in neural drive and corticospinal activity, whilst inhibitory TBS attenuates exercise performance through an exacerbation of central fatigue and intracortical inhibition. Whilst further studies are required to elucidate the mechanisms underlying these observations, this work provide the premise for exploring the potential of TBS in helping to modulate motor performance rather than neuromuscular fatigue. Furthermore, this study directs future research on the potential application of TBS in disease populations where fatigue and motor limitations represent some of the most common symptoms to be improved.

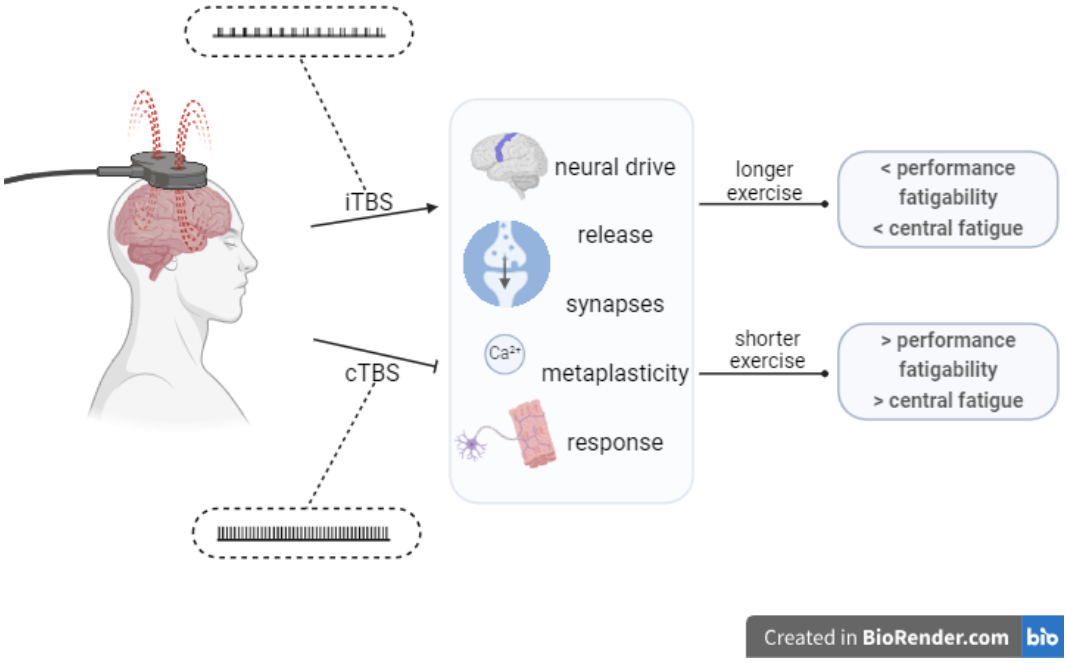

Figure 3.7. *The illustration presents how the paradigms of intermittent theta burst stimulation (iTBS), and continuous theta burst stimulation (cTBS) positively or negatively modulate cortical activity, the release of neurotransmitters and Calcium ions, the status of synapses, and the motoneuronal output, rather than the peripheral muscular structures, to determine the level of performance fatigability and central fatigue.*

Study II - The cross-over effect of theta-burst stimulation: another strategy to modulate exercise performance

Based on preliminary data

Camilla Martignon, Chiara Barbi, Gianluca Vernillo, Simranjit K Sidhu, Mehran Emadi Andani, Federico Schena, Massimo Venturelli

Abstract

Interhemispheric connections allow the exercise induced-fatigue to be transferred from a muscle group to a homologous or heterologous limb at rest, but little is known about the cross-over effect of theta-burst stimulation (TBS) on neuromuscular fatigue and its correlates. This study investigated the effects of TBS applied on the right primary motor cortex on neuromuscular parameters and exercise performance of the right arm.

Ten young healthy participants randomly completed three TBS sessions (facilitatory/intermittent: iTBS; inhibitory/continuous: cTBS, and sham: sTBS) and a fatiguing sustained contraction at 35% of the maximal voluntary isometric contraction (MVIC) of the elbow flexor. Neuromuscular function evaluations were performed with transcranial magnetic stimulation, cervicomedullary stimulation, and peripheral stimulations at baseline, after TBS, and after the fatiguing task on the contralateral arm.

The time-to-task failure was shorter for cTBS (115±30s) and longer for iTBS (147±39s) compared with sTBS (136±39s) ($P<.05$).

cTBS, after stimulation, deteriorated MVIC, voluntary activation (VA), motor-evoked potential, and silent period (SP) ($P<.05$), while iTBS did not cause any change.

In the expected fatigued status, only twitch response decreased in all TBS sessions ($P=.025$). However, no changes appeared for MVIC, VA, MEP, CMEP, and SP after the fatiguing task nor during the recovery ($P>.05$).

The findings suggest that the facilitatory and inhibitory effects given by iTBS and cTBS are transmitted from one hemisphere to the other, increasing and shortening the amount of exercise performance, respectively. However, although inhibitory TBS attenuates central drive and corticospinal mechanisms, TBS does not appear to influence neuromuscular fatigue or recovery after exercise.

Introduction

Brain interactions are complex. The model of interhemispheric cross-talk proposed a continuous interconnection between the right and left hemispheres where functional cooperation and competition reign. This phenomenon is attributed to the spreading of stimuli between the two hemispheres throughout transcallosal or spinal connectivity (Carson *et al.*, 2004; Carroll *et al.*, 2006). In the field of neuromuscular fatigue (i.e., a decline induced by exercise in the ability of a muscle to generate force (Gandevia, 2001) the effect of interhemispheric connection comes out when exercise-induced fatigue in a muscle group transiently impairs neuromuscular function in another homologous or heterologous limb at rest (Halperin *et al.*, 2015). Interestingly, when peripheral fatigue does not appear within the resting muscle and force expression decreases, only central mechanisms should play a decisive role in influencing neuromuscular function and cross-over fatigue phenomenon (Doix *et al.*, 2013). Indeed, several evidence documented a transferred fatigue response from the group III/IV afferent fibers of the exercising muscle to the central circuits that trigger inhibiting signals in muscles not directly involved in the task (Amann *et al.*, 2013; Kennedy *et al.*, 2015). For instance, cross-over fatigue has been detected in heterologous muscle groups after a unilateral lower limb task that compromised force production and motor performance (Halperin *et al.*, 2014). Other evidence found neither a significant force impairment nor alterations of the determinant of central fatigue in the contralateral homologous muscle following a fatiguing protocol (Todd *et al.*, 2003; Aboodarda *et al.*, 2016). This contrasting finding suggests that unilateral exercise-induced fatigue could increase the responsiveness of the neural circuitries rather than inducing cross-over central fatigue (Todd *et al.*, 2003; Halperin *et al.*, 2014). However, to our knowledge, there is no evidence about the neuromodulatory effect of the cortical area of the resting upper limb on the homologous contralateral muscle subjected to exercise in terms of exercise performance, central fatigue, and corticospinal responses. The spreading of a newer form of repetitive transcranial magnetic stimulation (rTMS), the theta burst paradigm, can induce positive or negative changes in cortical activity by facilitatory (intermittent theta burst stimulation [iTBS]) or inhibitory (continuous theta burst stimulation [cTBS]) paradigms. In addition, TBS was effective in

verifying long-term potentiation/depression-like long-lasting after-effects in both stimulated and non-stimulated cortices (Di Lazzaro *et al.*, 2008; Suppa *et al.*, 2008). Indeed, multiple cortical areas (e.g., primary, secondary, and association) are implicated in signal alterations, and, most of all, both sides of the motor cortex seem to exhibit substantial changes during a task. Nevertheless, it is still unclear if and to what extent the modulation of cortical activity and mainly the onset of neuromuscular fatigue from exogenous stimulation (e.g., TBS) could be transferred from the ipsilateral primary motor cortex (M1) to the contralateral area of the exercising limb.

Therefore, this study aimed to quantify the effects of facilitatory and inhibitory TBSs delivered to the M1 (right hemisphere) on exercise performance, neuromuscular fatigue, and excitability/inhibition of the corticospinal pathway of the ipsilateral elbow flexor (right muscle). The tested hypothesis was that alterations induced by iTBS and cTBS within the brain circuits (right hemisphere) could improve and deteriorate, respectively, the neuromuscular determinants, exercise performance, and the corticospinal parameters in the exercising ipsilateral elbow flexor (right muscle).

Methods

Ethical approval

The study received institutional ethical approval from the University of Verona Research Ethics Committee (12.R1_2021_em/2023) and was conducted according to all aspects of the Declaration of Helsinki, apart from registration in a database. Participants provided written, informed consent to volunteer for the study.

Participants

Ten young healthy male subjects (age: 24 ± 1 yr; height: 180 ± .08 cm; body mass: 79 ± 11 kg) volunteered and participated in this study (Table 2). The sample size was determined based on the effect of the TBS protocols on exercise performance related to our previous study, with an α level of 0.05 and a required power (1-β) of 0.80. Eligibility criteria were absence of any neurological or cardiovascular disease; and negative outcome to the TMS questionnaire (Rossi *et al.*, 2021). Participants were instructed to avoid the consumption of caffeine on the

day of the experiment and avoid performing any strenuous exercise during the 48 hours before testing.

Study design

The study consisted of 4 non-consecutive days of examination that were held at the same time of day for each participant to control for within-participant diurnal variation (Ly *et al.*, 2016). On day 1, participants underwent an interview and filled out TMS and physical activity questionnaires (Lee *et al.*, 2011; Rossi *et al.*, 2021). The handedness dominance was verified with the Waterloo Handedness Questionnaire (Steenhuis *et al.*, 1990). All the participants were right-handed. Then participants were familiarized with maximal and submaximal elbow flexor contractions with TMS, cervicomedullary stimulation, and electrical peripheral stimulation. On days 2-to-4, each participant was asked to carry out a standardized warm-up that consisted of three voluntary contractions at 10%, 30%, 50%, and one at 70% of their MVIC assessed during the familiarization. Each warm-up contraction was 5 s long, with 5 s of rest in between. The warm-up was followed by two 5-s isometric MVICs with 2 min of rest in between. Then participants performed eight neuromuscular evaluations (see "Neuromuscular function evaluation"). The first three neuromuscular evaluations (PRE) were interspersed by at least 1 minute of rest. The fourth neuromuscular evaluation (POST_TBS) was then performed before the fatiguing exercise and soon after the TBS protocol, randomly chosen among iTBS, cTBS, and sTBS to verify the effect of TBS alone without the influence of fatigue. Immediately at the end of the fatiguing sustained contractions at 35% of MVIC, the fifth neuromuscular evaluation was conducted (POST_1). Two, four, and eight minutes after the end of the task other neuromuscular evaluations were performed (POST_2, POST_3, POST_4) (Figure 4.1 A, B). Participants were highly motivated and instructed during all sessions to perform real MVICs, and strongly encouraged during the experiments by the investigators.

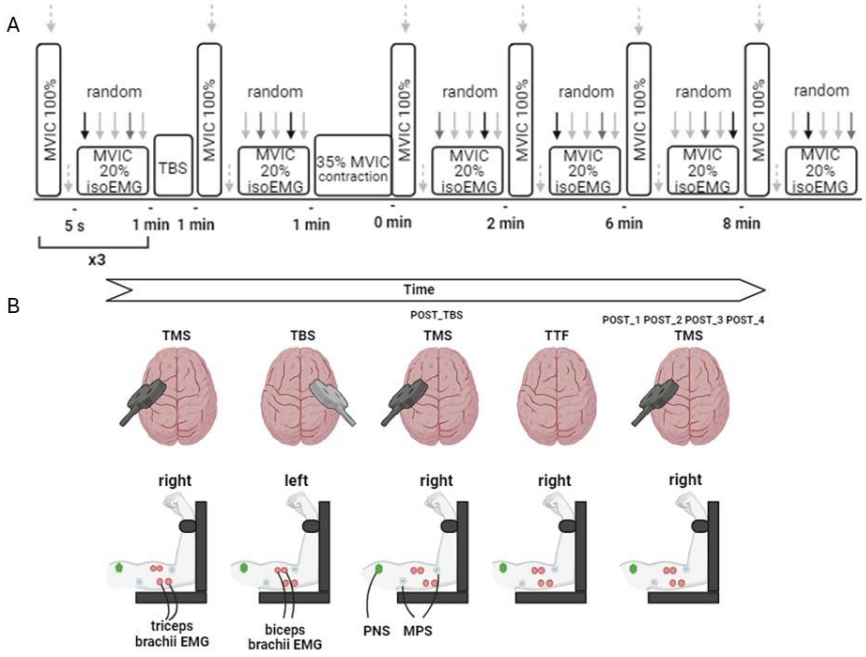

Figure 4.1. *Schematic (A) and illustrated (B) protocol design. MVIC, maximal voluntary isometric contraction; TBS, theta burst stimulation; Black arrows, peripheral nerve stimulation (PNS); Grey dashed arrows, motor point stimulation (MPS); Grey arrow, transcranial magnetic stimulation (TMS); Dark grey arrow, cervicomedullary stimulation; electromyography, EMG.*

Measurements

Force recording. Force was measured by a force transducer (DBBSE-100 kg, A2829; Applied Measurements Limited, Aldermaston Berkshire, UK) previously calibrated and connected to a high-speed acquisition system (PowerLab 16/30; ML880, ADInstruments, Colorado Springs, CO). The signal output was amplified (INT2-L, London Electronics Limited, Sandy, Bedfordshire, United Kingdom) and sampled at 2000 Hz. The participants were seated in an upright position with back support. Both shoulder and elbow joints of the right and left arms were flexed at 90° and the wrists were attached, via a strap and a rigid steel bar, to the force transducer (Figure 4.1, B). The force transducer was connected to a fixed bar and located in front of the participants' joints. The participant's dominant arm was placed in a U-shaped hold to ensure that the wrists were connected to the load cell that was to be pulled.

Surface electromyography. EMG of biceps brachii and triceps brachii on the right and left arm was recorded continuously using two pairs of self-adhesive surface electrodes (Ambu Neuroline 715, Ambu A/S, Ballerup, Denmark) in a bipolar configuration with a 20-mm interelectrode distance and the reference on the medial epicondyle of the humerus. Placement of EMG electrodes for biceps brachii (left and right arm) was on the line between the medial acromion and the cubital fossa at 1/3 the distance from the cubital fossa and placement for triceps brachii was at 50% of the line posterior crista of acromion-olecranon, 2 fingers medial to this line (Hermens *et al.*, 2000). Before the electrode's application, the skin was shaved, prepared by gentle local abrasion using an abrasive paste, and cleaned with an alcohol swab to minimize electrical impedance. The electrodes' location was marked on the skin with indelible ink to maintain the electrode's placement consistent across visits.

Acquisition of the EMG data was done using a Quad Bio Amp (ML135, ADInstruments, Australia), and all signals were integrated and digitalized with a computer-based data acquisition and analysis system (hardware: PowerLab 16/30; ML880, ADInstruments, Bellavista, NSW, Australia, and software: LabChart8, ADInstruments, Bellavista, NSW, CO Australia). The raw EMG signals, acquired at 2 kHz sampling frequency, were band-pass filtered (10-500 Hz).

Transcranial magnetic stimulation. Single TMS pulses were manually delivered to elicit MEP during voluntary contractions of the elbow flexor. The left M1 was stimulated by a magnetic stimulator (Magstim Rapid^2; The Magstim Company Ltd, Whitland, UK) with a 70 mm figure-8 air-cooled coil (Magstim; D70 Air Film Coil; The Magstim Company Ltd, Whitland, UK) to induce a posteroanterior current. To identify the vertex, participants wore a swim cap on which lines were drawn between the preauricular points and from nasion to inion. The coil was held tangentially on the scalp and moved 5 cm from the vertex and rotated 45 degrees from the midline. The exact hotspot for the biceps brachii was identified (and marked on the participants' cap) as the coil location and orientation evoking the largest peak-to-peak amplitude MEP using the lowest stimulation intensity from a biphasic current oriented posterior to anterior across the central sulcus during voluntary isometric contractions at 20% MVIC. The stimulation

intensity was defined during brief voluntary isometric contractions at 20% MVIC and increased until the amplitude of biceps brachii MEP matched 20% of M_{max} amplitude (Sidhu *et al.*, 2017). Stimulus intensity was determined at the start of each session. Mean stimulus intensities were 61% ± 8%, 58% ± 6%, and 61% ± 13% during iTBS, sTBS, and cTBS, respectively (all $P > .05$). TMS was always delivered once the participant had contracted to the appropriate force level and the force had stabilized during voluntary contractions. Participants were also instructed to re-contract to the prestimulus force level as quickly as possible after TMS delivery (Mathis *et al.*, 1998).

Spinal stimulation. The corticospinal tract was stimulated with single electrical stimuli of 500-μs duration via a constant-current stimulator (DS7A; Digitimer, Welwyn Garden City, Hertfordshire, UK). For BB, corticospinal responses [cervicomedullary motor-evoked potential (CMEP)] were evoked by electrical stimulation at the transmastoid level during voluntary contractions of EF. The electrical stimulus passed between two electrodes of 10-mm diameter (Meditrace 100) fixed to the skin over the left (cathode) and right (anode) mastoid processes. Biceps brachii was the main muscle of interest and stimulation intensity was determined for this muscle. The stimulus intensity was found during brief voluntary isometric contractions at 20% MVIC and increased until the amplitude of biceps brachii CMEPs matched 20% of M_{max} amplitude (Sidhu *et al.*, 2017). Mean stimulus intensities were 622 ± 227 mA, 694 ± 274 mA, and 432 ± 159 mA during iTBS, sTBS, and cTBS, respectively (all $P > .05$).

Theta burst stimulation. For each session, to identify the exact hotspot for TBS, the coil was held tangentially on the scalp and moved 5 cm from the vertex, and rotated 45 degrees from the midline in the cortical area of the right hemisphere (opposite to the hotspot for TMS). It was identified (and marked on the participants' cap) as the coil location and orientation evoking the largest peak-to-peak amplitude MEP using the lowest stimulation intensity from a biphasic current oriented posterior to anterior across the central sulcus during voluntary isometric contractions at 20% MVIC. TBS was delivered after the baseline neuromuscular function evaluations on the site drawn on the swim cap, by a magnetic stimulator (Magstim Rapid^2; The Magstim Company Ltd, Whitland, UK) with a 70 mm figure-

8 air-cooled coil (Magstim; D70 Air Film Coil; The Magstim Company Ltd, Whitland, UK), used in repetitive mode. Each TBS protocol consisted of bursts containing 3 pulses at 50 Hz and an intensity of 80% AMT repeated at 200 ms intervals. For the iTBS, a 2 s train of TBS was repeated every 10 s for a total of 190 s (600 pulses). For the cTBS, a 40 s train of uninterrupted TBS was given (600 pulses) (Huang *et al.*, 2005). sTBS was also delivered through the same coil to which a polystyrene panel was attached, and the intensity of stimulation was set to 80% AMT to provide some discharge noise to ensure participants remained blinded to the sham intervention.

Peripheral nerve stimulation. To evoke maximal M_{wave} (M_{max}) in biceps brachii, single electrical stimuli of 200-μs duration were delivered via a constant-current stimulator (DS7AH, Digitimer; Welwyn Garden City, UK) to the brachial plexus (M_{wave} measurements) and the motor point of biceps brachii (force measurements). For biceps brachii, stimuli were delivered to the brachial plexus trunk at Erb's point with a cathode (32 mm G0451, Globus Corporation, TV, Italy) in the supraclavicular fossa and the anode (32 mm G0451, Globus Corporation, TV, Italy) on the acromion. For motor point stimulation, the cathode (32 mm G0451, Globus Corporation, TV, Italy) was placed on the motor point (on the biceps brachii muscle belly, midway between the anterior edge of the deltoid and the proximal elbow crease with the elbow flexed at 90°), and the anode (32 mm G0451, Globus Corporation, TV, Italy) over the bicipital tendon.

Single stimuli were delivered incrementally in the relaxed muscle until M wave and twitch amplitudes plateaued. A stimulus intensity of 125% of the intensity to elicit M wave area (M_{max}) and maximal twitch responses was used throughout the rest of the experiment. Stimulus intensity was determined at the start of each session. The supramaximal stimulus intensity was 124 ± 22 mA, 126 ± 22, and 126 ± 31 for brachial plexus stimulation during iTBS, cTBS, and sTBS, respectively (all $P > .05$). The supramaximal stimulus intensity was 145 ± 37 mA, 137 ± 33, and 144 ± 40 for elbow flexor motor point stimulation during iTBS, cTBS, and sTBS, respectively (all $P > .05$).

Neuromuscular function evaluation. The neuromuscular function evaluation started with three sets of evaluations, separated by 60 s (PRE). Each evaluation

consisted of two contractions of the elbow flexor. The 5 s MVIC was superimposed with a single muscle stimulation delivered at the force plateau (superimposed twitch, SIT) and another stimulation evoked 2 s after the MVIC on the relaxed muscle (i.e. potentiated twitch, $Q_{tw,pot}$). All the peak forces from the MVIC trials were within 5% of each other. Five seconds after the MVIC, participants performed a 15 s contraction at the EMG corresponding to 20% of the first MVIC force (20% isoEMG). The root mean square (RMS) EMG of the biceps brachii during the 200 ms plateau of baseline MVIC was quantified and then the 20% of this value was calculated and shown on the computer screen by a reference line. Participants were asked to contract at this intensity while they received three TMS, one cervicomedullary stimulation, and one nerve stimulation every 3 s in random order. One neuromuscular function evaluation was then repeated following the TBS protocol (POST_TBS), as well as after the fatiguing task (POST_1, POST_2, POST_3, POST_4). Visual feedback of the force produced was provided to the participants using a custom-written MATLAB toolbox (The MathWorks, Inc., Natick, MA).

Fatiguing task. Participants were asked to perform a TTF exercise consisting of a submaximal sustained isometric elbow flexor contraction at 35% of the MVIC, previously recorded during the warm-up. Visual feedback of the force produced, as well as the target force levels, were provided to the participants using a real-time display on a computer screen. Participants contracted to the target force, and once force was attained, they were strongly encouraged by the investigators to maintain that target as long as possible during the session. The task ended when the force dropped by more than 10% compared to the target for more than 5 seconds (Cogiamanian *et al.*, 2007).

Data analysis

Force values were measured for the duration of the entire neuromuscular testing protocol. All data of force expression during the MVIC contractions and at rest were normalized to the lever arm of each participant. Area values for M_{max},

MEPs, and CMEPs were measured between cursors marking the initial deflection from the baseline to the second crossing of the horizontal axis (Martin *et al.*, 2006*a*). To account for any changes in the compound muscle action potential, MEPs and CMEPs were normalized to M_{max} values (MEP/M_{max}, $CMEP/M_{max}$) recorded during the same contraction. The duration of the SP after TMS and CMEP was measured by visually inspecting the interval from the stimulus to the return of continuous voluntary EMG (Taylor *et al.*, 1996). During the fatiguing task, the RMS of EMG values were averaged every 10 seconds and normalized for the M_{max} of the first 10-second interval.

For the calculation of the voluntary elbow flexors activation (VA), SIT and $Q_{tw,pot}$ were used as previously described (Merton, 1954):

$$VA = [1 - (SIT/Q_{tw,pot})] \times 100$$

Statistical analysis

Results are given as individual and mean ± SD unless otherwise stated. For the non-normally distributed variable (i.e., TTF), the Friedman test was used to compare the TTF across the TBS protocols (iTBS, cTBS, and sTBS). Normal Gaussian distribution of data was confirmed using the Shapiro-Wilk test. If a violation was detected, the data were analysed using non-parametric analysis. A two-way (3×2) repeated measures ANOVA (PRE *versus* POST_TBS, POST_TBS *versus* POST_TTF) was used to assess differences over time and between the TBS protocols for the following variables: MVIC, $Q_{tw,pot}$, VA, MEP/M_{max}, $CMEP/M_{max}$, MEP/CMEP and SP. A two-way ANOVA (3x4) was used to assess differences during the recovery and between the TBS protocols for the same variables. In case the statistical significance with $P < .05$ differed from the expected physiological behavior of a variable, the interquartile range analysis between the 25^{th} and 75^{th} percentile was adopted to verify the realm of variability and to determine if these changes were due to the TBS as a meaningful result, or rather to the participant's variability. The results of the interquartile range analysis were specified only when statistical significance was not meaningful compared to the physiological one.

For the EMG variable assessed during the TTF a two-way (2×2) repeated measures ANOVA was used to assess differences between the TBS protocol (iTBS, cTBS,

and sTBS) during the TTF. The compound symmetry, or sphericity, was checked using the Mauchly's test. If significant main or interaction effects were observed, these were followed up by *post-hoc* Bonferroni-corrected pairwise comparisons. The precision of estimates is indicated by the 95% confidence intervals (CIs). The significance level was set at $\alpha \leq .05$. Statistical analysis was conducted using IBM™ SPSS™ Statistics (version 26, IBM Corp., Somers, New York).

Results

Effects of theta burst stimulation on the time-to-task failure

Compared to the sTBS (136 ± 39 s), time-to-task failure (TTF) was longer for the iTBS (147 ± 39 s, +11%, P < .001) and shorter for the cTBS (115 ± 30 s, -21%, P = .005) (Figure 4.2A).

There was an effect of time (P < .001; $\eta^2_p = .817$), but not an interaction between time and TBS intervention P = .036; $\eta^2_p = .477$) on RMS/M_{max} of the electromyographic (EMG) signal during the TTF (Figure 4.2B). However, the change in RMS/M_{max} was significantly different only between iTBS and cTBS at the end (198 ± 161 *versus* 238 ± 248 %; P = .049) of the TTF (Figure 4.2B).

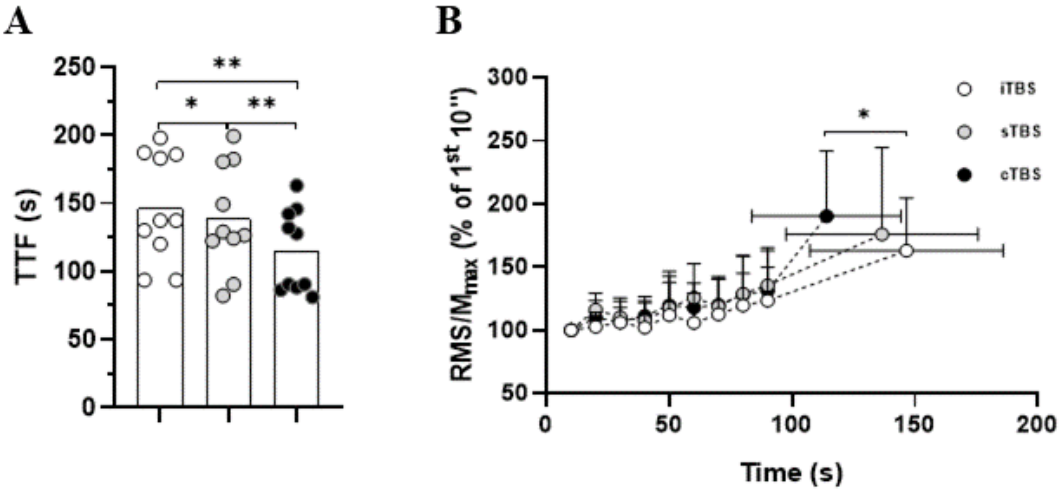

Figure 4.2. *Individual and mean data of the time-to-task failure (TTF) for each protocol (A). Root mean square (RMS) of the electromyographic data normalised for the maximal M-wave (M_{max}) in the percentage of the first 10 seconds interval where the maximal end interval of iTBS significantly differs from cTBS (B) sTBS, sham theta burst stimulation; iTBS, intermittent theta burst stimulation; cTBS, continuous theta burst stimulation. * P ≤ .05, ** P ≤ .01, *** P ≤ .001 (n = 10).*

Effects of theta burst stimulation on force and central fatigue

Maximal Voluntary Isometric Contraction

Figure 4.3A shows the MVIC force before, after each TBS protocol, immediately after each TTF task, and during the recovery. From PRE to POST_TBS, there was not an effect of TBS intervention (P = .136; η^2_p = .221) on MVIC; however, there was an effect of time (P = .022; η^2_p = .501), and an interaction between time and TBS intervention (P = .037; η^2_p = .338). MVIC significantly decreased from PRE to POST_TBS for cTBS only (from 103 ± 14 to 95 ± 20 N·m, P = .014), although MVIC for cTBS at POST_TBS was lower than iTBS (P = .038) but not different from sTBS (P = .051). Between POST_TBS and POST_1 there was an effect of time (P < .001; η^2_p = .948), but not an interaction between time and

TBS intervention (P = .181; η^2_p = .386) on MVIC. Moreover, MVIC had a similar trend during the whole recovery among the three protocols (P > .05).

Voluntary Activation

Figure 4.3B shows the VA before, after each TBS protocol and immediately after each TTF task, and during the recovery. Between PRE and POST_TBS there was an effect of time (P = .004; η^2_p = .671), and an interaction between time and TBS intervention (P = .011; η^2_p = .431) on VA. VA significantly decreased from PRE to POST_TBS for cTBS only (from 98% ± 2% to 92% ± 5%, P < .001), although VA for cTBS differed from iTBS (P < .001) and not from sTBS (P = .055) in POST_TBS. Between POST_TBS and POST_1 there was an effect of time (P = .001; η^2_p = .745), but not an interaction between time and TBS intervention (P = .069; η^2_p = .534) on VA. During the recovery there was an effect of time (P = .001; η^2_p = .500), and an interaction between time, but not an effect of TBS intervention (P = .251; η^2_p = .159). The significances obtained during the recovery are shown in Table 4.1A-C.

A iTBS	PRE	$POST_{TBS}$	POST1	POST2	POST3	POST4
MVIC (N m)	102 ± 14	102 ± 14 *	81 ± 13	86 ± 14	89 ± 11	94 ± 11
VA (%)	98 ± 2	97 ± 2 *	94 ± 4	92 ± 4	92 ± 6	95 ± 4
MEP/M_{max}	0,43 ± 0,24	0,43 ± 0,20 *	0,95 ± 0,31	0,47 ± 0,30	0,43 ± 0,26	0,43 ± 0,23
$CMEP/M_{max}$	0,47 ± 0,29	0,41 ± 0,35	0,10 ± 0,11	0,38 ± 0,30	0,39 ± 0,30	0,51 ± 0,60
SP_{TMS} (ms)	59 ± 16	52 ± 13 *	62 ± 17	57 ± 14	62 ± 18	58 ± 19
SP_{CMEP} (ms)	65 ± 15	63 ± 14	79 ± 15	70 ± 21	70 ± 15	66 ± 14
MEP/CMEP	0,99 ± 0,26	2,22 ± 2,21	16,21 ± 19,50	1,89 ± 1,92	1,78 ± 1,52	1,43 ± 0,79
$Q_{tw,pot}$ (N m)	15 ± 5	14 ± 5 §	6 ± 4 *	13 ± 5 §	14 ± 5 §	13 ± 4 §°
B sTBS	**PRE**	**$POST_{TBS}$**	**POST1**	**POST2**	**POST3**	**POST4**
MVIC (N m)	103 ± 14	102 ± 13	77 ± 11	81 ± 11	92 ± 17	97 ± 16
VA (%)	97 ± 1	97 ± 2	86 ± 9	92 ± 5 §	93 ± 5	95 ± 5 §
MEP/M_{max}	0,44 ± 0,21	0,51 ± 0,18	0,76 ± 0,28	0,55 ± 0,30	0,49 ± 0,26	0,49 ± 0,26
$CMEP/M_{max}$	0,49 ± 0,33	0,43 ± 0,37	0,14 ± 0,15	0,37 ± 0,43	0,42 ± 0,44	0,53 ± 0,69
SP_{TMS} (ms)	59 ± 11	61 ± 12	72 ± 15	61 ± 10	60 ± 10	64 ± 10
SP_{CMEP} (ms)	67 ± 9	67 ± 18	83 ± 16	75 ± 15	78 ± 14	72 ± 14
MEP/CMEP	1,19 ± 0,78	1,95 ± 1,44	19,06 ± 26,20	4,39 ± 6,16	2,20 ± 1,69	2,19 ± 2,50
$Q_{tw,pot}$ (N m)	16 ± 5	15 ± 5 §	9 ± 4	15 ± 4 §	14 ± 4 §	14 ± 4
C cTBS	**PRE**	**$POST_{TBS}$**	**POST1**	**POST2**	**POST3**	**POST4**
MVIC (N m)	103 ± 13	94 ± 17 *#	77 ± 20	88 ± 11	90 ± 16	94 ± 14
VA (%)	98 ± 1	91 ± 4 *#	84 ± 7	91 ± 5 §	93 ± 4 §°	94 ± 3 §+
MEP/M_{max}	0,44 ± 0,29	0,68 ± 0,28 *#	0,56 ± 0,26	0,65 ± 0,40	0,51 ± 0,29	0,46 ± 0,31
$CMEP/M_{max}$	0,43 ± 0,35	0,45 ± 0,42	0,20 ± 0,18	0,44 ± 0,24	0,57 ± 0,37	0,49 ± 0,38
SP_{TMS} (ms)	59 ± 15	74 ± 14 *#	84 ± 14	66 ± 17	64 ± 14	67 ± 13
SP_{CMEP} (ms)	66 ± 13	75 ± 11	92 ± 13	85 ± 21	74 ± 14	67 ± 16
MEP/CMEP	1,18 ± 0,57	2,37 ± 1,65	14,93 ± 16,78	1,94 ± 1,52	1,42 ± 1,57	1,42 ± 1,57
$Q_{tw,pot}$ (N m)	16 ± 4	15 ± 4 §	10 ± 3 *	13 ± 3 §	13 ± 2 §	12 ± 2 +°

Table 4.1. *Mean absolute data for iTBS (A), sTBS (B), and cTBS (C) during each time interval. POST_TBS, post theta burst stimulation; MVIC, maximal voluntary isometric contraction; $Q_{tw,pot}$, potentiated twitch force at rest; VA, voluntary activation; MEP, motor evoked potential; SP, silent period; CMEP, cervicomedullary motor evoked potential; sTBS, sham theta burst stimulation; iTBS, intermittent theta burst stimulation; cTBS,*

*continuous theta burst stimulation. * among protocols P ≤ .05; # from PRE P ≤ .05; § from POST_1 P ≤ .05; ° from POST_2 P ≤ .05; + from POST_3 P ≤ .05 (n = 10).*

Effects of theta burst stimulation on corticospinal excitability and inhibition

Figures 4.3E-H show the MEP/M_{max}, SP_{TMS}, CMEP/M_{MAX}, and SP_{CMEP} during the sustained contraction at 20% isoEMG before, after each TBS protocol, immediately after each TTF task, and during the recovery.

Corticospinal excitability

There was an effect of time (P = .005; η^2_p = .645), an interaction between time and TBS intervention (P = .002; η^2_p = .534) on MEP/M_{max}. MEP/M_{max} significantly increased from PRE to POST_TBS for cTBS only (from 0.44 ± 0.29 to 0.68 ± 0.28, P < .001), although the MEP/M_{max} for cTBS differed from iTBS (P = .037) and not from sTBS condition (P = .146) in POST_TBS. Between POST_TBS and POST_1 there was an effect of time (P < .001; η^2_p = .805), but not an interaction between time and TBS intervention (P = .543; η^2_p = .073) on MEP/M_{max}. During the recovery, there was an effect of time (P < .001; η^2_p = .652), but not an interaction between time and TBS intervention (P = .136; η^2_p = .177) (Figure 4.3E).

There was no effect of time (P = .461; η^2_p = .070), nor an interaction between time and TBS intervention (P = .828; η^2_p = .023) on CMEP/M_{MAX}. Between POST_TBS and POST_1 there was an effect of time (P = .004; η^2_p = .714), but not an interaction between time and TBS intervention (P = .975; η^2_p = .004) on CMEP/M_{MAX}. During the recovery there was an effect of time (P = .006; η^2_p = .490), but not an interaction between time and TBS intervention (P = .977; η^2_p = .031) on CMEP/M_{MAX} (Figure 4.3G).

Corticospinal inhibition

There was an interaction between time and TBS intervention (P < .001; η^2_p = .636), but not an effect of time (P = .236; η^2_p = .170) on SP_{TMS}. SP_{TMS} significantly increased from PRE to POST_TBS for cTBS only (from 59 ± 15 to 74 ± 14, P = .002), although the SP_{TMS} for cTBS differed from iTBS (P = .008) and not from sTBS condition (P = .146) in POST_TBS. Between POST_TBS and POST_1 there was an effect of time (P = .006; η^2_p = .633), but not an interaction between time and

TBS intervention (P = .945; η^2_p = .007) on SP_{TMS}. During the recovery there was an effect of time (P = .003; η^2_p = .476), but not an interaction between time and TBS intervention (P = .060; η^2_p = .240), on SP_{TMS} (Figure 4.3F).

There was no effect of time (P = .135; η^2_p = .257), nor an interaction between time and TBS intervention (P = .092; η^2_p = .257) on SP_{CMEP}. Between POST_TBS and POST_1 there was an effect of time (P = .002; η^2_p = .774), but not interaction between time and TBS intervention (P = .360; η^2_p = .064) on SP_{CMEP}. During the recovery there was an effect of time (P = .001; η^2_p = .484), but not an interaction between time and TBS intervention (P = .278; η^2_p = .139) on SP_{CMEP} (Figure 4.3H).

Effects of theta burst stimulation on the peripheral function

Figure 4.3C shows the $Q_{tw,pot}$ before, after each TBS protocol, and immediately after each TTF task, and during the recovery. There was an effect of time (P = .021; η^2_p = .507), but not an interaction between time and TBS intervention (P = .211; η^2_p = .177) on $Q_{tw,pot}$. Between POST_TBS and POST_1 there was an effect of time (P = .001; η^2_p = .743), and an interaction between time and TBS intervention (P = .025; η^2_p = .371) on MVIC. $Q_{tw,pot}$ significantly decreased from POST_TBS to POST_1 for iTBS (from 13.9 ± 5.0 to 5.8 ± 9.9 N·m, P < .001), and sTBS (from 15.2 ± 5.4 to 8.6 ± 4.4 N·m, P < .001), and for cTBS (from 14.6 ± 4.2 to 9.9 ± 3.3 N·m, P < .001). However, the reduction in $Q_{tw,pot}$ was greater for iTBS (-58% ± 23%) when compared to cTBS (-31% ± 16%, P = .015) but not to sTBS (-45% ± 18%, P = .090). However, $Q_{tw,pot}$ from POST_TBS to POST_1 significantly decreased for iTBS, sTBS (P < .001), but not cTBS (P = .093). During the recovery there was an effect of time (P = .001; η^2_p = .657), an interaction between time and TBS intervention (P = .001; η^2_p = .360), but not an effect of TBS intervention (P = .604; η^2_p = .061). The significances obtained during the recovery are shown in Table 4.1A-C.

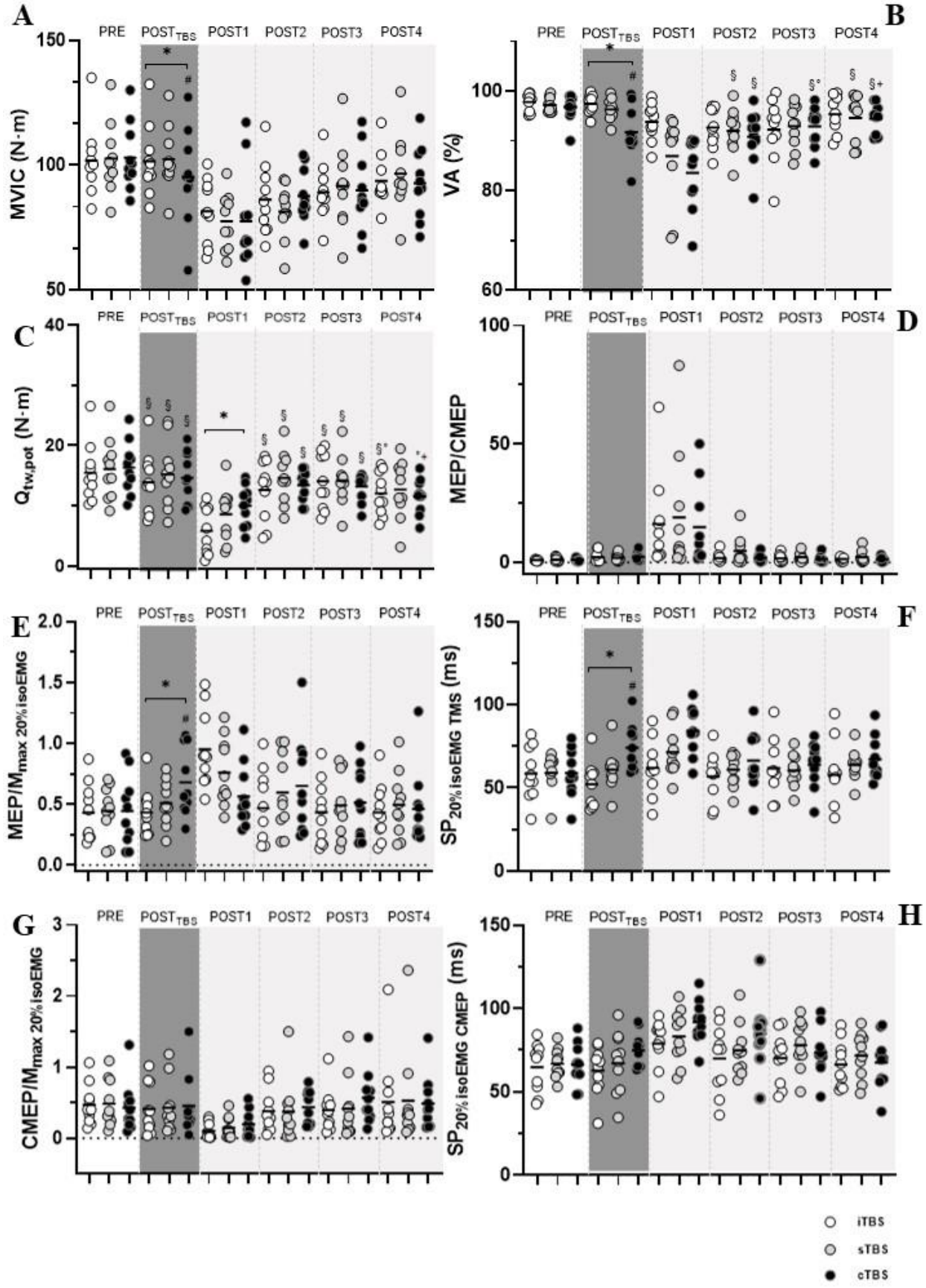
A
B
C
D
E
F
G
H
PRE
POST$_{TBS}$
POST1
POST2
POST3
POST4
MVIC (N·m)
VA (%)
$Q_{tw,pot}$ (N·m)
MEP/CMEP
MEP/$M_{max\ 20\%isoEMG}$
$SP_{20\%isoEMG\ TMS}$ (ms)
CMEP/$M_{max\ 20\%isoEMG}$
$SP_{20\%isoEMG\ CMEP}$ (ms)
iTBS
sTBS
cTBS

Figure 4.3 *Individual and mean data for MVIC (A), VA (B), $Q_{tw,pot}$ (C), MEP/CMEP (D), MEP/M_{MAX} (E), SP_{TMS} (F), CMEP/M_{MAX} (G), and SP_{CMEP} (H) before (PRE), after TBS (POST_TBS), after the fatiguing task, and during the recovery for each TBS protocol. MVIC, maximal voluntary isometric contraction; $Q_{tw,pot}$, potentiated twitch force at rest; VA, voluntary activation; MEP, motor evoked potential; SP, silent period; CMEP, cervicomedullary motor evoked potential; sTBS, sham theta burst stimulation; iTBS, intermittent theta burst stimulation; cTBS, continuous theta burst stimulation. * among protocols P ≤ .05; # from PRE P ≤ .05; § from POST_1 P ≤ .05; ° from POST_2 P ≤ .05; + from POST_3 P ≤ .05 (n = 10).*

Discussion

The current study aimed to investigate the effects of facilitatory and inhibitory TBS delivered to the right M1 on exercise performance, neuromuscular fatigue, and excitability/inhibition of the corticospinal pathway in the unfatigued (i.e. fresh) and fatigued ipsilateral right elbow flexor. We observed that in an unfatigued muscle, iTBS had no effect, while cTBS attenuated maximal muscle force and central drive, increased intracortical inhibition, and corticospinal excitability. In addition, while iTBS increased the performance of the fatiguing task, it had no effect on central fatigue, corticospinal excitability, and $GABA_B$ mediated intracortical inhibition. In contrast, cTBS lowered the performance of the isometric task, increased the development of central fatigue, and increased corticospinal excitability and $GABA_B$ mediated intracortical inhibition. Overall, these results suggest that TBS works on both the hemispheres due to interhemispheric crosstalk, managing the amount of exercise performance rather than the development of central and peripheral fatigue between hemispheres.

The modulatory effects of TBS in an unfatigued muscle

Examining the responses obtained after the application of each TBS protocol provides an engaging approach to studying interhemispheric crosstalk. The corpus callosum is located between the brain hemispheres and it represents an active structure full of connections that transmit both inhibitory and excitatory stimuli. This could mean that at any moment of life, there is no “control

hemisphere" since any plastic changes in one hemisphere influence the functioning of the opposite. Although the effects of the feedback relations between the hemispheres require further exploration, our results clearly show that the stimulated M1 responses rearrange the motor cortical map of the other hemisphere.

Thus, we assess the effects on the neuromuscular function as well as corticospinal excitability in a relatively unfatigued right muscle by applying iTBS and cTBS over the right M1 of the other resting upper limb. Differently from what we assumed, facilitatory TBS did not induce any positive effect on expressed force capacity, neural drive, or corticospinal excitability/inhibition. The current literature provides evidence of the mutual inhibitory influence that both M1s exhibit on each other (Wassermann *et al.*, 1991; Kobayashi *et al.*, 2004; Grefkes *et al.*, 2008). Indeed, a sort of interhemispheric competition could be detected during unilateral tasks to prevent interferences from the opposite cortical area (Grefkes *et al.*, 2008). In our study, iTBS was delivered at rest and we could imagine that the facilitatory stimulation on the right M1 of the resting arm increased its neurophysiological parameters mimicking some sort of cortical activity. This may have induced a non-response mediated by transcallosal inhibition on the other hemisphere. Unfortunately, the effects of TBS were only verified in the arm that did not directly receive the stimulation and only speculations can be made regarding the present findings.

In agreement with our initial hypothesis, after the inhibitory TBS our data depicted a reduced maximal force, as well as both central drive and corticospinal excitability and an increased intracortical inhibition. Despite the idea of interhemispheric competition described above, we propose a more simplistic view. Thus, the increase in GABA levels and the induction of LTD plasticity in the cTBS-stimulated hemisphere (right) were shifted to the opposite cortical motor area causing a limitation in the neural drive and the amount of force generated from the testing limb (right muscle). However, different susceptibility to facilitatory/inhibitory stimulation by both M1s can also be added to this scenario made of both facilitatory and inhibitory drives (Cabibel *et al.*, 2020; Chettouf *et al.*, 2020). In this regard, the contralateral motor cortex received a greater inhibitory drive from the ipsilateral one or was more susceptible to an inhibitory drive and less

responsive to facilitatory inputs from the ipsilateral motor cortex. Indeed, while facilitation has not always been observed, the inhibitory flows are more effective (Kobayashi *et al.*, 2004). We can only speculate on these underlying mechanisms, but we do not exclude that, in addition to the transcallosal cortico-cortical connections, mediations are coming from other levels of the axis we did not verify. However, regarding both iTBS and cTBS, our data obtained from the CMEP and the contraction force at rest did not present alterations induced by TBS and this is in line with all the literature which reports changes only at the cortical level after the stimulation.

Effect of facilitatory TBS combined with TTF

The iTBS stimulation led to a longer TTF compared to the sTBS and the cTBS. To our knowledge, no study applied TBS to the cortex of the resting limb to verify alterations in exercise performance on the exercising limb. However, the evidence provides the cross-limb transfer theory when learning new skills with one limb through task repetition can improve the ability to perform the same task with the opposite limb (Lee *et al.*, 2010). Explanations derive from the callosal access hypothesis in which the motor information in the dominant hemisphere can be accessed by the opposite one via the corpus callosum to facilitate task performance. Another similar mechanism depends on the bilateral motor activity produced during unilateral training that contributes to adaptations in both hemispheres and improved performance (Lee *et al.*, 2010). Thus, if we consider facilitatory TBS as a sort of unilateral training that reinforces the activation of the underneath tissue, we might think that its effects also influence the cortical activity of the other hemisphere, improving its performance. Nevertheless, studies in support of the previous theories detected TMS-related changes in the excitability of the corticospinal pathway after rTMS conditioning (Carroll *et al.*, 2008; Lee *et al.*, 2010) underlining that motor performance should always parallel changes in cortical and subcortical neural activity (Houweling *et al.*, 2010). Instead, our findings regarding exercise performance are not accompanied by corticospinal modifications. Also, the neural drive after iTBS, although visually less reduced than other conditions, does not show significant differences compared to the sTBS and the cTBS and compared to

the previous unfatigued muscle. At this point, the results of our study do not appear to demonstrate the presence of neuromuscular fatigue at the central level. However, we did not find other works that applied a study design like ours to assess the onset of fatigue. Therefore, due to associations found between TBS and the activity of remote cortical regions (Noh *et al.*, 2015), we can only speculate that iTBS acted on deeper brain structures not evaluated by us or it is possible that our tools were not very sensitive to some negligible physiological alterations induced by TBS.

Effect of inhibitory TBS combined with TTF

The application of the cTBS led to a shortening of the TTF, compared to iTBS and sTBS. The suppressive effect of cTBS is now known, although the mechanisms are still to be verified. Even more difficult is to explain the physiological alterations after cTBS applied on the M1 ipsilateral to the exercising limb. Indeed, the balance of neuronal activity between the two hemispheres is based on the reciprocal inhibition that acts as a regulatory coordination mechanism (Schindler *et al.*, 2008). The activation of one hemisphere should result in an inhibition of the opposite to avoid conflicting results and integrate performance. Therefore, an inhibitory current applied on the right motor cortex should have caused facilitation of the left motor cortex with positive effects on exercise. Our data, in contrast to this theory, show an inhibition in the hemisphere not stimulated (left) by cTBS. Evidence shows that muscle activation and voluntary contractions before or suddenly after stimulation can influence the outcome, reversing it (Huang *et al.*, 2011; Giboin *et al.*, 2016). Our study design involved the delivery of TBS followed by a maximal contraction and a sustained submaximal fatiguing isometric task. Therefore, we can consider the possibility that previous cortical activation switched excitation into depression. Although counterintuitive, a recent work suggests that cTBS may provide neuroprotection, and neurogenesis, and improve neurological function in pathological conditions (Zong *et al.*, 2022). However, this study helps us hypothesize that cTBS caused a positive effect leading to a facilitation of the stimulated hemisphere which favored inhibitory currents on the hemisphere involved in the exercise and on the exercise itself. Unfortunately, the

effects of TBS were only verified in the arm that did not directly receive the stimulation and only speculations can be made regarding the present findings.

As happened with facilitatory TBS, also in this case the presence of neuromuscular fatigue at a central level after the TTF was not found, as well as no changes in corticospinal excitability/inhibition. To the best of our knowledge, there are no comparable studies but we agree that parallel changes in cortical and subcortical neural activity should match the results of the exercise performance (Houweling *et al.*, 2010). Our study presents only preliminary data, and it is possible that by increasing the size of the sample the effects will be more consistent with what we expected. On the other hand, even peripheral fatigue does not appear to be strictly modulated by the type of TBS applied. The detected difference between the contraction force at rest in iTBS and cTBS sessions is solely dependent on the duration of the TTF and, thus, on the level of muscle contractile impairments induced (Hunter *et al.*, 2006; Yang *et al.*, 2009).

Conclusion

Our study observed that, while applying facilitatory and inhibitory TBS on the hemisphere ipsilateral to the exercising arm, it is possible to modulate exercise performance thanks to the mediation of intercallosal connections. Such TBS-induced modifications have nothing to do with changes in neural drive and corticospinal activity. However, this work coupled with further in-depth research would empower the practice of cross-education interventions mediated by TBS.

General Discussion

Recap

The purpose of this dissertation was to give new insights into the efficacy of TBS on exercise performance, neuromuscular fatigue, and corticospinal excitability. It is well known that TBS plays a crucial role in modulating brain networks, positively and negatively influencing muscle output depending on the applied protocol. The studies in this dissertation provide new insights into this potential effect, by verifying the results on exercise performance, neuromuscular fatigue, and corticospinal excitability applying TBS on the contralateral (Chapter 3) and ipsilateral (Chapter 4) M1 of the upper limb subjected to exercise.

In the first study, we delivered facilitatory and inhibitory TBS to directly influence the behavior and the output of a relatively unfatigued (before exercise) and fatigued muscle (after exercise). In the second study, we applied facilitatory and inhibitory TBS on the primary motor area of the resting upper limb to test the contribution of interhemispheric connections in affecting the exercising arm. In the last study of this dissertation, the limitations and advantages of both previous studies were examined to determine their greatest benefits in a possible clinical context as well as future research.

Discussion of the main findings

The results of each study (detailed discussed in respective chapters) can be summarized as follows:

In Chapter 3, it was shown that exercise performance can be modulated by facilitatory and inhibitory TBS applied on the M1, increasing and reducing the duration of the exercise, respectively. Specifically, time-to-task failure was +41% longer and -31% shorter after iTBS and cTBS, compared to sham condition. Interestingly, the levels of peripheral fatigue at the end of each fatiguing task were proportional to the duration of the exercise showing different levels of muscle contractile impairments not directly TBS-dependent. However, iTBS-induced alterations were detected regardless of changes in neural drive and corticospinal responses. On the other hand, cTBS-induced alterations in exercise performance were reflected in greater development of central fatigue and intracortical inhibition

phenomena. While most studies used facilitatory TBS in clinical populations or are limited to corticospinal changes, this is the first to compare the effects of both iTBS and cTBS in the field of neuromuscular fatigue and exercise performance and for this reason limitations cannot be ruled out, as outlined in the conclusion of Chapter 3. However, parts of our results contribute to evidence obtained from other forms of non-invasive stimulation (e.g., tDCS, conventional rTMS) on the plastic behavior of the brain networks giving a significant contribution to the field of neuroscience and neuromodulation.

In Chapter 4 we showed that exercise performance can also be modulated by altering the M1 not directly involved in the task. For this purpose, we applied iTBS and cTBS on the right M1, while the assessment in neuromuscular fatigue setup and the isometric fatiguing task were performed on the right arm ipsilateral to the stimulated hemisphere. Several studies tried to manage the functional connectivity and the rhythmic oscillations among cortical and subcortical brain areas (Kobayashi *et al.*, 2004; Noh *et al.*, 2015). Moreover, some of these used inhibitory TBS (Schindler *et al.*, 2008; Noh *et al.*, 2015) or other forms of stimulation (e.g., tDCS) corroborating the theory of interhemispheric inhibition and competition (Carroll *et al.*, 2008; Lee *et al.*, 2010). Following this idea, the action of one hemisphere can modulate the processing in its counterpart to refine the outcome without interfering signals (Grefkes *et al.*, 2008). However, no studies to our knowledge applied these experimental designs to investigate the cross-over effect of neuromuscular fatigue and its implications on performance. Our findings reflect the results of our previous study while seeming in contrast with the above-proposed theory. Indeed, exercise performance is longer (+11%) and shorter (-21%) when iTBS and cTBS are used, respectively. As partially for Chapter 3, iTBS-induced alterations were independent of changes in neural drive and corticospinal responses. Interestingly, cTBS-induced alterations did not correspond to the development of central fatigue and intracortical inhibition events. Rather, in no case does it appear that central fatigue developed, and we supposed that the high variability of these measures combined with the limited sample size has critically impacted the significance of the study.

In light of our results, finding which of the two study designs is more efficient in modulating cortical networks in favor of neuromuscular fatigue and

exercise performance may be particularly useful for detecting the neuroplastic mechanisms that govern the motor system. This may be crucial to determine the effectiveness of rehabilitation strategies in disease populations with severe neurological impairments, as well as in healthy subjects. Therefore, to make an unbiased comparison, ten out of twenty and ten out of ten participants were considered for Study I (Chapter 3) and Study II (Chapter 4), respectively. In addition, only common time intervals (PRE, POST_TBS, and TTF [Figure 5.1]), and neurophysiologic variables (MVIC, VA, $Q_{tw,pot}$, MEP/$M_{maxTMS\ 20\%isoEMG}$, $SP_{TMS\ 20\%isoEMG}$ [Figures 5.2 and 5.3]) were compared.

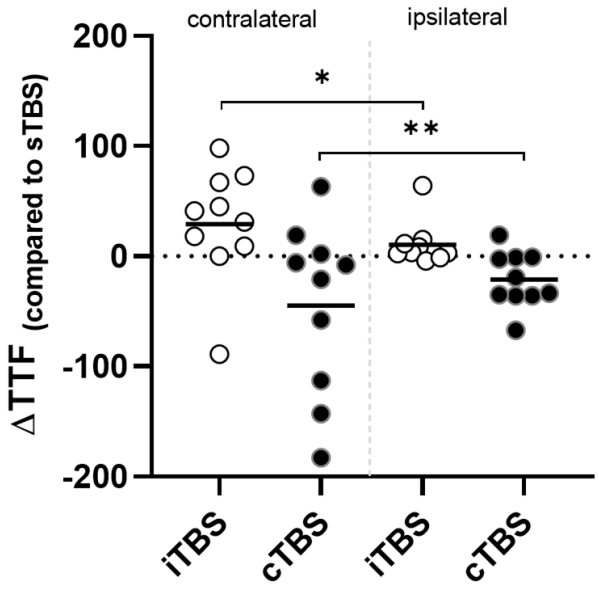

Figure 5.1. *Individual and mean data of the time-to-task failure (TTF) presented as percentage change compared to sham theta burst stimulation for study I (contralateral stimulation) and study II (ipsilateral stimulation). iTBS, intermittent theta burst stimulation; cTBS, continuous theta burst stimulation. * P ≤ .05, ** P ≤ .01 (n = 20).*

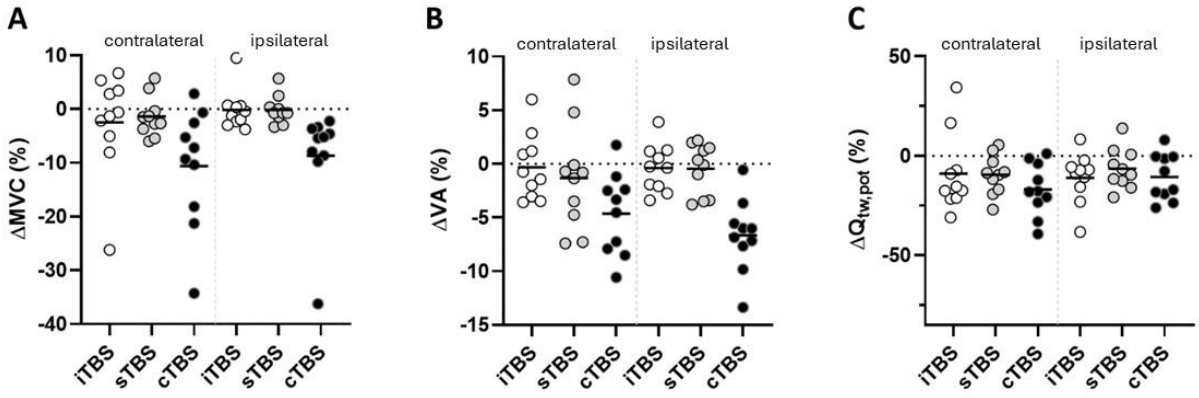

Figure 5.2. *Individual and mean data for percentage change between PRE and POST_TBS of MVIC (A), VA (B), $Q_{tw,pot}$ (C). MVIC, maximal voluntary isometric contraction; VA, voluntary activation; $Q_{tw,pot}$, potentiated twitch force at rest; sTBS, sham theta burst stimulation; iTBS, intermittent theta burst stimulation; cTBS, continuous theta burst stimulation. (n = 20).*

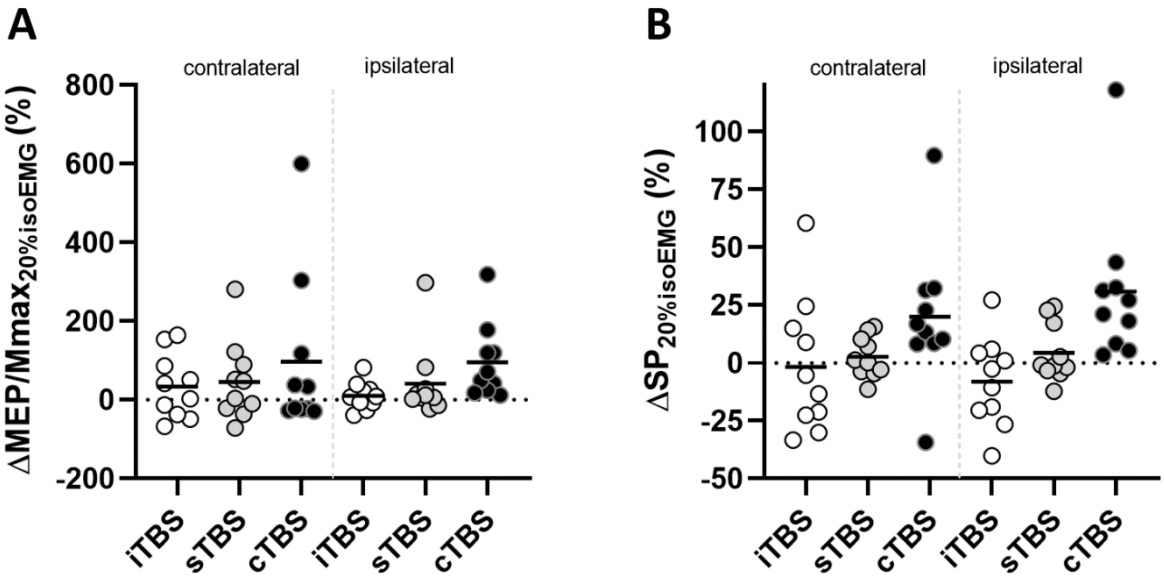

Figure 5.3. *Individual and mean data for percentage change between PRE and POST_TBS of MEP/M_{MAX} (A), SP_{TMS} (B) during the isometric contraction at the 20% isoEMG. MEP/M_{MAX}, motor evoked potential normalized for the maximal M_{wave}; SP, silent period; sTBS, sham theta burst stimulation; iTBS, intermittent theta burst stimulation; cTBS, continuous theta burst stimulation. (n = 20).*

	Δ% iTBS		Δ% sTBS		Δ% cTBS	
	Study I	**Study II**	**Study I**	**Study II**	**Study I**	**Study II**
MVIC	-2.5 ± 9.5	-2.0 ± 4.9	-1.4 ± 3.7	0.3 ± 3.2	-10.6 ± 11.1	-4.6 ± 13.3
VA	-0.3 ± 3.1	-2.2 ± 4.5	-1.3 ± 4.9	-1.9 ± 4.1	-4.6 ± 3.8	-6.9 ± 8.6
MEP/M_{maxTMS}	33.6 ± 81.4	10.7 ± 34.6	45.8 ± 101.7	41.1 ± 94.6	97.1 ± 204.9	95.4 ± 94.9
SP_{TMS}	-1.7 ± 29.3	-8.2 ± 19.3	2.6 ± 8.9	4.3 ± 12.6	19.8 ± 30.8	30.9 ± 33.2
$Q_{tw,pot}$	-8.9 ± 19.8	-16.4 ± 10.7	-9.6 ± 9.9	-5.5 ± 5.5	-16.8 ± 13.3	-10.6 ± 26.6

Table 5.1. *Mean ± SD data for percentage change between PRE and POST_TBS of MVIC, VA, MEP/M_{MAX}, SP_{TMS}, $Q_{tw,pot}$ for each protocol. MVIC, maximal voluntary isometric contraction; VA, voluntary activation; MEP/M_{MAX}, motor evoked potential normalized for the maximal M_{wave}; SP, silent period; $Q_{tw,pot}$, potentiated twitch force at rest; sTBS, sham theta burst stimulation; iTBS, intermittent theta burst stimulation; cTBS, continuous theta burst stimulation. (n = 20).*

However, as this is a pure results comparison, no additional measurements and data collection were included. Interestingly, regardless of neurophysiological parameters, the most effective cortical location for TBS stimulation to obtain greater changes in exercise performance is the contralateral M1 of the exercising limb, compared to the ipsilateral one (Figure 5.1). Our strategies, though, provide the possibility to apply TBS on the contralateral and ipsilateral cortical area of the exercising limb obtaining comparable effects from the direct and indirect stimulation towards the descending motor pathway (Figures 5.2 and 5.3). This comparative analysis concluded that both articles to some extent have weaknesses in common, such as the use of not entirely sufficient instruments and limited latency times between stimulations and neuromuscular assessments. Due to this, further research is needed. Nevertheless, at this stage studies I (Chapter 3) and II (Chapter 4) agree that iTBS and cTBS could increase and shorten the amount of exercise performance, respectively. On one hand, facilitatory TBS works independently of central drive and corticospinal mechanisms, on the other hand, inhibitory TBS

attenuates exercise performance increasing the development of central fatigue and intracortical inhibition.

Experimental considerations and Future perspectives

The number of studies using TBS on exercise performance and, most of all, on neuromuscular fatigue is certainly minor compared to others that use different types of non-invasive stimulations. Thus, our investigations in Chapters 3 and 4 provide an interesting approach that can be applied in broader contexts, for example in clinical research where it is necessary to evaluate the extent of neurological pathologies causing motor limitations in patients.

However, the works included in this thesis are not without limitations. Study I could highlight some methodological issues. Both male and female young adults were included with a larger number of participants in the female group. Hence, these results may not be well balanced. Indeed, possible gender differences in terms of brain activation, circulating hormones, brain and muscle anatomy, and contribution of the afferent feedback may have masked interesting implications. In other words, there may be a sex difference in the relative from the working muscles and other districts, such as the heart and the lungs, in the achievement of the sensory tolerance limit. In addition, these results may not be completely transferable and reproducible in a group of elderly people, although a future study could investigate this aspect further. Indeed, it is well known that differences in neurophysiological and motor outcomes are often age-dependent.

About study II, the limited sample size could undermine the validity of our results and also weaken the comparison with study I in which there were twice as many participants. Finally, for both studies I and II, the need for more adequate instruments for monitoring cortical activity in the target area and those associated with it could help reveal the involvement of broader neuronal networks and increase the sensitivity to spot differences in sensitivity to TBS.

However, these limitations, added to the great variability of results provided by the literature, pave the way for future studies to find valid and reliable protocols for assessing exercise performance and all aspects of fatigue.

References

Aboodarda SJ, Šambaher N & Behm DG (2016). Unilateral elbow flexion fatigue modulates corticospinal responsiveness in non-fatigued contralateral biceps brachii. *Scand J Med Sci Sport* **26,** 1301–1312.

Allen DG, Lamb GD & Westerblad H (2008). Skeletal muscle fatigue: Cellular mechanisms. *Physiol Rev* **88,** 287–332.

Allen DG & Westerblad H (2001). Role of phosphate and calcium stores in muscle fatigue. *J Physiol* **536,** 657–665.

Amann M & Calbet J (2008). Convective oxygen transport and fatigue. *J Appl Physiol* **104,** 861–870.

Amann M, Eldridge M, Lovering A, Stickland M, Pegelow D & Dempsey J (n.d.). Arterial oxygenation influences central motor output and exercise performance via effects on peripheral locomotor muscle fatigue in humans. *J Physiol* **575,** 937–952.

Amann M, Proctor L, Sebranek J, Eldridge M, Pegelow D & Dempsey J (2008). Somatosensory feedback from the limbs exerts inhibitory influences on central neural drive during whole body endurance exercise. Journal of Applied Physiology 105, 1714- 1724. *J Appl Physiol* **105,** 1714–1724.

Amann M, Proctor LT, Sebranek JJ, Pegelow DF & Dempsey JA (2009). Opioid-mediated muscle afferents inhibit central motor drive and limit peripheral muscle fatigue development in humans. *J Physiol* **587,** 271–283.

Amann M, Romer L, Pegelow D, Jacques A, Hess C & Dempsey J (2006). Effects of arterial oxygen content on peripheral locomotor muscle fatigue. *J Appl Physiol* **101,** 119–127.

Amann M, Venturelli M, Ives SJ, McDaniel J, Layec G, Rossman MJ & Richardson RS (2013). Peripheral fatigue limits endurance exercise via a sensory feedback-mediated reduction in spinal motoneuronal output. *J Appl Physiol* **115,** 355–364.

Angius L, Hopker J & Mauger AR (2017). The ergogenic effects of transcranial

direct current stimulation on exercise performance. *Front Physiol*; DOI: 10.3389/fphys.2017.00090.

Ansdell P, Brownstein CG, Škarabot J, Hicks KM, Simoes DCM, Thomas K, Howatson G, Hunter SK & Goodall S (2019). Menstrual cycle-associated modulations in neuromuscular function and fatigability of the knee extensors in eumenorrheic women. *J Appl Physiol* **126,** 1701–1712.

Bachtiar V & Stagg CJ (2014). The role of inhibition in human motor cortical plasticity. *Neuroscience* **278,** 93–104.

Bailey S, Davis J & Ahlborn E (1993). Serotonergic agonists and antagonists affect endurance performance in the rat. *Int J Sports Med* **14,** 330–333.

Baranyi A & Fehér O (1978). Conditioned changes of synaptic transmission in the motor cortex of the cat. *Exp Brain Res* **33,** 283–298.

Barcroft H & Dornhorst A (1949). The blood flow through the human calf during rhythmic exercise. *J Physiol* **109,** 402–411, pl.

Barcroft H & Millen J (1939). The blood flow through muscle during sustained contraction. *J Physiol* **97,** 17–31.

Barker A, Jalinous R & Freeston H (1985). Non-invasive stimulation of the human motor cortex. *Lancet* **1,** 1106–1107.

Behm D, St-Pierre D & Perez D (1996). Muscle inactivation: assessment of interpolated twitch technique. *J Appl Physiol* **81,** 2267–2273.

Behm DG & St-Pierre DMM (1997). Effects of fatigue duration and muscle type on voluntary and evoked contractile properties. *J Appl Physiol* **82,** 1654–1661.

Bigland-Ritchie B, Johansson R, Lippold O & Woods J (1983). Contractile speed and EMG changes during fatigue of sustained maximal voluntary contractions. *J Neurophysiol* **50,** 313–324.

Bigland-Ritchie B & Woods J (1984). Changes in muscle contractile properties and neural control during human muscular fatigue. *Muscle Nerve* **7,** 691–

699.

Bilodeau M (2006). Central fatigue in continuous and intermittent contractions of triceps brachii. *Muscle & Nerve& Nerve* **34,** 205–213.

Blain G, Mangum T, Sidhu S, Weavil J, Hureau T, Jessop J, Bledsoe A, Richardson R & Amann M (2016). Group III/IV muscle afferents limit the intramuscular metabolic perturbation during whole body exercise in humans. *J Physiol* **594,** 5303–5315.

Blazevich A & Babault N (2019). Post-activation Potentiation Versus Post-activation Performance Enhancement in Humans: Historical Perspective, Underlying Mechanisms, and Current Issues. *Front Physiol* **10,** 1359.

Boutiere C, Rey C, Zaaraoui W, Le Troter A, Rico A, Crespy L, Achard S, Reuter F, Pariollaud F, Wirsich J, Asquinazi P, Confort-Gouny S, Soulier E, Guye M, Pelletier J, Ranjeva JP & Audoin B (2017). Improvement of spasticity following intermittent theta burst stimulation in multiple sclerosis is associated with modulation of resting-state functional connectivity of the primary motor cortices. *Mult Scler J* **23,** 855–863.

Bradnam L V., Stinear CM & Byblow WD (2010). Theta burst stimulation of human primary motor cortex degrades selective muscle activation in the ipsilateral arm. *J Neurophysiol* **104,** 2594–2602.

Brighina F, Palermo A, Daniele O, Aloisio A & Fierro B (2010). High-frequency transcranial magnetic stimulation on motor cortex of patients affected by migraine with aura: a way to restore normal cortical excitability? *Cephalgia* **30,** 46–52.

Brownstone R (2006). Beginning at the end: repetitive firing properties in the final common pathway. *Prog Neurobiol* **78,** 156–172.

Broxterman R, Ade C, Wilcox S, Schlup S, Craig J & Barstow T (2014). Influence of duty cycle on the power-duration relationship: observations and potential mechanisms. *Respir Physiol Neurobiol* **192,** 102–111.

Burke M, Fried P & Pascual-Leone A (2019). Transcranial magnetic stimulation:

Neurophysiological and clinical applications. *Handb Clin Neurol* **163,** 73–92.

Cabibel V, Hordacre B & Perrey S (2020). Implication of the ipsilateral motor network in unilateral voluntary muscle contraction: the cross-activation phenomenon. *J Neurophysiol* **123,** 2090–2098.

Carroll T, Lee M, Hsu M & Sayde J (2008). Unilateral practice of a ballistic movement causes bilateral increases in performance and corticospinal excitability. *J Appl Physiol* **104,** 1656–1664.

Carroll TJ, Herbert RD, Munn J, Lee M & Gandevia SC (2006). Contralateral effects of unilateral strength training: Evidence and possible mechanisms. *J Appl Physiol* **101,** 1514–1522.

Carson RG, Riek S, Mackey DC, Meichenbaum DP, Willms K, Forner M & Byblow WD (2004). Excitability changes in human forearm corticospinal projections and spinal reflex pathways during rhythmic voluntary movement of the opposite limb. *J Physiol* **560,** 929–940.

Cash R, Murakami T, Chen R, Thickbroom G & Ziemann U (2016). Augmenting Plasticity Induction in Human Motor Cortex by Disinhibition Stimulation. *Cereb Cortex* **26,** 58–69.

Cheeran B, Talelli P, Mori F, Koch G, Suppa A, Edwards M, Houlden H, Bhatia K, Greenwood R & Rothwell J (2008). A common polymorphism in the brain-derived neurotrophic factor gene (42 BDNF) modulates human cortical plasticity and the response to rTMS. *J Physiol* **586,** 5717–5725.

Chen R, Classen J, Gerloff C, Celnik P, Wassermann E, Hallett M & Cohen L (1997). Depression of motor cortex excitability by low-frequency transcranial magnetic stimulation. *Neurology*; DOI: 10.1212/WNL.48.5.1398.

Chervyakov A V., Chernyavsky AY, Sinitsyn DO & Piradov MA (2015). Possible mechanisms underlying the therapeutic effects of transcranial magnetic stimulation. *Front Hum Neurosci* **9,** 1–14.

Chettouf S, Rueda-Delgado LM, Vries R de, Ritter P & Daffertshofer A (2020). Are unimanual movements bilateral? *Neurosci Biobehav Rev* **113,** 39–50.

Chieffo R, Straffi L, Inuggi A, Gonzalez-Rosa JJ, Spagnolo F, Coppi E, Nuara A, Houdayer E, Comi G & Leocani L (2016). Motor cortical plasticity to training started in childhood: The example of piano players. *PLoS One* **11,** e0157952–e0157952.

Chin E & Allen D (1997). Effects of reduced muscle glycogen concentration on force, Ca2+ release and contractile protein function in intact mouse skeletal muscle. *J Physiol* **498,** 17–29.

Cho S & Strafella A (2009). rTMS of the Left Dorsolateral Prefrontal Cortex Modulates Dopamine Release in the Ipsilateral Anterior Cingulate Cortex and Orbitofrontal Cortex. *PLoS One* **4,** e6725.

Chung SW, Hill AT, Rogasch NC, Hoy KE & Fitzgerald PB (2016). Use of theta-burst stimulation in changing excitability of motor cortex: A systematic review and meta-analysis. *Neurosci Biobehav Rev* **63,** 43–64.

Cirillo G, Di Pino G, Capone F, Ranieri F, Florio L, Todisco V, Tedeschi G, Funke K & Di Lazzaro V (2017). Neurobiological after-effects of non-invasive brain stimulation. *Brain Stimul*.

Clausen T (2003). Na+ -K+ Pump Regulation and Skeletal Muscle Contractility. *Physiol Rev* **83,** 1269–1324.

Cogiamanian F, Marceglia S, Ardolino G, Barbieri S & Priori A (2007). Improved isometric force endurance after transcranial direct current stimulation over the human motor cortical areas. *Eur J Neurosci* **26,** 242–249.

Collingridge GL & Bliss T V. (1995). Memories of NMDA receptors and LTP. *Trends Neurosci* **18,** 54–56.

Davis NJ (2013). Neurodoping: Brain stimulation as a performance-enhancing measure. *Sport Med* **43,** 649–653.

Dempsey J, Johnson J & Wagner P (1996). Control of respiratory and

cardiovascular system. In *Handbook of Physiology Section 12: Exercise: Regulation and Integration of Multiple Systems*, pp. 331–838.

Doix ACM, Lefèvre F & Colson SS (2013). Time Course of the Cross-Over Effect of Fatigue on the Contralateral Muscle after Unilateral Exercise. *PLoS One* **8,** 1–8.

Dotan R, Woods S & Contessa P (2021). On the reliability and validity of central fatigue determination. *Eur J Appl Physiol* **121,** 2393–2411.

Dulhunty A (2006). Excitation-contraction coupling from the 1950s into the new millennium. *Clin Exp Pharmacol Physiol* **33,** 763–772.

Elfving B, Liljequist D, Dedering Å & Németh G (2002). Recovery of electromyograph median frequency after lumbar muscle fatigue analysed using an exponential time dependence model. *Eur J Appl Physiol* **88,** 85–93.

Enoka R & Duchateau J (2016). Translating fatigue to human performance. *Med Sci Sport Exerc* **48,** 2228–2238.

Fabiato A & Fabiato F (1978). Effects of pH on the myofilaments and the sarcoplasmic reticulum of skinned cells from cardiace and skeletal muscles. *J Physiol* **276,** 233–255.

Fitzgerald PB, Chen L, Richardson K, Daskalakis ZJ & Hoy KE (2020). A pilot investigation of an intensive theta burst stimulation protocol for patients with treatment resistant depression: Short Title: Intensive TBS in depression. *Brain Stimul* **13,** 137–144.

Fuglevand A & Keen D (2003). Re-evaluation of muscle wisdom in the human adductor pollicis using physiological rates of stimulation. *J Physiol* **549,** 865–868.

Fung PK & Robinson PA (2014). Neural field theory of synaptic metaplasticity with applications to theta burst stimulation. *J Theor Biol* **340,** 164–176.

Gamboa OL, Antal A, Moliadze V & Paulus W (2010*a*). Simply longer is not better: reversal of theta burst after-effect with prolonged stimulation. *Exp*

Brain Res **204,** 181–187.

Gamboa OL, Antal A, Moliadze V & Paulus W (2010*b*). Simply longer is not better: Reversal of theta burst after-effect with prolonged stimulation. *Exp Brain Res* **204,** 181–187.

Gandevia S (2001). Spinal and Supraspinal Factors in Human Muscle Fatigue. *Physiol Rev* **81,** 1725–1789.

Gandevia S, Allen G, Butler J & Taylor J (1996). Supraspinal factors in human muscle fatigue: Evidence for suboptimal output from the motor cortex. *J Physiol* **490,** 529–536.

Garland S, Enoka RM, Serrano L & Robinson G (1994). Behavior of motor units in human biceps brachii during a submaximal fatiguing contraction. *J Appl Physiol* **76,** 2411–2419.

Gentner R, Wankerl K, Reinsberger C, Zeller D & Classen J (2008). Depression of human corticospinal excitability induced by magnetic theta-burst stimulation: Evidence of rapid polarity-reversing metaplasticity. *Cereb Cortex* **18,** 2046–2053.

Giboin LS, Thumm P, Bertschinger R & Gruber M (2016). Intermittent theta burst over M1 may increase peak power of a wingate anaerobic test and prevent the reduction of voluntary activation measured with transcranial magnetic stimulation. *Front Behav Neurosci* **10,** 1–10.

Gilio F, Conte A, Vanacore N, Frasca V, Inghilleri M & Berardelli A (2007). Excitatory and inhibitory after-effects after repetitive magnetic transcranial stimulation (rTMS) in normal subjects. *Exp Brain Res* **176,** 588–593.

Goodall S, Gonzalez-Alonso J, Ali L, Ross E & Romer L (2012). Supraspinal fatigue after normoxic and hypoxic exercise in humans. *J Physiol* **590,** 2767–2782.

Gould E, Beylin A, Tanapat P, Reeves A & Shors J T (1999). Learning enhances adult neurogenesis. *Nat Neurosci* **2,** 260–265.

Grefkes C, Eickhoff SB, Nowak DA, Dafotakis M & Fink GR (2008). Dynamic intra- and interhemispheric interactions during unilateral and bilateral hand movements assessed with fMRI and DCM. *Neuroimage* **41,** 1382–1394.

Halperin I, Chapman DW & Behm DG (2015). Non-local muscle fatigue: effects and possible mechanisms. *Eur J Appl Physiol* **115,** 2031–2048.

Halperin I, Copithorne D & Behm DG (2014). Unilateral isometric muscle fatigue decreases force production and activation of contralateral knee extensors but not elbow flexors. *Appl Physiol Nutr Metab* **39,** 1338–1344.

Heckman C & Enoka RM (2012). Motor unit. *Compr Physiol* **2,** 2629–2682.

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

Hilty L, Lutz K, Maurer K, Rodenkirch T, Spengler C, Boutellier U, Jancke L & Amann M (2011). Spinal opioid receptor-sensitive muscle afferents contribute to the fatigueinduced increase in intracortical inhibition in healthy humans. *Exp Physiol* **96,** 505–517.

Hoogendam JM, Ramakers GMJ & Di Lazzaro V (2010). Physiology of repetitive transcranial magnetic stimulation of the human brain. *Brain Stimul* **3,** 95–118.

Houweling S, van Dijk BW, Beek PJ & Daffertshofer A (2010). Cortico-spinal synchronization reflects changes in performance when learning a complex bimanual task. *Neuroimage* **49,** 3269–3275.

Huang Y, Lu M, Antal A, Classen J, Nitsche M, Ziemann U, Ridding M, Hamada M, Ugawa Y, Jaberzadeh S & Al E (2017). Plasticity induced by non-invasive transcranial brain stimulation: A position paper. *Clin Neurophysiol* **128,** 2318–2329.

Huang YZ, Edwards MJ, Rounis E, Bhatia KP & Rothwell JC (2005). Theta burst stimulation of the human motor cortex. *Neuron* **45,** 201–206.

Huang YZ, Rothwell JC, Chen RS, Lu CS & Chuang WL (2011). The theoretical model of theta burst form of repetitive transcranial magnetic stimulation. *Clin Neurophysiol* **122,** 1011–1018.

Hunter SK, Butler JE, Todd G, Gandevia SC & Taylor JL (2006). Supraspinal fatigue does not explain the sex difference in muscle fatigue of maximal contractions. *J Appl Physiol* **101,** 1036–1044.

Hunter SK, Griffith EE, Schlachter KM & Kufahl TD (2009). Sex differences in time to task failure and blood flow for an intermittent isometric fatiguing contraction. *Muscle and Nerve* **39,** 42–53.

Hureau T, Broxterman R, Weavil J, Lewis M, Layec G & Amann M (2022). On the role of skeletal muscle acidosis and inorganic phosphates as determinants of central and peripheral fatigue: A 31 P-MRS study. *J Physiol* **600,** 3069–3081.

Hureau T, Ducrocq G & Blain G (2016). Peripheral and Central Fatigue Development during All-Out Repeated Cycling Sprints. *Med Sci Sport Exerc* **48,** 391–401.

Hureau T, Olivier N, Millet G, Meste O & Blain G (2014). Exercise performance is regulated during repeated sprints to limit the development of peripheral fatigue beyond a critical threshold. Exp Physiol 99, 951-963. *Exp Physiol* **99,** 951–963.

Iezzi E, Conte A, Suppa A, Agostino R, Dinapoli L, Scontrini A & Berardelli A (2008). Phasic voluntary movements reverse the aftereffects of subsequent theta-burst stimulation in humans. *J Neurophysiol* **100,** 2070–2076.

Imperatori LS, Milbourn L & Garasic MD (2018). Would the Use of Safe, Cost-Effective tDCS Tackle Rather than Cause Unfairness in Sports? *J Cogn Enhanc* **2,** 377–387.

Isaac J (2001). Protein phosphatase 1 and LTD: synapses are the architects of depression. *Neuron* **32,** 963–966.

Jacobs KM & Donoghue JP (1991). Reshaping the cortical motor map by

unmasking latent intracortical connections. *Science (80-)* **251,** 944–947.

Johnson M, Sharpe G, Williams N & Hannah R (2015). Locomotor muscle fatigue is not critically regulated after prior upper body exercise. J Appl Physiol (1985). *J Appl Physiol* **119,** 840–850.

Juel C, Pilegaard H, Nielsen J & Bangsbo J (2000). Interstitial K+ in human skeletal muscle during and after dynamic graded exercise determined by microdialysis. *Am J Physiol Regul Integr Comp Physiol* **278,** R400-6.

Kaufman M & Forster H (1996). Reflexes Controlling Circulatory, Ventilatory and Airway Responses to Exercise. In *Handbook of Physiology Section 12: Exercise: Regulation and Integration of Multiple Systems*, ed. Rowell L, pp. 381–447. New York.

Kennedy DS, Fitzpatrick SC, Gandevia SC & Taylor JL (2015). Fatigue-related firing of muscle nociceptors reduces voluntary activation of ipsilateral but not contralateral lower limb muscles. *J Appl Physiol* **118,** 408–418.

Kernell D & Monster A (1982). Motoneurone properties and motor fatigue. An intracellular study of gastrocnemius motoneurones of the cat. *Exp Brain Res* **46,** 197–204.

Kew JNC & Kemp JA (2005). Ionotropic and metabotropic glutamate receptor structure and pharmacology. *Psychopharmacology (Berl)* **179,** 4–29.

Kleim JA, Hogg TM, Vandenberg PM, Cooper NR, Bruneau R & Remple M (2004). Cortical synaptogenesis and motor map reorganization occur during late, but not early, phase of motor skill learning. *J Neurosci* **24,** 628.

Kleim JA, Lussnig E, Schwarz ER, Comery TA & Greenough WT (1996). Synaptogenesis and Fos expression in the motor cortex of the adult rat after motor skill learning. *J Neurosci* **16,** 4529.

Klomjai W, Katz R & Lackmy-Vallée A (2015). Basic principles of transcranial magnetic stimulation (TMS) and repetitive TMS (rTMS). *Ann Phys Rehabil Med* **58,** 208–213.

Kluger BM, Krupp LB & Enoka RM (2013). Fatigue and fatigability in neurologic inllnesses. *Neurology* **80,** 409–416.

Kobayashi M, Hutchinson S, Théoret H, Schlaug G & Pascual-Leone A (2004). Repetitive TMS of the motor cortex improves ipsilateral sequential simple finger movements. *Neurology* **62,** 91–98.

Koc G, Gokcil Z, Bek S, Kasikci T, Eroglu E & Odabasi Z (2017). Effects of continuous theta burst transcranial magnetic stimulation on cortical excitability in patients with idiopathic generalized epilepsy. *Epilepsy Behav*; DOI: https://doi.org/10.1016/j.yebeh.2017.09.011.

Korzeniewski B & Rossiter H (2020). Exceeding a "critical" muscle Pi: implications for [Formula: see text] and metabolite slow components, muscle fatigue and the power-duration relationship. *Eur J Appl Physiol* **120,** 1609–1619.

Kuo I & Ehrlich B (2015). Signaling in muscle contraction. Cold Spring Harb Perspect Biol. 7:1–14. *Cold Spring Harb Perspect Med* **7,** 1–14.

Di Lazzaro V, Pilato F, Dileone M, Profice P, Oliviero A, Mazzone P, Insola A, Ranieri F, Meglio M, Tonali PA & Rothwell JC (2008). The physiological basis of the effects of intermittent theta burst stimulation of the human motor cortex. *J Physiol* **586,** 3871–3879.

Di Lazzaro V & Rothwell J (2014). Corticospinal activity evoked and modulated by non-invasive stimulation of the intact human motor cortex. *J Physiol*.

Lee M, Hinder MR, Gandevia SC & Carroll TJ (2010). The ipsilateral motor cortex contributes to cross-limb transfer of performance gains after ballistic motor practice. *J Physiol* **588,** 201–212.

Lee PH, Macfarlane DJ, Lam TH & Stewart SM (2011). Validity of the international physical activity questionnaire short form (IPAQ-SF): A systematic review. *Int J Behav Nutr Phys Act* **8,** 115.

Lefaucheur J, Aleman A, Baeken C, Benninger D, Brunelin J, Di Lazzaro V, Filipović S, Grefkes C, Hasan A, Hummel F & Al. E (2020). Evidence-based

guidelines on the therapeutic use of repetitive transcranial magnetic stimulation (rTMS): An update (2014–2018). *Clin Neurophysiol* **131,** 474–528.

Ly JQM, Gaggioni G, Chellappa SL, Papachilleos S, Brzozowski A, Borsu C, Rosanova M, Sarasso S, Middleton B, Luxen A, Archer SN, Phillips C, Dijk DJ, Maquet P, Massimini M & Vandewalle G (2016). Circadian regulation of human cortical excitability. *Nat Commun*; DOI: 10.1038/ncomms11828.

Marcora SM, Staiano W & Manning V (2009). Mental fatigue impairs physical performance in humans. *J Appl Physiol* **106,** 857–864.

Marsden C, Meadows J & Merton P (1983). "Muscular wisdom" that minimizes fatigue during prolonged effort in man: peak rates of motoneuron discharge and slowing of discharge during fatigue. *Adv Neurol* **39,** 169–211.

Martin PG, Gandevia SC & Taylor JL (2006*a*). Output of Human Motoneuron Pools to Corticospinal Inputs During Voluntary Contractions. 3512–3518.

Martin PG, Gandevia SC & Taylor JL (2006*b*). Theta burst stimulation does not reliably depress all regions of the human motor cortex. *Clin Neurophysiol* **117,** 2684–2690.

Mathis J, de Quervain D & Hess C (1998). Dependence of the transcranially induced silent period on the "instruction set" and the individual reaction time. *Electroencephalogr Clin Neurophysiol* **109,** 426–435.

McKenna M (1992). The role of ionic processes in muscular fatigue during intense exercise. *Sport Med* **13,** 134–145.

McKenna M, Bangsbo J & Renaud J-M (2007). Muscle K+, Na+, and Cl− disturbances and Na+ -K+ pump inactivation: implications for fatigue. *J Appl Physiol* **104,** 288295.

McNeil CJ, Giesebrecht S, Gandevia SC & Taylor JL (2011). Behaviour of the motoneurone pool in a fatiguing submaximal contraction. *J Physiol* **589,** 3533–3544.

Merton PA (1954). Voluntary strength and fatigue. *J Physiol* **123,** 553–564.

de Morree H & Marcora S (2015). Psychobiology of Perceived Effort During Physical Tasks. *Handb Biobehav Approaches to Self-Regulation*255–270.

Mosso A (1891). *La Fatica*. Milano.

Nielsen J, Schroder H, Rix C & Ortenblad N (2009). Distinct effects of subcellular glycogen localization on tetanic relaxation time and endurance in mechanically skinned rat skeletal muscle fibres. *J Physiol* **587,** 3679–3690.

Noakes T, St. Clair Gibson A & Lambert E (2004). From catastrophe to complexity: A novel model of integrative central neural regulation of effort and fatigue during exercise in humans. *Br J Sports Med* **38,** 511–514.

Noh NA, Fuggetta G & Manganotti P (2015). Theta-burst transcranial magnetic stimulation alters the functional topography of the cortical motor network. *Malaysian J Med Sci* **22,** 35–43.

Okano AH, Fontes EB, Montenegro RA, De Tarso Veras Farinatti P, Cyrino ES, Li LM, Bikson M & Noakes TD (2015). Brain stimulation modulates the autonomic nervous system, rating of perceived exertion and performance during maximal exercise. *Br J Sports Med* **49,** 1213–1218.

Olsson K, Cheng A, Al-Ameri M, Wyckelsma V, Rullman E, Westerblad H, Lanner J & JD. GT& B (2020). Impaired sarcoplasmic reticulum Ca(2+) release is the major cause of fatigue-induced force loss in intact single fibres from human intercostal muscle. *J Physiol* **598,** 773–787.

Ørtenblad N, Nielsen J, Saltin B & Holmberg H (2011). Role of glycogen availability in sarcoplasmic reticulum Ca2+ kinetics in human skeletal muscle. J Physiol. *J Physiol* **589,** 711–725.

Ørtenblad N, Westerblad H & Nielsen J (2013). Muscle glycogen stores and fatigue. *J Physiol* **591,** 4405–4413.

Pate E, Bhimani M, Franks-Skiba K & Cooke R (1995). Reduced effect of pH on skinned rabbit psoas muscle mechanics at high temperatures: implications for

fatigue. *J Physiol* **486,** 689–694.

Perrier J & Cotel F (2008). Serotonin differentially modulates the intrinsic properties of spinal motoneurons from the adult turtle. *J Physiol* **586,** 1233–1238.

Piacentini M, Meeusen R, Buyse L, De Schutter G, Kempenaers F, Van Nijvel J & De Meirleir K (2002). No effect of a noradrenergic reuptake inhibitor on performance in trained cyclists. *Med Sci Sport Exerc* **34,** 1189–1193.

Place N (2021). Quantification of central fatigue: a central debate. *Eur J Appl Physiol* **121,** 2375–2376.

Place N, Bruton JD & Westerblad H (2009). Mechanisms of fatigue induced by isometric contractions in exercising humans and in mouse isolated single muscle fibres. *Clin Exp Pharmacol Physiol* **36,** 334–339.

Place N, Yamada T, Bruton JD & Westerblad H (2010). Muscle fatigue: From observations in humans to underlying mechanisms studied in intact single muscle fibres. *Eur J Appl Physiol* **110,** 1–15.

Rassier D & Macintosh B (2000). Coexistence of potentiation and fatigue in skeletal muscle. *Brazilian J Med Biol Res* **33,** 499–508.

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. *Eur J Appl Physiol* **118,** 501–521.

Roelands B, Goekint M, Buyse L, Pauwels F, De Schutter G, Piacentini F, Meeusen R, Watson P & Hasegawa H (2009). Time trial performance in normal and high ambient temperature: Is there a role for 5-HT? *Eur J Appl Physiol* **107,** 119–126.

Roelands B & Meeusen R (2010). Alterations in central fatigue by pharmacological manipulations of neurotransmitters in normal and high ambient temperature. *Sport Med* **40,** 229–246.

Romeo S, Gilio F, Pedace F, Ozkaynak S, Inghilleri M, Manfredi M & Berardelli

A (2000). Changes in the cortical silent period after repetitive magnetic stimulation of cortical motor areas. *Exp Brain Res* **135,** 504–510.

Romer L & Polkey M (2008). Exercise-induced respiratory muscle fatigue: implications for performance. *J Appl Physiol Appl Physiol* **104,** 879–888.

Romero MC, Merken L, Janssen P & Davare M (2022). Neural effects of continuous theta-burst stimulation in macaque parietal neurons. *Elife* **11,** e65536.

Rosenthal J, Waller H & Amassian V (1967). An analysis of the activation of motor cortical neurons by surface stimulation. *J Neurophysiol* **30,** 844–858.

Rossi S et al. (2021). Safety and recommendations for TMS use in healthy subjects and patient populations, with updates on training, ethical and regulatory issues: Expert Guidelines. *Clin Neurophysiol* **132,** 269–306.

Rossini PM et al. (2015). Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application: An updated report from an I.F.C.N. Committee. *Clin Neurophysiol* **126,** 1071–1107.

Rounis E & Huang Y (2020). Theta burst stimulation in humans: a need for better understanding effects of brain stimulation in health and disease. *Exp Brain Res* **238,** 1707–1714.

Van De Ruit M, Perenboom MJL & Grey MJ (2015). TMS brain mapping in less than two minutes. *Brain Stimul* **8,** 231–239.

Schindler K, Nyffeler T, Wiest R, Hauf M, Mathis J, Hess CW & Müri R (2008). Theta burst transcranial magnetic stimulation is associated with increased EEG synchronization in the stimulated relative to unstimulated cerebral hemisphere. *Neurosci Lett* **436,** 31–34.

Shepard RJ (2009). Is it Time to Retire the 'Central Governor'? *Sport Med* **39,** 709–721.

Sidhu SK, Weavil JC, Mangum TS, Jessop JE, Richardson RS, Morgan DE &

Amann M (2017). Group III/IV locomotor muscle afferents alter motor cortical and corticospinal excitability and promote central fatigue during cycling exercise. *Clin Neurophysiol* **128,** 44–55.

Søgaard K, Gandevia S, Todd G, Petersen N & Taylor J (2006*a*). The effect of sustained low-intensity contractions on supraspinal fatigue in human elbow flexor muscles. *J Physiol* **573,** 511–523.

Søgaard K, Gandevia SC, Todd G, Petersen NT & Taylor JL (2006*b*). The effect of sustained low-intensity contractions on supraspinal fatigue in human elbow flexor muscles. *J Physiol* **573,** 511–523.

Søgaard K, Gandevia SC, Todd G, Petersen NT & Taylor JL (2006*c*). The effect of sustained low-intensity contractions on supraspinal fatigue in human elbow flexor muscles. *J Physiol* **573,** 511–523.

Stagg C, Best J, Stephenson M, O'Shea J, Wylezinska M, Kincses Z, Morris P, Matthews P & Johansen-Berg H (2009). Polarity-sensitive modulation of cortical neurotransmitters by transcranial stimulation. *J Neurosci* **29,** 5202–5206.

Steenhuis RE, Bryden MP, Schwartz M & Lawson S (1990). Reliability of hand preference items and factors. . *Clin Exp Neuropsychol* **12,** 921–930.

Sundberg CW, Prost RW, Fitts RH & Hunter SK (2019). Bioenergetic basis for the increased fatigability with ageing. *J Physiol* **597,** 4943–4957.

Suppa A, Huang Y, Funke K, Ridding M, Cheeran B, Di Lazzaro V, Ziemann U & Rothwell J (2016). Ten Years of Theta Burst Stimulation in Humans: Established Knowledge, Unknowns and Prospects. *Brain Stimul*.

Suppa A, Ortu E, Zafar N, Deriu F, Paulus W, Berardelli A & Rothwell J (2008). Theta burst stimulation induces after-effects on contralateral primary motor cortex excitability in humans. *J Physiol* **586,** 4489–4500.

Taylor JL (2009). Point: the interpolated twitch does/does not provide a valid measure of the voluntary activation of muscle. *J Appl Physiol* **107,** 354–355.

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

Taylor JL & Gandevia SC (2008). A comparison of central aspects of fatigue in submaximal and maximal voluntary contractions. *J Appl Physiol* **104,** 542–550.

Temesi J, Gruet M, Rupp T, Verges S & Millet GY (2014). Resting and active motor thresholds versus stimulus-response curves to determine transcranial magnetic stimulation intensity in quadriceps femoris. *J Neuroeng Rehabil* **11,** 1–13.

Teo JTH, Terranova C, Swaynethwell JCO & Greenwood RJ (2009). Differing effects of intracortical circuits on plasticity. *Exp Brain Res* **193,** 555–563.

Todd G, Petersen NT, Taylor JL & Gandevia SC (2003). The effect of a contralateral contraction on maximal voluntary activation and central fatigue in elbow flexor muscles. *Exp Brain Res* **150,** 308–313.

Tornero-Aguilera JF, Jimenez-Morcillo J, Rubio-Zarapuz A & Clemente-Suárez VJ (2022). Central and Peripheral Fatigue in Physical Exercise Explained: A Narrative Review. *Int J Environ Res Public Health*; DOI: 10.3390/ijerph19073909.

Torta DME, Legrain V, Algoet M, Olivier E, Duque J & Mouraux A (2013). Theta Burst Stimulation Applied over Primary Motor and Somatosensory Cortices Produces Analgesia Unrelated to the Changes in Nociceptive Event-Related Potentials. *PLoS One* **8,** e73263.

Vanhatalo A, Fulford J, DiMenna F & Jones A (2010). Influence of hyperoxia on muscle metabolic responses and the power-duration relationship during severe-intensity exercise in humans: a 31P magnetic resonance spectroscopy study. *Exp Physiol* **95,** 528–540.

Vernillo G, Temesi J, Martin M & Millet G (2018). echanisms of Fatigue and Recovery in Exercise, Upper versus Lower Limbs in Men. *Med Sci Sports Exerc* **50,** 334–343.

Vitor-Costa M, Okuno N, Bortolotti H, Bertollo M, Boggio P, Fregni F & Altimari L (2015). Improving Cycling Performance: Transcranial Direct Current Stimulation Increases Time to Exhaustion in Cycling. *PLoS One* **10,** e0144916.

Wang Z & Van Praag H (2012). Functional neuroimaging in exercise and sport sciences. Exercise and the brain: neurogenesis, synaptic plasticity, spine density, and angiogenesis. In *Springer*. New York.

Wassermann EM, Fuhr P, Cohen LG & Hallett M (1991). Effects of transcranial magnetic stimulation on ipsilateral muscles. *Neurology* **41,** 1795–1799.

Weavil J & Amann M (2018). Corticospinal excitability during fatiguing whole body exercise. *Prog Brain Res* **240,** 219–246.

Weir JP, Beck TW, Cramer JT & Housh TJ (2006). Is fatigue all in your head? A critical review of the central governor model. *Br J Sports Med* **40,** 573–586.

Westerblad H, Allen D & Lannergren J (2002). Muscle fatigue: lactic acid or inorganic phosphate the major cause? *News Physiol Sci* **17,** 17–21.

Williams C & Ratel S (2009). *Human Muscle Fatigue. Taylor & Francis.*ed. Francis T&.

Williams PS, Hoffman RL & Clark BC (2013). Preliminary evidence that anodal transcranial direct current stimulation enhances time to task failure of a sustained submaximal contraction. *PLoS One*; DOI: 10.1371/journal.pone.0081418.

Williamson J (2010). The relevance of central command for the neural cardiovascular control of exercise. *Exp Physiol* **95,** 1043–1048.

Wilson W & Maughan R (1992). Evidence for a possible role of 5-hydroxytryptamine in the genesis of fatigue in man: administration of paroxetine, a 5-HT re-uptake inhibitor, reduces the capacity to perform prolonged exercise. *Exp Physiol* **77,** 921–924.

Woody CD, Gruen E & Birt D (1991). Changes in membrane currents during

Pavlovian conditioning of single cortical neurons. *Brain Res* **539,** 76–84.

Yang Q, Fang Y, Sun C, Siemionow V, Ranganathan VK, Khoshknabi D, Davis MP, Walsh D, Sahgal V & Guang H (2009). Weakening of Functional Corticomuscular Coupling during Muscle Fatigue. *brain Res* **1250,** 101–112.

Yu F, Tang X, Hu R, Liang S, Wang W, Tian S, Wu Y, Yuan TF & Zhu Y (2020). The After-Effect of Accelerated Intermittent Theta Burst Stimulation at Different Session Intervals. *Front Neurosci* **14,** 1–9.

Ziemann U, Ilic T V., Pauli C, Meintzschel F & Ruge D (2004). Learning modifies subsequent induction of long-term potentiation-like and long-term depression-like plasticity in human motor cortex. *J Neurosci* **24,** 1666–1672.

Ziemann U, Muellbacher W, Hallett M & Cohen LG (2001). Modulation of practicedependent plasticity in human motor cortex. *Brain* **124,** 1171–1181.

Zong X, Gu J, Zhou S, Ding D, Hu Y, Tucker L, Huang Z, Geng D & Gao D (2022). Continuous theta-burst stimulation enhances and sustains neurogenesis following ischemic stroke. *Theranostics* **12,** 5710–5726.