The use of transcranial magnetic stimulation in locomotor function: methodological issues and application to extreme exercise

Utilisation de la stimulation magnétique transcrânienne dans l'évaluation de la fonction motrice : aspects méthodologiques et application à l'exercice extrême

Submitted in fulfillment of the requirements for the degree of Doctor of Philosophy

Soumis pour obtenir le grade de Docteur

de l'Université Jean Monnet de Saint-Etienne

John Temesi

Laboratoire de Physiologie de l'Exercice
Faculté de Médecine
Université Jean Monnet
Saint-Etienne, France

28 October 2013

Jury :

Olivier David, Université Joseph Fourier, Grenoble, France (rapporteur)
Julien Duclay, Université Paul Sabatier, Toulouse, France (examinateur)
Stuart Goodall, Northumbria University, Newcastle upon Tyne, UK (examinateur)
Marc Jubeau, Université de Nantes, France (examinateur)
Romuald Lepers, Université de Bourgogne, Dijon, France (rapporteur)
Guillaume Millet, University of Calgary, Canada (Directeur)
Samuel Vergès, INSERM U 1042, Grenoble, France (Co-directeur)

TABLE OF CONTENTS

TABLE OF FIGURES

TABLE OF TABLES

LIST OF ABBREVIATIONS

AMT	active motor threshold
ANOVA	analysis of variance
BOLD	blood oxygenation level dependent
BF	*biceps femoris*
CAR	central activation ratio
CMEP	cervicomedullary-evoked potential
CSP	cortical silent period
Db10	potentiated doublet (10 Hz)
Db100	potentiated doublet (100 Hz)
EEG	electroencephalography
EMG	electromyography
ERT	estimated resting twitch
GABA	gamma-aminobutyric acid
HR	heart rate
HR_{max}	maximal heart rate
ICF	intracortical facilitation
ITT	interpolated twitch technique
LICI	long-interval intracortical inhibition
MAP	maximal aerobic power output
MEP	motor-evoked potential
Mmax	maximal M-wave
MRI	magnetic resonance imaging
Msup	maximal M-wave during MVC
MT	motor threshold
MVC	maximal voluntary force/contraction
NR	not reported
N/A	not applicable
PNS	peripheral nerve stimulation
RF	*rectus femoris*
RMS	root mean square
RMT	resting motor threshold
RPE	rating of perceived exertion

RT	reaction time
SD	sleep deprivation
SICI	short-interval intracortical inhibition
SIT	superimposed twitch
TF	task failure
TMS	transcranial magnetic stimulation
TwPot	potentiated twitch
VA	voluntary activation
VAc	cortical voluntary activation
VAp	peripheral voluntary activation
VL	*vastus lateralis*
VM	*vastus medialis*
VO_2	oxygen consumption
VO_{2max}	maximal oxygen consumption

STUDY-SPECIFIC ABBREVIATIONS

Study 1

CT	contraction time
CV	coefficient of variation
DEC	contraction where the force decreased to the target
INC	contraction where the force increased to the target
PLA	contraction where there was a plateau at the target force

Study 2

IFCN	International Federation of Clinical Neurophysiology
k	slope parameter (Boltzmann)
MEPmax	estimated maximal MEP amplitude (Boltzmann)
S	stimulus intensity (Boltzmann)
*S*50	stimulus intensity to produce a response half MEPmax (Boltzmann)

Study 3

CO	control condition
D1	day 1 evaluations

$CYCL_{20\text{-}40}$	cognitive evaluations during submaximal cycling
POST 40	neuromuscular evaluations after submaximal cycling
POST TF	cognitive and neuromuscular evaluations post-cycling task failure
PRE	cognitive and neuromuscular measures before cycling
TTF	timed exercise to task failure

Study 4

PRE	evaluation before the ultra-trail
POST	evaluation after the ultra-trail

ACKNOWLEDGEMENTS

I wish to express my enormous gratitude to my supervisor Guillaume Millet for taking a chance on me. It has definitely been a crazy adventure these last three years. Your support and guidance has been incredible, as has the opportunity to fumble around in the dark as I am prone to do. I now know that he has a bit of a soft spot for Canadians, especially those unafraid of Jurassiens.

I am exceptionally grateful to my co-supervisor, Samuel Vergès for all his support. He was also kind enough to aid me with my difficulties *avec la langue française* and to not ban me from Grenoble. He also consented to lodge and feed me (no small task) whenever I was in Grenoble.

To Alain Belli, the director of the Laboratoire de Physiologie de l'Exercice in St-Etienne, thank you for welcoming me and making me feel that I was a valuable member of the team.

To Pierrick Arnal, Dr Léonard Féasson, Cyrille Bankolé and Thomas Lapole, my principal collaborators at the Laboratoire de Physiologie de l'Exercice, it has been a pleasure to work and learn with you.

To Thomas Rupp, Mathieu Gruet, Vincent Martin, Karen Davranche, Marc Jubeau, Damien Bachasson, Dr Bernard Wuyam, Daria Neyroud, Nicolas Place and all the researchers at other institutions that I have had the opportunity to work with and learn from over the last few years, I have greatly appreciated the experience.

To Régis Bonnefoy, without your technical assistance this study would have been impossible. I would be sitting in the middle of hefty and technologically-advanced paper weights wondering what to do. You made my life much easier, and I am sure the lives of everyone else.

To Marion Ravelojaona, Philippe Gimenez, Marlène Giandolini, Pierre Samozino, Victorien Picolet and all the other PhD students and post docs during my time in St-Etienne, thank you for all your assistance, and also for keeping me somewhat sane (it is a tough job but some people have to do it).

To Jean-Benoit Morin, Thierry Busso, Xavier Devillard, and all the other professors and researchers that have shared their expertise with me, I have learnt a great deal.

I am tremendously grateful to Dr Roger Ouillon, Dr Pascal Edouard, Rodolphe Testa and all the rest of the medical, laboratory, technical and administrative staff that have gone out of their way to be of assistance in every way possible and who are too numerous to mention.

To Isabelle Lévaï-Daudel and Anne-Marie Milhau at Pôle Intenational for making my interactions with the infamous French administration a great deal less painful than they could have been.

To the Rhône-Alpes region for sponsoring my PhD and enabling me to travel and share what I have learnt during my PhD with researchers from around the world.

To the organizers and medical commission of the North Face® Ultra-Trail du Mont-Blanc® 2012 and the Institut Fédératif de Recherche en Sciences et Ingénierie de la Santé for their assistance in making Study 4 a reality.

To my parents, for still thinking that I am crazy but getting starting to get used to the idea that I will not change and supporting me all the same.

For donating brain matter, sweat and lonely nights to the cause of research, I am indebted to my subjects. Without you none of this would have been possible.

PUBLICATIONS AND PRESENTATIONS

Journal articles published from this thesis:

Temesi J, Arnal PJ, Davranche K, Bonnefoy R, Levy P, Vergès S, Millet GY. Does central fatigue explain reduced cycling after complete sleep deprivation? *Med Sci Sports Exerc* doi: 10.1249/MSS.0b013e31829ce379.

Gruet M*, **Temesi J***, Rupp T, Millet GY & Vergès S. (2013). Effect of different approaches to target force on transcranial magnetic stimulation responses. *Muscle Nerve* doi: 10.1002/mus.23786.

Journal articles under review from this thesis:

Temesi J, Gruet M, Rupp T, Vergès S, Millet GY. Resting and active motor thresholds versus stimulus-response curves to determine transcranial magnetic stimulation intensity in quadriceps femoris. *J NeuroEng Rehab*.

Temesi J, Rupp T, Martin V, Arnal PJ, Féasson L, Vergès S, Millet GY. Central fatigue assessed by transcranial magnetic stimulation in ultra-trail running. *Med Sci Sports Exerc*.

Conference abstracts from thesis:

Temesi J, Rupp T, Arnal PJ, Martin V, Vergès S & Millet GY. Effect of a 103-km mountain trail-running race on corticospinal voluntary activation and excitability. *ECSS 2013*, Barcelona, Spain.

Temesi J, Rupp T, Martin V, Arnal PJ, Féasson L, Vergès S, Millet GY. Central fatigue assessed by transcranial magnetic stimulation in ultra-endurance trail running. *Exercise Neuroscience Group 2013*, Oshawa, Canada.

Temesi J, Rupp T, Vergès S & Millet GY. Effect of a 103-km mountain trail-running race on neuromuscular function. *ACSM 2013*, Indianapolis, USA.

Temesi J, Rupp T, Arnal PJ, Martin V, Vergès S & Millet GY. Effect of a 103-km mountain trail-running race on corticospinal activation and excitability. *UJM Journée de la Recherche 2013*, St-Etienne, France.

Temesi J, Arnal PJ, Vergès S & Millet GY. Effect of 30-h sleep deprivation on cycling performance and central fatigue. *ECSS 2012*, Bruges, Belgium.

Temesi J, Arnal PJ, Vergès S & Millet GY. Effect of 30-h sleep deprivation on cycling performance and central fatigue. *UJM Journée de la Recherche 2012*, St-Etienne, France.

Other journal articles published during completion of this thesis:

Gruet M, **Temesi J**, Rupp T, Levy P, Millet GY & Vergès S. (2013). Stimulation of the motor cortex and corticospinal tract to assess human muscle fatigue. *Neuroscience* **231**, 384-399.

Bachasson D, **Temesi J**, Bankole C, Lagrange E, Boutte C, Millet GY, Vergès S, Levy P, Féasson L & Wuyam B. (2013). Assessment of quadriceps strength, endurance and fatigue in FSHD and CMT: benefits and limits of femoral nerve magnetic stimulation. *Clin Neurophysiol*. DOI: 10.1016/j.clinph.2013.08.001.

Millet GY, Bachasson D, **Temesi J**, Wuyam B, Féasson L, Vergès S & Levy P. (2012). Potential interests and limits of magnetic and electrical stimulation techniques to assess neuromuscular fatigue. *Neuromuscul Disord* **22**, S181-186.

Other journal articles under review from work during completion of this thesis:

Gruet M, **Temesi J**, Rupp T, Levy P, Millet GY & Vergès S. Dynamics of corticospinal changes during and after a high-intensity quadriceps exercise. *J Physiol*.

Jubeau M, Rupp T, Perrey S, **Temesi J**, Wuyam B, Levy P, Vergès S & Millet GY. Changes in cortical voluntary activation during prolonged cycling exercise. *Exp Physiol*.

Other conference abstracts during completion of this thesis:

Jubeau M, Rupp T, Perrey S, **Temesi J**, Wuyam B, Vergès S & Millet GY. Changes in cortical activation and excitability during prolonged cycling exercise. *ECSS 2012*, Bruges, Belgium.

Invited presentations:

Temesi J. Central and supraspinal fatigue evaluation with endurance exercise. Faculty of Kinesiology and Physical Education, University of Toronto, Toronto, Canada. 10 June 2013.

LITERATURE REVIEW

Fatigue can be categorized in many ways and generally refers to a decrement in measureable performance. Physical fatigue may present as decreased maximal voluntary force (Merton, 1954; Hakkinen, 1994) or decreased maximal power (Beelen & Sargeant, 1991), and in extreme cases complete exercise cessation. This is referred to as task failure. For an athlete, this can be recognized by changes such as a reduction in running speed, jump height or endurance time. Fatigue has also been identified as a primary symptom in a large number of diseases, particularly neuromuscular disorders (Feasson *et al.*, 2006) and their impact in clinical populations can be enormous. Decrements in physical performance as determined by objective or self-reported feelings of fatigue or weakness can have an important impact on daily living and quality of life. Individuals may be unable to perform activities of daily living at the requisite level such as domestic chores, work responsibilities or child care. It may also impair the ability to have an active social life, for example, to participate in leisure activities and take holidays.

As fatigue develops, the energy cost of performing physical activity increases regardless of whether this is during short (Candau *et al.*, 1998; Borrani *et al.*, 2003) or long (Gimenez *et al.*, 2013) duration exercise bouts. Fatigue during physical activity is also associated with higher subjective feelings of effort, or ratings of perceived exertion (RPE) (Borg, 1970). RPE incorporates a variety of factors including physical and mental components (Millet, 2011). As exercise duration at a given intensity increases, RPE increases in parallel until the exercise can no longer be maintained. This may result in decreased exercise intensity (*e.g.* (Martin *et al.*, 2010), or exercise cessation if speed/work is not self-selected (Presland *et al.*, 2005; Pires *et al.*, 2011). Mental fatigue has also been shown to influence physical performance, resulting in decreased time to task failure as higher initial RPE reaches a maximal value sooner (Marcora *et al.*, 2009) or the production of a lower power output at a given RPE (Brownsberger *et al.*, 2013). It has been suggested that higher RPE during a given task may be indicative of the greater cognitive effort required to plan the on-going activity (Berchicci *et al.*, 2013), thus highlighting the importance of the brain and all related inputs and outputs.

CENTRAL FATIGUE AND CEREBRAL PERTURBATIONS WITH EXERCISE

CENTRAL FATIGUE

The concept of fatigue has been of interest to researchers for many years. The precise meaning of fatigue however has undergone tremendous change. The idea of fatigue began as a very vague concept that meant something different to everyone. Originally, fatigue was characterized as an inability to continue working at a given intensity or maintain a required force; however this definition implied that fatigue only occurred at task failure. The fact that the capacity to produce maximal force is impaired almost from the time exercise begins, has led to a more accepted definition of fatigue in the neuromuscular research domain that fatigue is any decrease in the ability to apply muscular force or power caused by exercise whether or not the task can be sustained (Bigland-Ritchie & Woods, 1984).

Until the 1800s, it was not possible to qualify fatigue since there were no accurate methods of assessment. In 1890, Prof Alessandro Mosso investigated the effects of university lecturing on performance of finger movement with a weight (Mosso, 1904). Mosso concluded that the decreased exercise performance, or fatigue, after either prior physical (exercise) or mental (lecturing) activity was due to central nervous system deficiencies. Subsequently, Bainbridge (1919) proposed that there were both central nervous system and muscular components to fatigue, having been influenced both by the work of Mosso and A.V. Hill. Unlike Mosso, Hill supported the widely-held belief that young athletes were able to go all out in their exercise endeavours, implying that fatigue in this group should not have a central component. As such Hill focused his research on the role of carbohydrate and lactic acid on muscular activity (Hill, 1924). The understanding that fatigue is caused by both the central nervous system and muscular factors was furthered by Reid (1928), who observed that mechanical responses to peripheral stimulation of the muscle or its innervating nerve were unaffected after voluntary task failure at some voluntary contraction frequencies (12-80 contractions·min^{-1}) but not others (120-160 contractions·min^{-1}). This suggested that at the lower contraction frequencies, fatigue was exclusively of central nervous system origin but at higher contraction frequencies, there was an additional muscular component.

These two components have become categorized as central and peripheral (**Figure 1**).

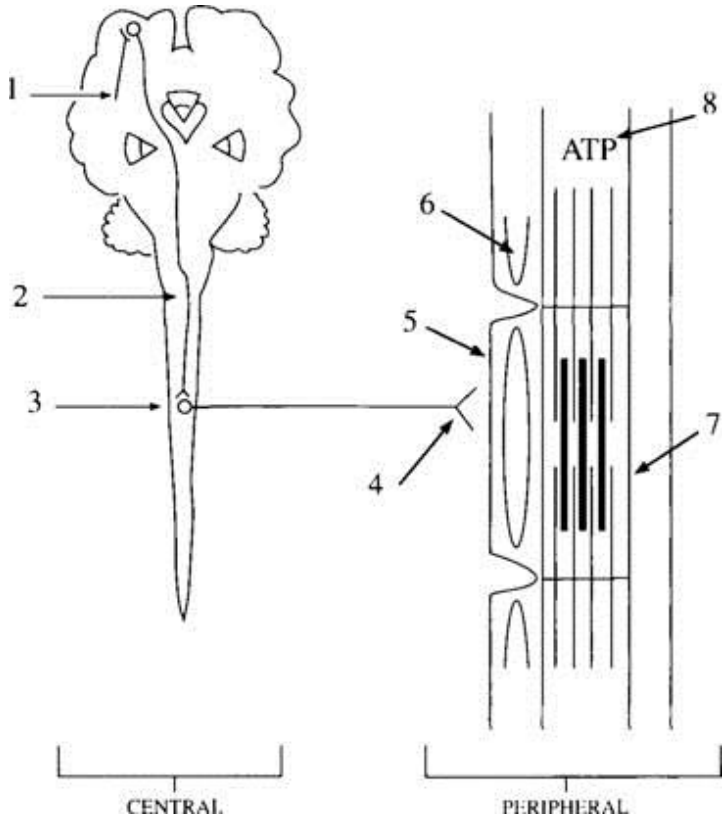

Figure 1. Principal potential sites of fatigue, as first described by Bigland-Ritchie (1984). The central components are (1) excitatory input to higher motor centres; (2) excitatory drive to lower motoneurons; (3) motoneuron excitability; and (4) neuromuscular transmission. The peripheral factors within the muscle include (5) sarcolemmal excitability; (6) excitation-contraction coupling, including T-tubular and sarcoplasmic reticulum Ca^{2+} release and reuptake; (7) contractile mechanisms; and (8) metabolic energy supply and metabolic accumulation. Adapted from Fitts (2011).

The peripheral component is now interpreted to be everything distal to the neuromuscular junction while the central component is everything proximal to the neuromuscular junction. Thus, the central component includes everything that happens in the brain and both the upper and lower motoneurons. This includes decrements to both cognitive performance and motor control and increased RPE (see above). Motor control studies have demonstrated that in a fatigued state subjects employ compensatory systems to try to achieve the same levels of competence or efficiency as in an unfatigued state (Forestier & Nougier, 1998; Berger *et al.*, 2010). While exercise, including maximal exercise, induces benefits in cognitive performance at all exercise intensities (Chang *et al.*, 2012), this benefit may be short-lived. Any benefit is mitigated the longer the exercise continues and eventually cognitive impairments may develop. For example, Grego *et al.* (2005) observed the disappearance of exercise-induced cognitive benefits in the third hour of a 3-h exercise bout and this decline may translate into

functional performance impairment during ultra-endurance events such as adventure racing (Lucas *et al.*, 2009). It is now accepted that both central and peripheral factors have roles in the development of fatigue. Research indicates that they are also interrelated since motoneuronal recruitment depends on the descending drive from supraspinal sites in the brain and central drive is controlled by various factors including excitatory and inhibitory afferents (Amann, 2011).

A major advancement in the evaluation of the central component of fatigue occurred when Merton (1954) demonstrated that the increment in supplementary force provided by electrical neural stimulation during voluntary contraction decreased as the contraction intensity increased, until at maximal voluntary force there was no supplementary increment in force. Merton (1954) concluded that at maximal voluntary effort, muscles are in fact contracting maximally, a finding corroborated by Bigland and Lippold (1954). Merton (1954) also concluded that the linear relationship between supplementary electrically-induced force and voluntary force permits the determination of the theoretical maximal voluntary force by extrapolating this linear relationship.

The potential of twitch interpolation was unfulfilled until Belanger and McComas (1981). Since then, the use of twitch interpolation to assess the ability to voluntarily contract the muscle maximally, voluntary activation (VA), has been extensively employed. Two principal methods have been used; central activation ratio (CAR) and the interpolated twitch technique (ITT) (**Figure 2**). CAR is evaluated by comparing maximal voluntary force with the force produced by tetanic stimuli delivered to the peripheral nerve at maximal force and calculated by the following equation:

$$CAR = (\text{maximal voluntary force} \cdot \text{maximal force}^{-1}) \times 100$$

With ITT, a stimulus (single-, paired- or quadruple-pulse) is delivered to the peripheral nerve at maximal voluntary force and immediately after while the muscle is in the relaxed state. The evoked force increment (superimposed twitch, SIT) at maximal force and the evoked potentiated twitch amplitude are compared by the following equation:

$$ITT = (1-(\text{superimposed twitch amplitude} \cdot \text{potentiated resting twitch amplitude}^{-1})) \times 100$$

Both CAR and ITT may also be evaluated by directly stimulating the muscle. At present, the ITT is the most common method of investigating VA. This method operates on the presumption that descending drive from the motor cortex is the most important factor determining the strength and timing of voluntary contractions. Although both CAR and ITT are expressed as percentages, it is incorrect to interpret these percentages as being a precise or

accurate assessment of the maximal capability of the brain to drive a muscle or group of muscles to make a movement. Instead, VA is a qualitative or semi-quantitative measure indicating motoneuronal drive to the muscle and the (in)ability for this to be converted to force. It does not quantify the source of the motoneuronal drive, motoneuronal firing rates, or motoneuronal input or output. It also cannot account for changing VA of other muscles contributing to a movement or of antagonists. The benefits and limitations of this widely used method have been extensively debated (de Haan *et al.*, 2009; Taylor, 2009). Other methods of evaluating central fatigue include measuring changes in the ratio of maximal voluntary force to induced tetanic force (Bigland-Ritchie *et al.*, 1978) or the ratio of root mean square (RMS) EMG to M-wave amplitude (*e.g.* (Boerio *et al.*, 2005; Garrandes *et al.*, 2007)) or the presence of increase SIT during maximal contractions (Bigland-Ritchie *et al.*, 1983; Gandevia *et al.*, 1996).

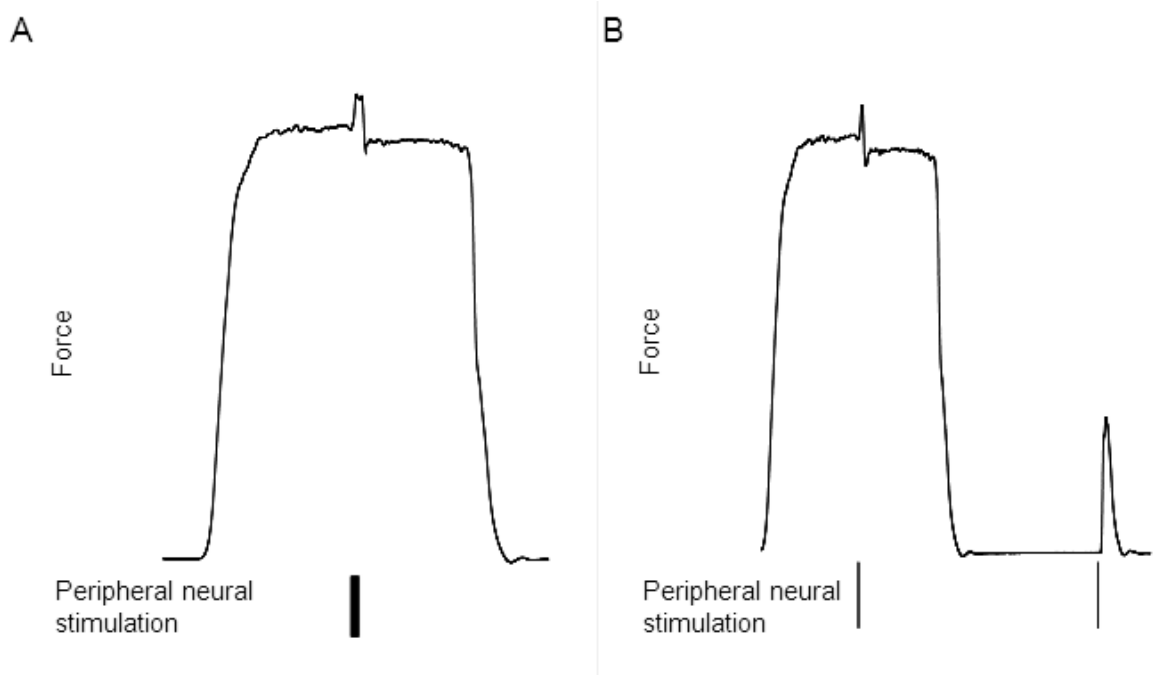

Figure 2. The central activation ratio (CAR) and interpolated twitch technique (ITT). Panel **A**) A high-frequency tetanic stimulation is delivered once the force plateaus during a maximal voluntary contraction (MVC). Panel **B**) A stimulus (*i.e.* usually a single or high-frequency paired pulse) is delivered once the force plateaus during a MVC. A second identical stimulus is delivered when the muscle is in the relaxed and potentiated state immediately after the MVC.

Central fatigue has been observed during and after a variety of exercise protocols. It has been observed after intermittent (Goodall *et al.*, 2010) and sustained (Sogaard *et al.*, 2006; Smith *et al.*, 2007) submaximal and intermittent (Nordlund *et al.*, 2004) and sustained

(Gandevia *et al.*, 1996) maximal isometric voluntary contractions (MVCs). Central fatigue has also been observed after running (Millet *et al.*, 2002; Millet *et al.*, 2003a; Place *et al.*, 2004; Martin *et al.*, 2010; Ross *et al.*, 2010a; Millet *et al.*, 2011c) and cycling (Lepers *et al.*, 2002; Ross *et al.*, 2010b; Decorte *et al.*, 2012). It has been observed more consistently after running than cycling or cross-country skiing of similar duration/intensity (Lepers *et al.*, 2002; Millet *et al.*, 2003a; Millet *et al.*, 2003b; Place *et al.*, 2004), indicating that exercise mode is important in the development of central fatigue.

More recently, attempts have been made to divide the central component of fatigue into several sections to better understand where and how fatigue manifests itself. In addition to stimulation of the lower motoneuron innervating a muscle, stimuli can be delivered to the motor cortex (transcranial stimulation), at the cervicomedullary junction (cervicomedullary stimulation) and at the spinal nerve roots. The stimulation techniques can all be conducted with magnetic stimulation. Before detailing these techniques, methods to investigate motoneuron excitability and other techniques employed to examine central alterations will be described.

Three methods have principally been employed to investigate motoneuronal excitability. These are the H-reflex (Hoffmann reflex), F-waves and V-waves (**Figure 3**).

The H-reflex is a reflex response elicited by a low-intensity stimulus delivered to the peripheral nerve when the muscle is in the relaxed state, or occasionally during weak voluntary contraction. This weak stimulus evokes a single volley from large-diameter muscle Ia afferents that is be modified by pre-synaptic inhibition before recruiting motoneurons according to the Henneman size principle (from small to large). The amplitude of the H-reflex increases with increasing stimulus intensity until maximal excitatory input to the motoneuron is reached. At higher stimulus intensities, the H-reflex response decreases due to increasing collisions with antidromic volleys as the M-wave amplitude increases.

The F-wave is a late response to a supramaximal stimulus delivered to the peripheral nerve in response to motoneurons reactivated by antidromic impulses. When the antidromic impulse reaches the motoneuron body, a small number of normally large motoneurons backfire, initiating an orthodromic pulse that presents as an F-wave. The F-wave is normally evaluated at rest since collisions between antidromic pulses and voluntary orthodromic pulses will permit transmission of an H-reflex and conceal the F-wave. At higher contraction intensities, the resultant response would likely be a V-wave.

The V-wave is a response to a supramaximal stimulus delivered to the peripheral nerve during a maximal or near-maximal voluntary contraction. Collisions between the evoked

antidromic and voluntary orthodromic pulses allow motor axons to conduct a reflex response that can be measured from EMG recordings. Only motor axons actively involved in the contraction contribute to the V-wave because in other motor axons the reflex will either collide with the antidromic pulse or arrive after the antidromic pulse, during which time these motoneurons are refractory. The strength of the voluntary contraction and maximal firing rates within the motoneuron pool are important determinants of V-wave amplitude. Although proposed to be indicative of the amount of descending motoneuronal voluntary drive, motoneuron discharge rate is a reflection of all inputs to the motoneuron, including supraspinal inputs, making it difficult to isolate changes to V-wave amplitude.

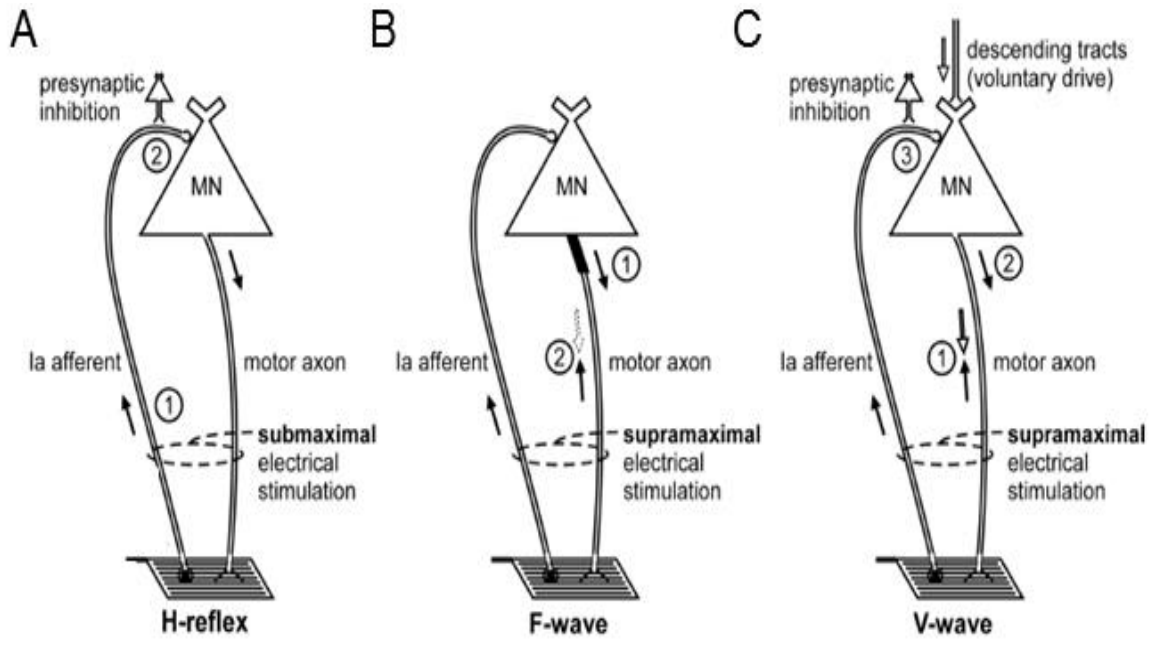

Figure 3. The mechanisms of the H-reflex, F-wave and V-wave. Panel **A**) The H-reflex is elicited by a submaximal neural stimulus [(1)] that evokes a single afferent volley that recruits motoneurons. The response is modified by presynaptic inhibition [(2)]. Panel **B**) The F-wave is elicited by the reactivation of a limited number of motoneurons in response to antidromic pulses evoked by a supramaximal neural stimulus [(1)]. Reflex activation of small motoneurons and their collision with the antidromic volley at rest results in F-waves limited to large motoneurons [(2)]. Panel **C**) The V-wave is elicited by an antridromic pulse from a supramaximal neural stimulus colliding with orthodromic voluntary drive [(1)] and the subsequent reflex response along this pathway [(2)]. The response is modified by presynaptic inhibition [(3)]. Adapted from McNeil *et al.* (2013).

TECHNIQUES OTHER THAN NEURAL STIMULATION TO INVESTIGATE MECHANISMS OF CENTRAL FATIGUE

The presence or absence of central fatigue and the development of central fatigue as evaluated by stimulation of the nerves and muscles are among the most commonly discussed effects of exercise on the brain. However, there are other techniques frequently employed to investigate central perturbations associated with acute exercise bouts. This section provides an introduction to these techniques.

Electromyography

Electromyography (EMG) is a technique used to record the electrical activity in muscles. This activity is the propagation of action potentials along the sarcolemma in individual muscle fibers. The recorded signal represents the sum of all the propagating action potentials in the area. Fine wire electrodes only record electrical activity from several muscle fibers while surface electrodes record from many muscle fibers. This signal is influenced by the number of muscle fibers recruited and their firing rates in addition to other physiological (*e.g.* fibre membrane and motor unit properties, muscle temperature), anatomical (*e.g.* thickness of subcutaneous tissue, pennation angle) and technical (*e.g.* skin-electrode contact, filter, amplification) parameters (Farina *et al.*, 2004).

Central motor command can be evaluated during voluntary muscular contractions from the EMG activity. During constant power output submaximal exercise, raw EMG activity has been observed to increase (Amann *et al.*, 2006; Amann & Dempsey, 2008; Decorte *et al.*, 2012). While raw EMG has been presented as an index of central drive (Viitasalo *et al.*, 1982; Nicol *et al.*, 1991), this may not reflect the reality and numerous methodological limitations must be considered (Dimitrova & Dimitrov, 2003). When normalized to the response to a supramaximal stimulus of the peripheral nerve (*i.e.* maximal M-wave, Mmax), EMG activity provides an indication of central motor drive, including in a fatigued state (Millet *et al.*, 2011b). During isometric MVCs compared before and after a ski marathon (Millet *et al.*, 2003b) and in young adults during series of maximal concentric and eccentric contractions (Baudry *et al.*, 2007), differences in EMG normalized to maximal M-wave were not observed despite decreased EMG activity.

Near-infrared spectroscopy

Near-infrared spectroscopy involves observation of the changes in emitted light of wavelengths within the near-infrared region (800-2500 nm) of the electromagnetic spectrum. Near-infrared light transmits through tissue and it can be used as a non-invasive method to identify local hemodynamic changes. This method employs measurement of radiation intensity and any change indicates hemodynamic (*i.e.* combined myoglobin and hemoglobin) oxygenation changes. Despite the limited penetration of near-infrared light, its inability to differentiate between venous and arterial changes and the influence of bones (skull) and other non-brain tissues on near-infrared signals, this technique is capable of identifying changes in cerebral oxygenation.

Changes in cerebral oxygenation due to exercise can be identified by near-infrared spectroscopy because of the correspondence between brain activity and blood flow within the brain (Rupp & Perrey, 2008). It has been shown that oxygenation of the prefrontal cortex changes as a function of exercise intensity and/or duration (Ide *et al.*, 1999; Rupp & Perrey, 2008). During a maximal incremental cycling test, cerebral oxygenation increased during the first minutes of exercise before plateauing. At exercise intensities near maximum, cerebral deoxygenation occurs just prior to task failure (Rupp & Perrey, 2008; Timinkul *et al.*, 2008). This may occur because of changes in local cerebral blood flow and increased cerebral oxygen consumption and metabolic rate, and thus neither activation nor inhibition can be differentiated.

Doppler ultrasound

Doppler ultrasound is an imaging technique based on the principles of ultrasound, employing oscillating sound pressure waves at a frequency above 20 kHz. In research and diagnostic settings, these waves are generally emitted at frequencies from 2 to 18 MHz. The emitted sound waves are partially reflected and partially transmitted at the limit of two different tissues. The reflected return signal is sampled repeatedly because the time to the return of the signal is related to the depth of the reflecting tissue. Due to the scattering of the signal, tissues perpendicular to the sound waves will better reflect the signal and be easier to identify. This technique permits the visualisation of subcutaneous tissues including blood vessels, muscles, tendons and internal organs.

Cerebral blood flow has generally been assessed by transcranial Doppler. This method measures the velocity of blood flow in proximal intracranial arteries or arteries in the neck. Cerebral blood flow changes are a function of blood flow velocity and blood vessel diameter. Therefore, because of the relative stability of the middle cerebral artery diameter (Secher *et al.*, 2008), this artery is most frequently used to represent changes throughout the brain under a variety of conditions. Blood velocity in the medial cerebral artery increases from rest to submaximal intensities in whole-body exercise (Madsen *et al.*, 1993; Ide *et al.*, 1999). Conversely, cerebral blood flow decreases during high-intensity exercise just prior to task failure (Gonzalez-Alonso et al., 2004). Mechanisms responsible for increased cerebral blood flow during exercise include cerebral autoregulation, the physiological mechanisms maintaining appropriate cerebral blood flow despite changes in perfusion pressure, and carbon dioxide reactivity (Secher *et al.*, 2008).

Cerebral blood flow and arterio-venous differences can be used to estimate mitochondrial oxygenation and metabolic brain responses to exercise (Rasmussen *et al.*, 2007). Reductions in mitochondrial oxygenation have been proposed to be indicative of deficient cerebral aerobic metabolism from a combination of decreased cerebral flow and decreased arterial oxygenation. It is only during high-intensity and maximal exercise that cerebral mitochondrial oxygen tension and metabolism are affected. Maximal exercise could induce decreased cerebral mitochondrial oxygen tension (Rasmussen *et al.*, 2010) and cerebral metabolic substrate preferences could be affected by the marked increase in cerebral lactate (Volianitis *et al.*, 2008).

Magnetic resonance imaging

Magnetic resonance imaging (MRI) is a technique that permits the visualization of internal body structures. The premise of this technique is that a magnetic field causes magnetization of the nuclei of atoms. Radio frequency magnetic fields can be applied to change the alignment of magnetization that causes rotating magnetic fields to be produced by atomic nuclei. The gradients in the magnetic field cause differential nucleic rotational speeds and through Fourier analysis their spatial information can be converted into an image

Functional magnetic resonance imaging can be used to detect the blood oxygenation level dependent (BOLD) signal. The BOLD signal can be used to determine changes in blood oxygenation, flow and/or volume because of the difference in spin states of haemoglobin with and without bound oxygen. Simple exercises such as finger-tapping and sustained

submaximal hand-grip contractions showed increases in the BOLD signal during exercise (Kastrup *et al.*, 2002; Liu *et al.*, 2003; Sander *et al.*, 2010). Although use of MRI to evaluate central changes during whole-body exercise is not feasible, the BOLD signal may open up opportunities to evaluate more types of exercise (Mehta *et al.*, 2009).

Electroencephalography

Electroencephalography (EEG) is a technique employed to record the electrical activity in the brain. Electrodes placed on the scalp are used to record fluctuations in voltage resulting from cerebral intra-neuronal ionic current flow. Rhythmic EEG signals fall into one of six wave patterns; delta, theta, alpha, beta, gamma and mu. Transient EEG signals may appear for a variety of reasons. Electroencephalographic investigations may also include the use of evoked or event-related potentials. The former technique averages electroencephalographic activity time-locked to the presentation of a stimulus while the latter is time-locked to stimuli processing.

Initial investigations into EEG changes and exercise compared pre- to post-intervention changes. Generally, increased post-exercise electroencephalographic activity was observed after moderate- to high-intensity exercise with differences related to exercise familiarity and preference. Interestingly, athletes experienced reduced frontal beta activity after high-intensity exercise in their chosen sport, indicating deactivation of emotional brain regions (Brummer *et al.*, 2011). Recently, EEG has begun to be measured during exercise. Across the spectrum increased EEG activity was observed in incremental cycling exercise at high intensities and task failure (Bailey *et al.*, 2008). Increased theta activity, even at low exercise intensites suggests that differential EEG increases may be related to exercise intensity. Post-exercise EEG activity returned to baseline by 10 min post-exercise. Further evidence for a link between exercise intensity and EEG comes from the correlation between EMG and EEG at higher cycling power outputs (Schneider *et al.*, 2013). A recent study reported high prefrontal cortex activity in subjects experiencing fatigue that correlated to increased RPE, suggesting that increased RPE may lead to detriments in attentional focus and abstract planning, and thus play a role in central fatigue (Berchicci *et al.*, 2013).

NEUROMUSCULAR STIMULATION

ELECTRICAL STIMULATION

In electrical circuits, there is a flow of electrons from the anode to the cathode. This flow of electric current, as with electrical stimulation of peripheral nerves, must sufficiently alter the flow of current within the axon of the nerve to induce stimulation. Transmembrane current flow is essential to change the electric field along the axon, and thus for there to be a change in the current flowing through the axon. The ability to induce a response, or stimulate the nerve, is proportional to the rate of electric field change, or spatial derivative. The spatial derivative of the electric field along nerve cannot be zero for stimulation to occur. In electrical stimulation, peripheral stimulation occurs easily once a certain threshold is reached because the current passes through the body/limb and bisects the nerve. **Figure 4** illustrates the mechanism of electrical stimulation through transmembrane current flow.

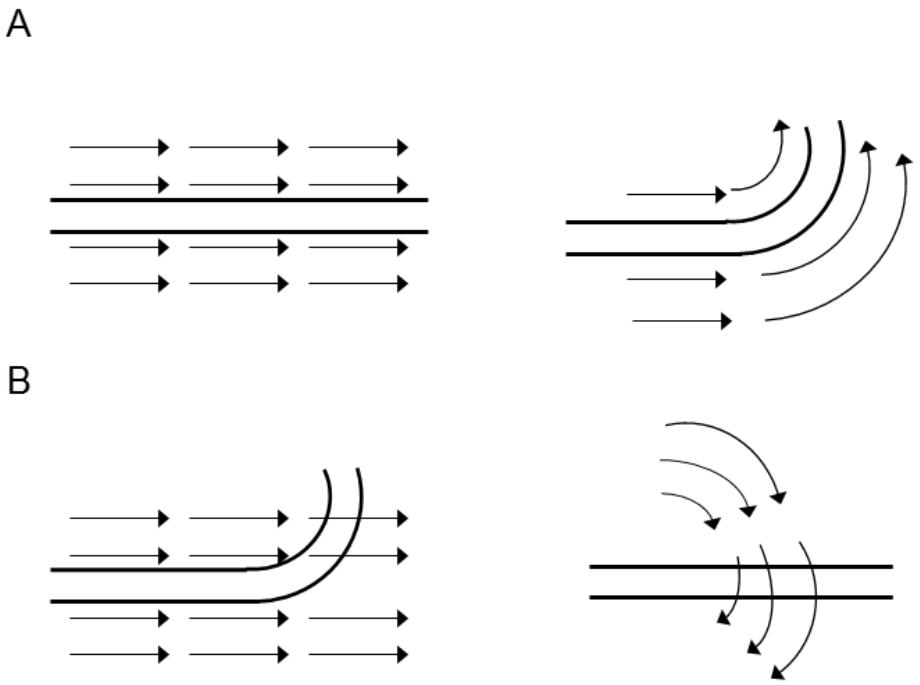

Figure 4. The mechanism of neural stimulation. Panel **A**) Stimulation of the axon cannot occur regardless of current intensity because the induced current does not cross the axon (*i.e.* no transmembrane current flow). Panel **B**) The axon will be stimulated if the induced transmembrane current flow is of sufficient intensity to alter the current flow along the axon. Adapted from (Barker, 1999).

MAGNETIC STIMULATION

In 1985 the first magnetic stimulation of the human motor cortex (Barker *et al.*, 1985) was documented. This represented a coming of age of magnetic stimulation. Modern magnetic stimulation is actually a simple feat extended from the basic principles of magnetic induction. In 1831, Michael Faraday took an iron ring and wound a coil of wire on opposite sides of the ring. When current to one of the coils was turned on or off, there was a brief flow of current in the other coil. Although facilitating the magnetic field between coils, the iron ring was quickly observed to be unnecessary since the air between the coils can act as the medium for magnetic field conduction. Prior to the demonstration of Barker *et al.* (1985), others had touched upon the potential of magnetic stimulation. Initial observations were that crude direct stimulation of the retina affected vision more than 100 years ago (d'Arsonval, 1896; Thompson, 1910). Developments then led to the stimulation of frog preparations of nerve and muscle (Oberg, 1973) and then recording of the first human M-waves elicited by peripheral nerve stimulation (Polson *et al.*, 1982).

Magnetic stimulation is based upon the rate of change of the magnetic field emitted from a coil. The differential rate of change of the magnetic field creates virtual anodes and cathodes, areas of depolarization and hyperpolarization, respectively. The rate of change of the induced magnetic field is the means by which an electric current is induced in the tissues of the body. This electric current, not the induced magnetic fields, if sufficiently strong, causes depolarization of cell membranes in human tissue and results in stimulation of the tissue.

The strength of the magnetic field decreases rapidly as the distance from the stimulating coil increases, thus it is most effective to stimulate with the coil in direct contact with the body. The rise time, the maximal energy delivered to the coil and the spatial distribution of the magnetic field affect magnetic pulse characteristics. The latter is dependent on coil form and the local anatomy at the site of induced electric current flow, while the former two depend on the coil and stimulator characteristics.

Coils

Circular coils

The first magnetic stimulation coils were circular in shape. These are the least complicated and easiest to manufacture; however, the stimulated region is not precise. The area stimulated with a circular coil is not below the centre (or hole in the middle). Instead it occurs around the coil winding (**Figure 5**). Any nerve that passes tangentially to the coil has an equal likelihood of being stimulated. Circular coils of 70 and 90 mm in diameter are frequently utilized, meaning that nerves passing through a large area may be stimulated at once. Decreasing the diameter of the coil increases precision (*i.e.* specificity of a single nerve in peripheral stimulation or focus on a certain brain area in transcranial magnetic stimulation, TMS); however, small coils have greater difficulty in diffusing the energies produced and thus overheat more readily. Furthermore, smaller coils have a reduced depth of penetration, thus requiring higher stimulus intensities to reach deep structures and this may cause increased discomfort for the subject. The use of circular coils is widespread because they can be placed over most areas of the body with relative ease.

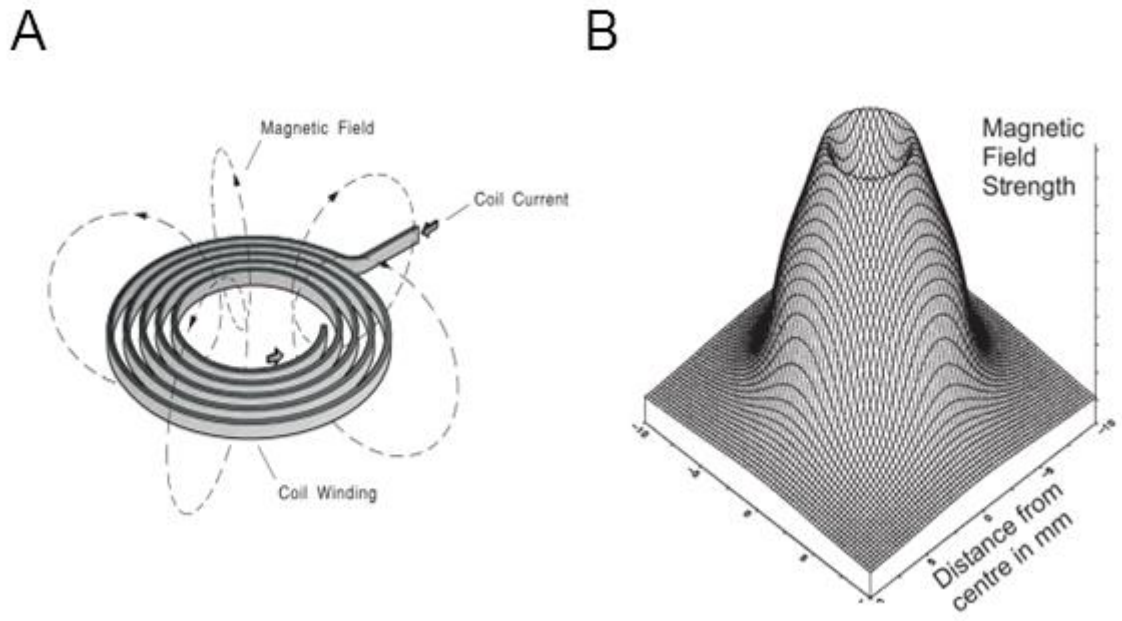

Figure 5. Magnetic field produced by a circular coil. Panel **A**) The lines of force produced as current flows through the windings of a circular coil. Panel **B**) The magnetic field strength from a 90-mm circular coil. The magnetic field strength is greatest underneath the coil winding and rapidly decreases towards the centre or further away from the coil Adapted from Hovey and Jalinous (2006).

Figure-of-eight coils

A major advancement from circular coils was the figure-of-eight coil. This type of coil is comprised of two circular coils with current rotating in opposite directions. This coil type permits greater precision since the induced electric field change is greatest along the central axis at the intersection of the two coil windings (*i.e.* primary virtual anode and cathode on either side of the intersection of the coil windings) (**Figure 6**). Although stimulation may occur at any point tangential to the coil, the likelihood of an induced response at a given stimulus intensity is much greater at the intersection point.

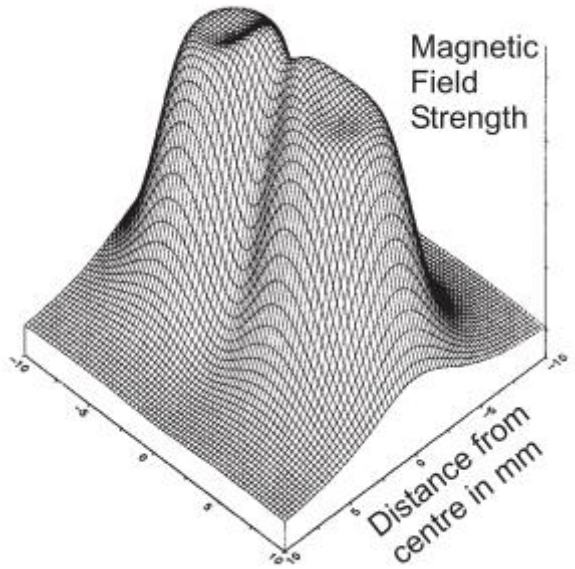

Figure 6. Magnetic field produced by a 70-mm figure-of-eight coil. The magnetic field strength is greatest where the coil windings meet. Adapted from Hovey and Jalinous (2006).

Double-cone coils

As denoted by Penfield's homunculus (Penfield & Rasmussen, 1950), the legs are of relatively minor importance in the motor cortex and their representation is close to or in the central sulcus. The imprecise nature of circular and figure-of-eight coils has limited investigations of motor cortical-induced responses in the lower limbs. By changing the position of the two coils, from side-by-side on the same plane to side-by-side with an acute angle in the middle, the coil conforms to the spherical nature of the head and can stimulate with both greater precision and to a greater depth (**Figure 7**). This facilitates the stimulation

of deeper brain structures and more inaccessible parts of the brain, including areas projecting to the lower limbs. The double-cone coil also induces greater current at the central axis than regular figure-of-eight coils.

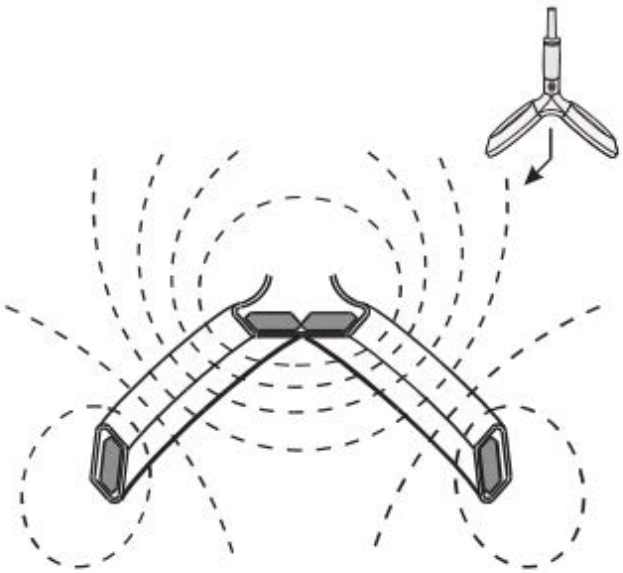

Figure 7. The lines of force of the magnetic field produced by a double-cone coil. The magnetic field strength is greatest on the underside (*i.e.* the side in contact with the head) where the coil windings meet. Adapted from Hovey and Jalinous (2006).

Stimulator types

Stimulators are capable of emitting two output waveforms. In some cases a stimulator can only produce one waveform while other stimulators have the option to produce both types of waveforms or polyphasic waveforms (*i.e.* repeated biphasic waveforms). The waveform produced by a stimulator is fundamental to the responses induced because the waveform is a function of the rate of change of the induced current, and thus the resulting magnetic field. The two commercially available stimulator waveform types are characterized as:

Monophasic

A monophasic waveform is characterized by a rapid rate of change of induced current during the initial quarter cycle followed by a gradual return to baseline. Examples of monophasic stimulators include MagPro X100 and Magstim 200/200².

Biphasic

A biphasic waveform is characterized by a rapid rate of change of induced current throughout the waveform. Initially, there is a rapid change and this is followed by an equally rapid change in the opposite direction before returning to baseline. Examples of biphasic stimulators include MagPro R30, MagPro X100, Magstim Rapid², Nexstim NBS System 4 and Cadwell MES-10.

Early studies employing magnetic stimulators paid little attention to the type of waveform employed. Investigations have since examined the effects of waveform on TMS responses since the type of waveform influences TMS-induced responses. In the muscle, TMS induces a compound muscle action potential, termed motor-evoked potential (MEP), observed in EMG recordings (**Figure 8**). Resting motor threshold (*i.e.* the minimum stimulus intensity to elicit MEPs in response to at least half the stimuli when the muscle is in the relaxed state, RMT) (Kammer *et al.*, 2001; Sommer *et al.*, 2006) is waveform dependent. For example, Sommer *et al.* (2006) observed increased RMT with monophasic waveforms compared to biphasic wave forms. Posterior-anterior brain current flow was also observed to result in a lower monophasic RMT while with a biphasic waveform, RMT was lower when current flowed in the anterior-posterior direction within the brain. A shorter cortical silent period was also observed after delivery of monophasic TMS compared to biphasic TMS. Differences in response to monophasic and biphasic waveforms have also been identified in repetitive TMS (Arai *et al.*, 2005; Hosono *et al.*, 2008) including a greater reduction in corticospinal inhibition after repetitive TMS with a monophasic waveform than biphasic waveform (Sommer *et al.*, 2002).

The decision to use magnetic stimulation for research or clinical purposes must first consider the advantages and disadvantages of this method in conjunction with the ability for magnetic stimulation to assess the required parameters. Compared to electrical stimulation, magnetic stimulation causes less discomfort (Barker, 1999). Magnetic stimulation also facilitates the use of human brain stimulation in applied environments. Conversely, the equipment utilized in magnetic stimulation (*i.e.* stimulator, coils) is expensive and it is impossible to use this technique to stimulate with the same precision as electrical stimulation. In addition, certain medical conditions (*e.g.* epilepsy for TMS) and the presence of ferro-magnetic implants in the area of stimulation preclude its use.

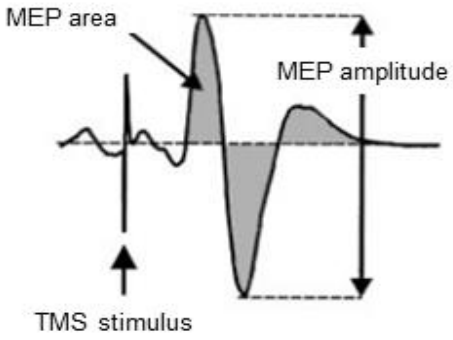

Figure 8. The motor-evoked potential (MEP). MEP amplitude is measured peak-to-peak and MEP area is shaded grey. Adapted from Taylor *et al.* (2000).

Magnetic stimulation, particularly TMS has applications in a variety of fields. TMS has been employed in the study of psychiatry (Fitzgerald *et al.*, 2002; Berlim *et al.*, 2013), vision (Vesia *et al.*, 2008; de Graaf *et al.*, 2012), language (Papeo *et al.*, 2013), emotion (Balconi & Ferrari, 2012), brain plasticity (Stefan *et al.*, 2000; Villamar *et al.*, 2012), mapping functions of cortical regions (Paiva *et al.*, 2012) and fatigue (Taylor *et al.*, 1999; Sogaard *et al.*, 2006; Sidhu *et al.*, 2009b; Goodall *et al.*, 2012).

MAGNETIC STIMULATION FOR FATIGUE

Magnetic stimulation has been used extensively as a substitute for electrical stimulation in research and clinical evaluation of fatigue. The use of magnetic stimulation has been employed to stimulate peripheral nerves, the cervicomedullary junction and the motor cortex (by TMS).

PERIPHERAL MAGNETIC STIMULATION

Peripheral magnetic stimulation has been used in fatigue evaluation in both healthy and clinical populations. To date only a small number of studies have employed peripheral magnetic stimulation, with most investigations continuing to opt for electrical stimulation. A limiting factor in the use of peripheral magnetic stimulation is the distance between the targeted nerve and the coil. In individuals with a substantial layer of adipose tissue over the stimulation site, it may be impossible to achieve stimulus intensity supramaximality (Tomazin *et al.*, 2011). When supramaximality is achieved, electrically- and magnetically-evoked

single- and paired-pulse responses are comparable as demonstrated before and after 30 min of downhill running (Verges *et al.*, 2009) (**Figure 9)**. Several lower-limb studies have evaluated CAR in the *quadriceps femoris* with a magnetic pulse train delivered at maximal force (Kremenic *et al.*, 2009; Glace *et al.*, 2013) before and after cycling bouts. A restraint to the use of magnetic stimulation to evaluate CAR is that there are limits to stimulus frequency and intensity and their interaction, thus making the use of supramaximal-intensity stimulus trains problematic. Specifically, these studies state that they employed a pulse train at 40 Hz at an intensity of 100% maximal stimulator output. This protocol also required 8 booster units, so while theoretically possible, it is not practical for most laboratories or hospitals. Most lower-limb studies employing magnetic stimulation have evaluated VA by ITT. These include

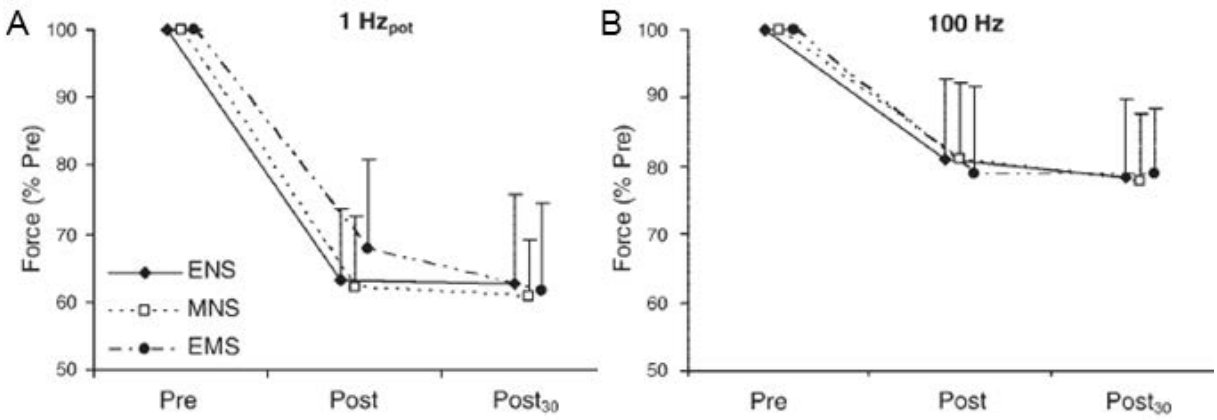

Figure 9. Peak evoked forces elicited in the relaxed muscle by electrical neural stimulation (ENS), magnetic neural stimulation (MNS) and electrical muscle stimulation (EMS) before (Pre), immediately after (Post), and 30 min after (Post30) exercise. All values are means ± standard deviations and presented as a percentage of Pre values. Panel **A**) Potentiated twitch amplitude (single pulse) and Panel **B**) potentiated doublet amplitude (paired pulse at 100 Hz). Adapted from Verges *et al.* (2009).

whole-body exercise protocols such as before and after a treadmill running marathon (Ross *et al.*, 2007), before, during and after an intermittent cycling protocol (Decorte *et al.*, 2012) and before and after a 6-min walk test in chronic obstructive pulmonary disease patients (Mador *et al.*, 2001). Peripheral magnetic stimulation has also been used to investigate the effects of fatigue before, during and after isometric contraction protocols in both healthy (Bachasson *et al.*, 2013b; Decorte *et al.*, 2013) and clinical populations (Bachasson *et al.*, 2013a; Bachasson *et al.*, 2013c).

CERVICOMEDULLARY JUNCTION MAGNETIC STIMULATION

Cervicomedullary junction stimulation at the level of the foramen magnum and mastoids is employed to stimulate the corticospinal axons at the point closest to the brain that is not influenced by cortical excitability. Usually conducted by electrical stimulation, this painful method (McNeil *et al.*, 2013) evokes single volleys in descending axons of upper motoneurons and elicits cervicomedullary-evoked potentials (CMEPs) in the muscle (Berardelli *et al.*, 1991). Although it is recognised that ascending pathways and descending pathways in addition to the corticospinal tract will be triggered by a stimulus at the cervicomedullary junction, existing research suggests that these will have little if any influence on the production of CMEPs (Berardelli *et al.*, 1991; Gandevia *et al.*, 1999). The cervicomedullary junction can also be stimulated magnetically by placing the coil approximately over the inion (Taylor, 2006) although this is relatively rare since the distance from the spinal cord to the coil is large (~7-8 cm) causing the induced magnetic current to be sub-optimal at this depth. This likely explains why in fatigue studies employing cervicomedullary junction stimulation to elicit CMEPs, electrical stimulation of the cervicomedullary junction has usually (*e.g.* (Gandevia *et al.*, 1999; Butler *et al.*, 2003; McNeil *et al.*, 2009; McNeil *et al.*, 2011a; McNeil *et al.*, 2011b; Sidhu *et al.*, 2012a)) but not always (Levenez *et al.*, 2008; Hoffman *et al.*, 2009; Giesebrecht *et al.*, 2011) been employed.

TRANSCRANIAL MAGNETIC STIMULATION

Transcranial magnetic stimulation is a non-invasive, safe and relatively painless technique to investigate the motor cortex. Unlike with peripheral stimulation, there are important differences between TMS and transcranial electrical stimulation. Transcranial electrical stimulation directly excites pyramidal tract axons at either the initial portion of the neuron or at proximal internodes within the subcortical white matter, eliciting descending D-waves. Conversely, TMS trans-synaptically excites the pyramidal neurons although direct excitation of pyramidal tract axons is believed to occur to various degrees depending on a variety of factors such as stimulus intensity (Houlden *et al.*, 1999; Terao *et al.*, 2000), coil orientation (Sakai *et al.*, 1997; Terao *et al.*, 2000) and muscle investigated (Day *et al.*, 1989; Awiszus & Feistner, 1994; Houlden *et al.*, 1999). The response to TMS is predominantly that of descending I-waves. D-waves indicate the degree of direct pyramidal tract stimulation. I-

waves may also appear at short intervals and this is believed to represent repeated firing of pyramidal tract neurons after a cortical stimulus.

Transcranial magnetic stimulation can elicit both excitatory and inhibitory responses that present in EMG. These include both the MEP and cortical silent period (CSP) elicited by single-pulse TMS. MEPs are the recorded electrical responses in muscle elicited by TMS (**Figure 8**) and are a direct result of the descending D and I waves. Due to the possibility for TMS to cause multiple discharges of a single motoneuron, MEP amplitude/area can exceed that of Mmax. Changes in MEP amplitude/area are indicative of changes in cortical excitability (*i.e.* efficiency in motor command generation). Conversely, the CSP is the TMS-induced period of EMG near-silence after the MEP (**Figure 10**) and in the evaluation of fatigue is generally measured as the duration from TMS delivery to the resumption of continuous voluntary EMG (Taylor *et al.*, 2000). Changes to CSP duration are proposed to be indicative of changes in intracortical inhibition. Additionally, paired TMS pulses can be used to assess changes in cortical facilitation (*e.g.* intracortical facilitation, ICF) and inhibition (*e.g.* short- (SICI) and long- (LICI) interval intracortical inhibition) (see sections below).

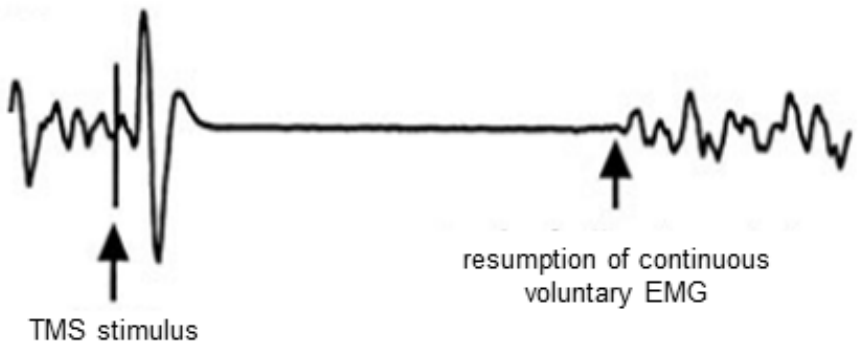

Figure 10. The cortical silent period (CSP). CSP is the duration from the delivery of TMS to the resumption of continuous voluntary EMG. Adapted from Taylor *et al.* (2000).

Initial investigations with TMS delivered single and then paired pulses while the muscle was in the relaxed state. Unlike peripheral nerve stimulation which stimulates the lower motoneurons that are unaffected or only marginally affected by voluntary contraction intensity (Todd *et al.*, 2003; Lee & Carroll, 2005), TMS-induced motoneuronal output is greatly affected by the rapid increase in corticospinal excitability from rest to weak and moderate voluntary muscular contractions (Ugawa *et al.*, 1995). Therefore, the investigation of central parameters (*e.g.* MEP, CSP, SICI, ICF, LICI) measured in contracting muscle

before, during and after an exercise intervention permits greater understanding of the origins of corticospinal changes with fatigue than SIT alone. In isolation, TMS can only identify corticospinal changes. In conjunction with cervicomedullary junction stimulation, TMS can be used to partition responses and changes into cortical/supraspinal and spinal components.

Methodological issues

A major difficulty in interpreting the results of different protocols employing TMS is that there are many technical and methodological differences. For example, different stimulators cannot be compared due to differences in stimulator properties. Kammer *et al.* (2001) showed a difference in RMT between two stimulator systems and also between monophasic and biphasic waveforms. Similarly, the differential induced magnetic fields created by different coil types (*i.e.* circular, figure-of-eight, double-cone) and different winding diameters may lead to stimulation of different brain structures at the same coil position and stimulus intensity. It is unknown whether differences in equipment are capable of producing conflicting or contradictory results in the evaluation of fatigue.

Fortunately, despite numerous companies manufacturing magnetic stimulators, most laboratories employing TMS to evaluate fatigue use Magstim stimulators, theoretically making comparison of stimulus intensities more feasible. It is also possible to employ two stimulators to deliver single TMS pulses at greater than 100% maximal stimulator output (The Magstim Co. Ltd., 2013). Furthermore, stimulus intensity is always presented as a percentage of maximal stimulator output. Without the use of standardized units, knowledge of the relationship between the percentage of maximal stimulator output and the resulting induced magnetic field or whether all stimulators of the same model induce identical magnetic fields under identical conditions (*i.e.* same stimulator intensity and same coil), comparison between studies remains difficult.

Determination of optimal coil position has been largely mysterious. Most studies have indicated that the optimal coil position was where the largest MEP was elicited. Information concerning such details as the stimulus intensity to determine the position, whether this was conducted with the muscle relaxed or during voluntary contractions and the number of responses considered for each site is generally lacking. Furthermore, the use of posterior-anterior current in the brain (Davey *et al.*, 1994; Rossini *et al.*, 1994; Kammer *et al.*, 2001; Groppa *et al.*, 2012) is standard in many TMS studies, including those investigating fatigue of the lower limbs (Goodall *et al.*, 2009; Sidhu *et al.*, 2009b; Goodall *et al.*, 2010; Goodall *et al.*,

2012; Iguchi & Shields, 2012; Sidhu *et al.*, 2012a; Sidhu *et al.*, 2013b). This is despite some studies suggesting that other coil orientations stimulate different muscles better than others (Mills *et al.*, 1992; Werhahn *et al.*, 1994), including differences between upper- and lower-limb muscles (Rosler *et al.*, 1989). In all cases, the rationale for utilization of a certain coil orientation is because studies have shown it to identify the lowest RMT (Davey *et al.*, 1994; Balslev *et al.*, 2007). The only apparent rationale for assessing the efficacy of coil orientation to minimize the intensity at RMT and not on the size of the elicited responses (*e.g.* MEP) is that this method permits the selection of lower TMS stimulus intensities since many studies have used RMT to determine stimulus intensity.

While most studies have used RMT as a basis to determine TMS intensity, the evaluation of fatigue inherently requires muscular contraction. Recently, other methods have been employed to determine TMS intensity and these include active motor threshold (*i.e.* the minimum stimulus intensity to elicit a MEP in at least half of responses when the muscle is contracted weakly, *e.g.* 3-10% MVC; AMT) (*e.g.* (Kalmar & Cafarelli, 2006; Iguchi & Shields, 2012)), stimulus-response curves (Rupp *et al.*, 2012) and a stimulus intensity to evoke MEP responses of a certain size in the target muscle during voluntary contraction (*e.g.* (Sidhu *et al.*, 2009b; Klass *et al.*, 2012)). The advantages and disadvantages of these methods have not yet been elucidated. **Table 1** details methodological aspects of TMS investigations in the lower limbs that have selected a specific TMS intensity for investigative purposes. These include the coil and stimulator used, the methods of determining both coil position and stimulator intensity and the stimulator intensity selected.

The best method of determining TMS intensity remains to be determined. It is unknown whether the different methods employed to determine TMS intensity result in selection of the same intensity. Furthermore, it is unknown whether selection of TMS stimulus intensity should always be conducted in the same manner. Current recommendations principally address evaluations for clinical purposes (Groppa *et al.*, 2012) and it remains to be determined if these can be applied to the evaluation of fatigue in a healthy population. It also remains to be investigated if the manner of approaching a target force influences elicited responses, particularly because of the importance of contraction intensity on corticospinal excitability.

Table 1. A summary of methodological characteristics of lower-limb TMS protocols in healthy populations.

Reference	Stimulator	Coil	Muscle(s) investgated[1]	Coil position selection	TMS intensity selection	Stimulator intensity (% maximum)	Fatigue protocol/acute exercise intervention
(Beck *et al.*, 2007)	Magstim 200	90-mm circular coil	TA, SOL, *gastrocnemius*[2]	motor hotspot for TA	80 (for condition pulse only) 90, 100 and 120% RMT	mean RMT ~60%[3]	N
(Fernandez-del-Olmo *et al.*, 2013)	Magstim 200[2]	70-mm figure-of-eight coil	VL (BF)	largest RF MEP and smallest BF MEP[4]	elicit RF MEP area of >90% Mmax at 50% MVC[4]	75-95%	Y
(Girard *et al.*, 2013)	Magstim 200	130-mm double-cone coil	VL (BF)	over left motor cortex to elicit largest VL MEPs and small BF MEPs (<20% VL MEP) during contractions at 50% MVC and 60% maximal stimulator output	140% AMT determined at 50% MVC[5]	58 ± 13% (42-87%)	Y
(Goodall *et al.*, 2009)	Magstim 200	110-mm double-cone coil	VL (BF)	over left motor cortex to elicit large VL MEPs and small BF MEPs	130% RMT	75±11%	N
(Goodall *et al.*, 2010)	Magstim 200	110-mm double-cone coil	VL (BF)	over left motor cortex to elicit large VL MEPs and small BF MEPs	130% RMT	73 ± 7%	Y
(Goodall *et al.*, 2012)	Magstim 200	110-mm double-cone coil	VL (BF)	over left motor cortex to elicit large VL MEPs and small BF MEPs	130% RMT	67 ± 9%	Y
(Griffin & Cafarelli, 2007)	MES-10 (Cadwell)	angled figure-of-eight coil	TA	over TA cortical motor area to produce the largest MEP in response to low-intensity TMS	120% RMT; 120% AMT determined at 10% MVC	means ~65-66%[3,6]	N

(Hilty *et al.*, 2011)	Magstim 200	130-mm double-cone coil	VM	over vertex to elicit largest VM MEPs	120% AMT determined at 3% MVC	62 ± 3%	Y
(Hoffman *et al.*, 2009)	Magstim 200²	120-mm double-cone coil	SOL, MG	slightly left of vertex; determined at 30% MVC	120% SOL AMT determined at 30% MVC	NR	Y
(Iglesias *et al.*, 2012)	Magstim 200	double-cone coil	VL, SOL, TA	in sagittal plane 0-5 cm posterior to the vertex with a rotation of 5-30° to elicit largest VL MEPs	90% AMT and intensity to elicit MEPs of ~10% M-wave amplitude (~140 AMT), both determined during walking and tonic VL contraction	means ~27% and 42-44%	N
	Magstim Rapid	double-cone coil	VL, SOL, TA	in sagittal plane 0-5 cm posterior to the vertex with a rotation of 5-30° to elicit largest VL MEPs	90-95% AMT determined during walking and tonic VL contraction	means ~28-29%	N
(Iguchi & Shields, 2012)	Magstim 200[2]	110-mm double-cone coil	SOL (TA)	largest SOL MEP from repeated trials via a grid	120% AMT determined at 10% MVC	71 ± 12%	Y
(Kalmar & Cafarelli, 2006)	MES-10 (Cadwell)	angled figure-of-eight coil	VL	over left vertex to elicit large VL MEPs at 80% maximal stimulator output	110% AMT determined at 3% MVC	66 ± 10%[3]	Y
(Kamibayashi *et al.*, 2009)	Magstim 200	110-mm double-cone coil	RF, BF, SOL, TA	over left motor cortex to elicit largest TA MEPs	to produce TA MEP amplitude of ~0.1 mV during upright standing with 40% body weight unloading	41-62%	N
(Klass *et al.*, 2012)	Magstim 200	130-mm double-cone coil	RF, VM (BF)	1-2 cm to the left of vertex to optimally stimulate RF and VM	to elicit large MEP in both RF and VM, small MEP in the BF, and biggest SIT at the different target torques in the protocol	30-60%	Y

(Krishnan & Dhaher, 2012)	Magstim 200	110-mm double-cone coil	AL, RF, VL, VM	over left motor cortex to elicit largest AL and VM MEPs	to elicit MEPs during 10% MVC hip adduction clear and distinguishable from background EMG	NR	N
(Lagerquist *et al.*, 2012)	Magpro R30	figure-of-eight (MC-B70) coil	SOL	over left and right motor cortices at site where clear MEPs elicited at lowest intensity	AMT and 120% AMT at 5% maximal SOL EMG activity	NR	N
(Lentz & Nielsen, 2002)	Magstim 200	130-mm double-cone coil	TA (SOL)	site of lowest threshold and shortest latency	120% RMT	55.1 ± 8.6%	Y
(Mang *et al.*, 2011)	Magpro R30	parabolic (MMC-140) or figure-of-eight (MC-B70) coil	TA, VM, SOL	optimal stimulus site for each muscle	120% RMT	NR	N
(McKay *et al.*, 1995)	Magstim 200	96-mm inside diameter double-cone coil (type 9902)	quadriceps, hamstrings, TA, SOL	centred over the scalp	indicates 80% maximal stimulator output required to elicit repeatable MEPs and 45-85% maximal stimulator output to elicit MEPs in all muscles, both at rest	NR	Y
(McKay *et al.*, 1996)	Magstim 200	96-mm inside diameter double-cone coil (type 9902)	TA	centred over the scalp vertex	Paradigm 1: RMT between 40% and 50% maximal stimulator output. 80% maximal stimulator output empirically determined to elicit MEPs easily differentiated from background EMG during MVCs Paradigm 2: 110% RMT	Paradigm 1: 80% Paradigm 2: 45-55%[7]	Y
(Mileva *et al.*, 2009)	Magstim Bistim 200	110° double-cone coil (P/N 9902-00)	SOL, TA	centred over scalp in the area of vertex	120% RMT	NR	Y

(Mileva *et al.*, 2012)	Magstim Bistim 200	110° double-cone coil (P/N 9902-00)	SOL, TA	centred over scalp in the area of vertex	120% RMT	NR	Y
(Racinais & Girard, 2012)	Magstim 200	120-mm double-cone coil	VL, RF	positioned over vertex	140% MT[8]	68 ± 7%	Y
(Ross *et al.*, 2007)	Magstim 200	70mm figure-of-eight coil	TA	mapping procedure performed for optimal TA activation (0–3 cm lateral to vertex)	120% RMT and 100% maximal stimulator output	NR and 100%	Y
(Ross *et al.*, 2010b)	Magstim 200	90-mm cone figure-of-eight coil	VL	mapping procedure performed for optimal VL activation (0–15 mm contralateral to vertex)	120% RMT	60 ± 8%	Y
(Ross *et al.*, 2012)	Magstim 200	90-mm cone figure-of-eight coil	VL	mapping procedure performed for optimal VL activation (0–10 mm contralateral to vertex)	130% RMT	88 ± 10%	N
(Rupp *et al.*, 2012)	Magstim 200	110-mm double-cone coil	VL, RF, VM (BF)	largest RF MEP and small BF MEP during 20% MVC contractions	lowest intensity to elicit maximal RF MEP from 50% MVC stimulus-response curve at 30, 40, 50, 60, 70, 80, 90 and 100% maximal stimulator output	60 ± 10%	N
(Sammut *et al.*, 1995)	Magstim 200	110-mm double-cone coil	SOL, TA	NR	120% RMT	mean ~ 55%[3]	N
(Sidhu *et al.*, 2009a)	Magstim 200²	130-mm double-cone coil	RF (BF)	to elicit RF MEPs during weak contractions	largest RF MEP (at least 50% Mmax) at 50% MVC and small BF MEP (< 10% of raw RF MEP amplitude)	40-60%	N

(Sidhu *et al.*, 2009b)	Magstim 200²	130-mm double-cone coil	RF (BF)	to elicit RF MEPs	largest RF MEP at 50% MVC and small BF MEP (< 10% of raw RF MEP amplitude)	30-60%	Y
(Sidhu *et al.*, 2013b)	Magstim 200²	130-mm double-cone coil	VL, BF, TA	largest VL MEPs during small tonic contraction while seated on cycle ergometer	10 stimuli at >AMT and sham each randomly delivered at selected crank angle during 75% MAP cycling. Average EMG with respect to stimuli for a 100-ms period beginning 20-ms before each stimulus overlaid to determine effect of TMS on EMG amplitude. TMS intensity then decreased by ~ 5%, and repeated until no facilitation observed in EMG trace	18.5 ± 0.8%	Y
(Sidhu *et al.*, 2012a)	Magstim 200²	130-mm double-cone coil	VL, RF	to elicit VL MEPs during a submaximal contraction at 20% of maximal EMG during MVC	to elicit a MEPs of similar size to CMEPs (*i.e.* ~10% of M max)	41.4 ± 0.9%	Y
(Sidhu *et al.*, 2012b)	Magstim 200²	130-mm double-cone coil	VL, RF, VM	optimal location to elicit MEPs in right quadriceps muscles	test pulse at 140% AMT determined at 50% maximal EMG and conditioning pulses at 70, 80, 90 and 95% AMT	Mean test stimulus 39-42% depending on condition	N
(Stevens-Lapsley *et al.*, 2013)	Magstim BiStim 200^2	NR	VL	largest and most consistent MEPs from a number of positions on a grid	80 and 120% RMT for paired pulses; 120% AMT determined at ~20 maximal EMG for single pulses	mean ~ 56%	N
(Tallent *et al.*, 2012)	Magstim 200^2	110-mm double-cone coil	TA (LG)	search for hotspot began 5-10 mm posterior and along anteroposterior plane of vertex	120% RMT	mean ~55%[9]	N

(Tallent *et al.*, 2013)	Magstim 200[2]	110-mm double-cone coil	TA	coil placed over primary motor cortex of contralateral hemisphere corresponding to the dominant leg area	120% RMT	mean ~54%[3]	N
(Tarkka *et al.*, 1995)	Magstim 200	96-mm diameter double-cone coil	SOL, TA, hamstring and quadriceps femoris	vertex	lowest of 20, 40, 60, 80 and 100% maximal stimulator output above early response threshold	80% for one subject; 60% for all others	N
(Verin *et al.*, 2004)	Magstim 200	45-mm figure-of-eight cone coil	RF, lowest intercostal space	largest MEPs in diaphragm and RF at rest at 100% maximal stimulator output	120% RMT for diaphragm	90 ± 12%[3]	Y
(Weier *et al.*, 2012)	Magstim BiStim 200[2]	90-mm circular coil	RF	largest MEP in area ~ 3–4 cm anterior to vertex	120% AMT determined at 10% MVC for single pulses; 70 and 120% AMT for paired pulses	mean ~53%[3]	N

AL, *adductor longus*, AMT, active motor threshold; BF, *biceps femoris*; CSP, cortical silent period; LG, *lateral gastrocnemius*; MAP, maximal aerobic power output; MEP, motor-evoked potential; MG, *medial gastrocnemius*; MT, motor threshold; RF, *rectus femoris*; RMT, resting motor threshold; SOL, *soleus*; TA, tibialis anterior; TMS, transcranial magnetic stimulation; VL, *vastus lateralis*; VM, *vastus medialis*.

[1] in cases where an agonist-antagonist pair was evaluated, the antagonist is indicated in parentheses
[2] head of the *gastrocnemius* not specified
[3] calculated from information in methods or results
[4] indicates that RF was used to determine coil position and TMS intensity although EMG was only recorded in VL and BF
[5] AMT determined by the presence of at least 3 of 5 MEPs (>50 μV) at a given stimulus intensity
[6] at 120% AMT only; stimulator intensity at RMT or 120% RMT not reported
[7] unclear if this is RMT or 110% RMT
[8] not clear whether this is AMT or RMT but appears to be determined during cycling
[9] calculation from RMT determined by article figure

Table 2. A summary of changes in VA, MEP and CSP with exercise in lower-limb protocols.

Reference	Type of effort	Protocol	Duration of effort	SIT change (PRE-POST or *kinetics*)	ERT (%)	ERT (pooled/ by series)	VA change (PRE-POST or *kinetics*)	Method of evaluation	MEP change (PRE-POST or *kinetics*)	CSP	CSP change (PRE-POST or *kinetics*)
ISOMETRIC PROTOCOLS and EVALUATIONS AT REST											
(Lentz & Nielsen, 2002)	isometric dorsiflexion	sustained contractions from 100 to75, 100 to 50, 100 to 25 and 50 to 25% MVC	means of 21-22 s, 55-57 s, 147-159 s, 204-221 s	NR	N/A	N/A	N/A	rest	increased	N/A	N/A
(McKay *et al.*, 1995)	isometric right dorsiflexion	sustained MVC until force decreased below 50% initial MVC	80.4 ± 6.6 s	NR	N/A	N/A	N/A	rest	decreased[4]	N/A	N/A
ISOMETRIC PROTOCOLS and ISOMETRIC EVALUATIONS											
(Goodall *et al.*, 2009)	isometric right knee extension	sustained MVC	2 min	increased MVC SIT[2]	25-50-75-80-100% or 50-75-80-100%	by series	decreased	isometric right knee extension	no change	N/A	N/A
(Goodall *et al.*, 2010)	isometric right knee extension	5 x initial 60% MVC + 1 MVC (5 s on/5 s off, 15 s between sets) until failure to reach the 60% target force 3 times in one set	24.7 ± 5.5 min	increased MVC SIT[2]	50-75-100%	pooled	decreased	isometric right knee extension	no change	interval from stimulus when post-stimulus EMG exceeded ± 2SD of pre-stimulus EMG for ≥100 ms	no change

(Hilty *et al.*, 2011)	isometric right knee extension	30 s at 3% MVC followed by sets of 8 contractions at ~63% MVC and 1 MVC (5 s on/5s off, 30 s between sets) until TF (<70% initial MVC or failure to maintain two consecutive submaximal contractions) and then 30 s at 3% MVC	mean 9-10 min	NR	N/A	N/A	N/A	isometric right knee extension	no change	interval from stimulus to resumption of spontaneous EMG	increased
(Hoffman *et al.*, 2009)	isometric right plantar flexion	sustained 30% MVC contraction to TF	434 ± 84 s	NR	N/A	N/A	N/A	isometric right plantar flexion	*increased*	N/A	N/A
(Iguchi & Shields, 2012)	isometric plantar flexion	45 MVCs (7 s on/3 s off) in 9 epochs of five contractions followed by 1 10% initial MVC contraction each epoch	1035 s	NR	N/A	N/A	N/A	isometric plantar flexion	*no change at MVC; increased at 10% MVC*	interval from stimulus to the return of continuous EMG	no change
(Kalmar & Cafarelli, 2006)	isometric right knee extension	MVC, 8 contractions at 50% MVC and MVC (4 s on 2 s off, sets separated by 12 s during which a 3% MVC was performed for delivery of 4 TMS pulses)	mean 5-6 min	NR[3]	N/A	N/A	N/A	isometric right knee extension	decreased	N/A	N/A

(McKay *et al.*, 1996)	isometric right knee extension	sustained MVC	2 min	NR	N/A	N/A	N/A	isometric right knee extension at 10% MVC	no change[5]	interval from MEP latency determined in relaxation to the return of recognizable EMG	increased
	isometric right knee extension	sustained MVC	2 min	NR	N/A	N/A	N/A	isometric right knee extension	*no change*[5]	interval from MEP latency determined in relaxation to the return of recognizable EMG	*increased*
(Mileva *et al.*, 2012)	isometric right dorsiflexion	repeated MVCs (2 s on/1 s off) until force decreases below 50% initial MVC	368 ± 51 s	increased; *increased*[6]	50-75-100%	by series	decreased	maximal isometric right dorsiflexion	decreased; *increased*[6,7]	interval from stimulus to return of EMG ≥50% pre-stimulus EMG	no change; *increased*[6,7]
DYNAMIC EXERCISE and EVALUATIONS AT REST or AT REST AND ISOMETRIC CONTRACTIONS											
(Ross *et al.*, 2007)	treadmill running	42.2 km treadmill run starting at -5% lactate threshold velocity (permitted to change ± 10%)	208 ± 22 min	NR	50-75-100%	pooled	decreased	rest	decreased raw MEP	interval from stimulus to return of EMG ≥50% pre-stimulus EMG	no change
(Ross *et al.*, 2010b)	cycling	2007 Tour de France (20 stages in 22 days)	165 ± 66 km·day^{-1}; 522 ± 111 min·day^{-1}	NR	N/A	N/A	N/A	rest	decreased raw MEP	N/A	N/A

(Verin *et al.*, 2004)	treadmill walking/ running	Bruce protocol	18 ± 4 min	NR	N/A	N/A	N/A	rest	raw MEP amplitude decreased	N/A	N/A
DYNAMIC EXERCISE and ISOMETRIC EVALUATION											
(Fernandez-del-Olmo *et al.*, 2013)	cycling	2 x 30-s Wingate with evaluation after each	60 s[1]	NR	50-75-100%	by series	decreased	isometric right knee extension	increased at 50 and 75% MVC; no change at 100% MVC	interval from stimulus to the return of background EMG	no change
(Girard *et al.*, 2013)	cycling	10 x 6-s sprints (30-s recovery), 6-min break and then 5 x 6-s sprints (30-s recovery)	90 s	NR	50-75-100%	by series	decreased	isometric right knee extension	no change	interval from stimulus to the return of background EMG	no change
(Goodall *et al.*, 2012)	cycling	77 ± 5% MAP to TF	8.1 ± 2.9 min; 3.6 ± 1.3 min	NR	50-75-100%	by series	decreased	isometric right knee extension	no change	interval from stimulus when post-stimulus EMG exceeded ±2SD of pre-stimulus EMG for ≥100 ms	no change
(Klass *et al.*, 2012)	cycling	60 min at 55% MAP then TT equivalent to 30 min at 75% MAP	60 min + 30.78 ± 2.08 min	NR	50-75-100%	pooled	no change	isometric right knee extension	no change	interval from stimulus to the return of continuous EMG	no change
(Sidhu *et al.*, 2009b)	cycling	8 x 5-min with 1 min rest at 80% MAP	47 min	NR	50-75-100%	by series	decreased	isometric right knee extension	no change	interval from stimulus to the return of continuous EMG	no change

DYNAMIC EXERCISE and DYNAMIC EVALUATIONS											
(Mileva *et al.*, 2009)	sustained squat	sustained squat at 30° knee flexion	330 s	NR	N/A	N/A	N/A	sustained squat	*no change*	N/A	N/A
(Racinais & Girard, 2012)	cycling	20 min cycling at 100 W and incremental cycling test to TF	20 min and mean time 14-17 min	N/A	N/A	N/A	N/A	cycling at 100 W when crank was at 45°	*no change*	N/A	N/A
(Sidhu *et al.*, 2012a)	cycling	30 min at 75% MAP then 105% MAP to task failure	31.3 ± 0.2 min	N/A	N/A	N/A	N/A	cycling	no change in MEPs normalized to Mmax; decreased MEPs normalized to EMG	N/A	N/A
(Sidhu *et al.*, 2013b)	cycling	75% MAP	30 min	N/A	N/A	N/A	N/A	cycling	N/A	start and end of EMG suppression (any period EMG lower than mean EMG for ≥4 ms from 20 and 50 ms for VL/ BF and 30–60 ms for TA) determined and amount of EMG suppression compared[8]	*increased EMG inhibition*

CSP, cortical silent period; ERT, estimated resting twitch; MAP, maximal aerobic power output; MEP, motor-evoked potential; MVC, maximal voluntary force contraction; N/A, not applicable (the parameter was not evaluated); POST, measure after the exercise intervention; PRE, measure before the exercise intervention; SIT, superimposed twitch; TF, task failure; VAc, cortical voluntary activation; MAP, maximal aerobic power output

[1] two times 30 s
[2] only reported for MVCs
[3] only PRE-POST caffeine capsule SIT changes reported
[4] raw MEPs only. M-waves reported to be unchanged by the intervention
[5] raw MEP amplitude increased during MVC
[6] only measured during the first and last MVCs of the fatiguing protocol
[7] raw MEP amplitude
[8] EMG suppression instead of CSP employed to quantify changes in intracortical inhibition

Upper limbs

TMS investigations began with muscles of the hand and arms. In the motor cortex, these muscles are much better represented than the muscles of the lower limbs. As previously described, magnetic stimulation began with circular coils that lacked precision, thus rendering TMS only feasible in the upper limbs.

Cortical voluntary activation

As in isometric MVCs with peripheral neural stimulation, the SIT evoked by TMS can increase, indicating that supraspinal mechanisms contribute to the observed fatigue (Gandevia *et al.*, 1996). While the presence of increased SIT indicates the presence of supraspinal fatigue, it does not eliminate the possibility of spinal contributions to central fatigue. The increased SIT only means that despite the increasing possibility for improved neural drive from the motor cortex, the brain is unable to provide it. Increased TMS-evoked SIT at maximal force has been observed in upper-limb muscle groups in both intermittent (Hunter *et al.*, 2008) and continuous (Todd *et al.*, 2005) fatiguing exercise protocols. During sustained submaximal contractions, there was a gradual development of supraspinal fatigue that was demonstrated by increasing SIT at the submaximal contraction intensity and confirmed during brief MVCs at regular intervals during low-intensity sustained elbow-flexor contractions of 5% (Smith *et al.*, 2007) and 15% (Sogaard *et al.*, 2006) MVC **(Figure 11**).

Cortical voluntary activation (VAc) assessed by TMS is more complicated than ITT with peripheral nerve stimulation (Todd *et al.*, 2003) since it is inappropriate to compare SIT elicited during MVCs to evoked responses in the relaxed muscle. The large increase in corticospinal excitability from rest to even weak voluntary muscular contractions (Ugawa *et al.*, 1995) means that TMS-induced motoneuronal output at rest is not representative of that at maximal voluntary force. Therefore a potentiated twitch induced by TMS delivered in the relaxed muscle would be greatly underestimated, and thus underestimate the cortical drive to the muscle. Todd et al. (2003) proposed the extrapolation of the linear relationship between SIT and voluntary force between 50 and 100% MVC to estimate the amplitude of the resting twitch that would be produced by TMS under comparable conditions of corticospinal excitability. Originally applied in the elbow flexors (Todd *et al.*, 2003), the validity and reliability of extrapolating the relationship between TMS-evoked SIT and voluntary forces at 50%, 75% and 100% MVC has also been confirmed in the wrist extensors (Lee *et al.*, 2008).

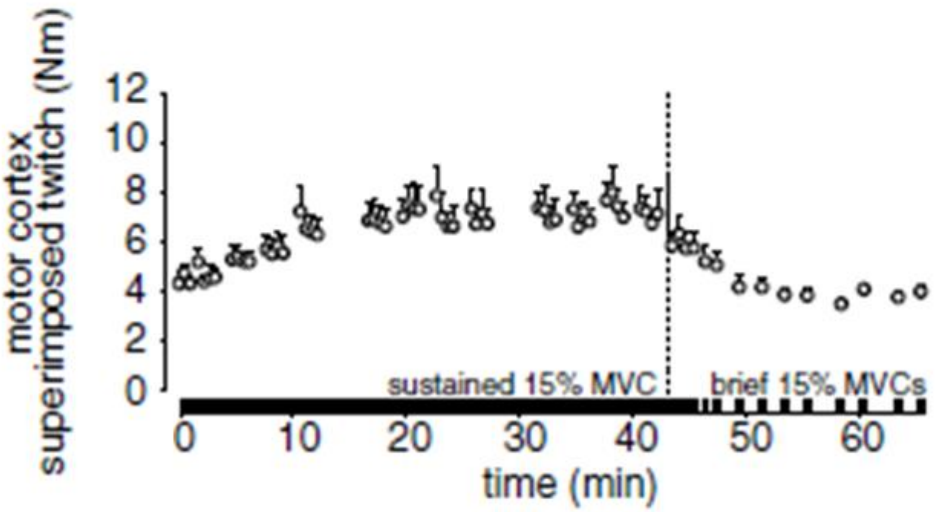

Figure 11. The evolution of SIT during a 43-min sustained isometric contraction at 15% MVC and during recovery contractions at 15% MVC. The broken vertical line denotes the end of the 43-min sustained contraction. Adapted from Sogaard *et al.* (2006).

It is acknowledged that VAc can be quantified by this method in fresh and fatigued muscles although there are some methodological concerns in addition to those associated with peripheral assessment of VA (VAp). The regression of voluntary force and the SIT is almost always linear in the unfatigued state, allowing estimation of resting twitch amplitude, and therefore VAc (Todd *et al.*, 2003; Hunter *et al.*, 2006; Cahill *et al.*, 2011). This relation may frequently be non-linear ($r < 0.9$) during or after a fatigue protocol (*e.g.* up to one-third of contraction sets in Hunter et al. (2006; 2008)), thus preventing the estimation of the resting twitch in some subjects (del Olmo *et al.*, 2006; Hunter *et al.*, 2006; Hunter *et al.*, 2008). To obtain a valid linear extrapolation, it is essential that the stimuli activate most of the motoneurons, which is possible at high levels of voluntary force (*i.e.* > 50% MVC in *biceps brachii* and *brachioradialis*) as demonstrated by MEPs of maximal amplitude (Taylor *et al.*, 1997; Todd *et al.*, 2003). Indeed, TMS is less effective at activating motoneurons at lower contraction intensities because of reduced corticospinal excitability (Todd *et al.*, 2003). This is demonstrated by a curvilinear relationship between SIT and voluntary force at contraction strengths below 50% MVC (del Olmo *et al.*, 2006; Lee *et al.*, 2008). It may also be impossible to obtain a SIT at high contraction intensities (>75% MVC) (del Olmo *et al.*, 2006), a phenomenon also observed in ITT with peripheral nerve stimulation (discussed in (de Haan *et al.*, 2009; Taylor, 2009)). Therefore, if a SIT can be evoked at near-maximal

contraction intensities and if the SIT-voluntary force relationship (50-100% MVC) is linear ($r \geq 0.9$), then it is appropriate to estimate resting twitch amplitude and calculate VAc.

During sustained maximal (Hunter *et al.*, 2006; Szubski *et al.*, 2007) and submaximal (Smith *et al.*, 2007) isometric fatiguing contractions, VAc decreases, suggesting that supraspinal fatigue develops progressively. The evaluation of VAc with dynamic upper-body exercise has never been conducted; therefore it is unknown whether VAc changes with a similar time-course during dynamic exercise.

As with the presence of increased SIT during sustained voluntary contractions, the decreased VAc observed in the aforementioned studies and indicating the presence of supraspinal fatigue does not eliminate the possibility of spinal contributions to central fatigue. Furthermore, the proportion of central fatigue corresponding to each level of the motor pathway cannot be completely elucidated without the combination TMS and cervicomedullary and spinal nerve root stimulation. Smith *et al.* (2007) attempted to quantify the amount of central fatigue originating solely at the supraspinal level. This was done by determining the post-intervention MVC if VAc had remained unchanged. The additional decrease in MVC was attributed to a decrease in VAc (*i.e.* supraspinal fatigue). After a 70-min 5% MVC sustained elbow flexion, they concluded that 66% of the decrease in MVC was due to supraspinal fatigue. It remains to be determined whether this is a valid method of quantifying supraspinal fatigue in isolation.

Motor-evoked potentials

Changes in MEP amplitude or area indicate changes in corticospinal excitability. Normalized MEP size (*i.e.* normalized to maximal M-wave) rather than raw MEP amplitude/area should be used since normalized MEPs take into account peripheral changes such as any change in the rate of action potential propagation along the sarcolemma. In conjunction with changes to CMEP amplitude/area, MEPs can be used to identify a change or lack of change at the cortical level. For example, similar changes in $CMEP \cdot Mmax^{-1}$ and $MEP \cdot Mmax^{-1}$ indicate that most or all of the difference observed occurs at the spinal level while differential responses indicate changes at the supraspinal level (**Figure 12**).

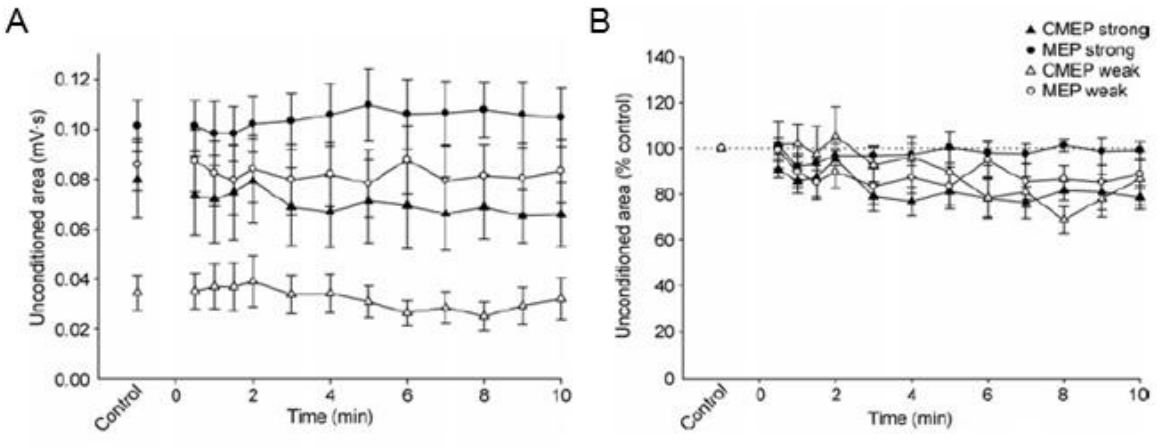

Figure 12. Motor-evoked potentials (MEPs) and cervicomedullary-evoked potentials (CMEPs) at two different stimulus intensities during a 10-min sustained iso-EMG elbow flexion at 25% maximal *biceps brachii* EMG. Panel **A**) Absolute MEP and CMEP area. Panel **B**) Normalized MEP and CMEP areas as a percentage of pre-exercise (control) values. There were no differences in the evolution of MEP and CMEP areas elicited by weak stimuli indicating that the decreased MEP and CMEP amplitude from 7-9 min was due to spinal changes. Conversely, in response to strong stimuli, MEP area remained unchanged while CMEP area decreased and was significantly smaller than baseline from 8-10 min, indicating corticospinal changes to compensate for the decreased spinal excitability. Adapted from McNeil *et al.* (2011a).

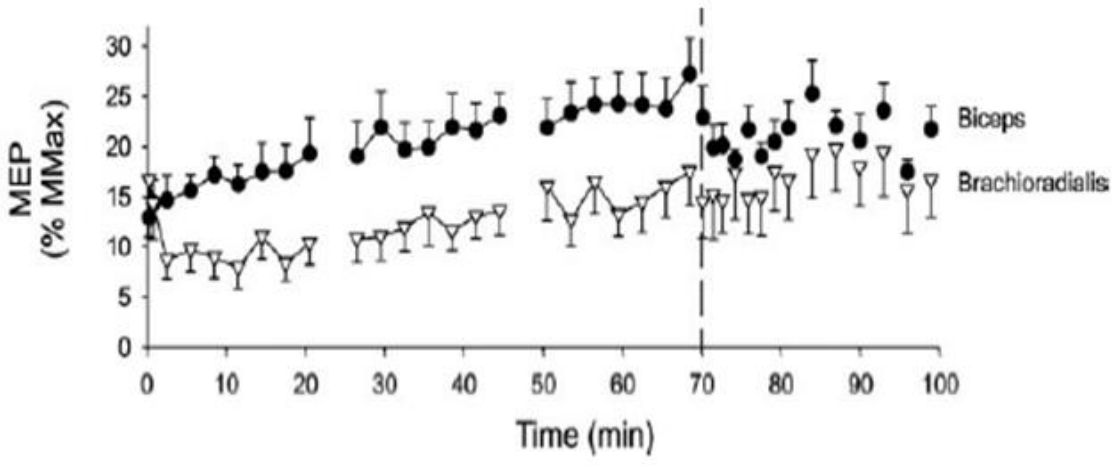

Figure 13. Changes in motor-evoked potential (MEP) area normalized to maximal M-wave (Mmax) in the *biceps brachii* and *brachioradialis* muscles during a 70-min sustained isometric contraction of the elbow flexors at 5% MVC and during recovery contractions at 5% MVC. Adapted from Smith *et al.* (2007).

During sustained iso-force submaximal isometric contractions, MEP·Mmax^{-1} has been observed to increase in the elbow flexors (Sogaard *et al.*, 2006; Smith *et al.*, 2007; Klass *et*

al., 2008; Levenez *et al.*, 2008; Yoon *et al.*, 2012) (**Figure 13**). In conjunction with a progressive increase in voluntary EMG activity, this has been interpreted as an augmentation of central drive to lower motoneurons to maintain a constant force level despite peripheral fatigue development (Sogaard *et al.*, 2006; Smith *et al.*, 2007). These observations are consistent with increased corticospinal excitability in submaximal fatiguing contractions. During a 50% MVC elbow-flexor contraction to task failure, similar MEP·Mmax^{-1} and CMEP·Mmax^{-1} kinetics were observed (*i.e.* increasing over the first 40% of the task and then a plateau to task failure) (Levenez *et al.*, 2008), suggesting that central changes almost entirely occurred at the spinal level. McNeil et al. (2011a) also investigated corticospinal changes with a sustained submaximal contraction; however, this was conducted at constant EMG activity (*i.e.* iso-EMG) with both strong and weak stimulus intensities. During a 10-min sustained elbow-flexor contraction at 25% of maximal EMG signal, MEP·Mmax^{-1} area did not change while CMEP·Mmax^{-1} area decreased and was lower than baseline values from 8 min of exercise in response to strong stimuli (*i.e.* TMS intensity of 155.8 ± 43.0% RMT, actual TMS intensity not reported). These results suggest a compensatory increase in cortical excitability to counteract decreased spinal excitability, in contrast with findings from investigation of MEP and CMEP kinetics during constant force contractions employing strong stimuli (*i.e.* TMS intensity of 70-90% maximal stimulator output) (Levenez *et al.*, 2008). Because voluntary EMG progressively increased during the constant force task in Levenez *et al.* (2008) while in McNeil *et al.* (2011a) it was unchanged, changes in evoked corticospinal responses in this type of protocol should be interpreted in relation to changes in volitional EMG since they may intrinsically influence evoked EMG responses. McNeil *et al.* (2011a) also investigated MEP and CMEP changes elicited by weak stimuli (*i.e.* TMS intensity of 124.2 ± 24.5% RMT, actual TMS intensity not reported) during another 10-min iso-EMG contraction. In this contraction, there were no differences in either MEP or CMEP evolution with decreased MEP and CMEP area observed from 7-9 min. These findings suggest the stimulus intensity may be an important factor influencing corticospinal excitability and that further research needs to be conducted to elucidate the impact stimulus intensity has on MEP amplitude/area changes with fatigue and the reasons for any observed differences.

During a sustained MVC, MEP size has been observed to increase, either progressively (Szubski *et al.*, 2007) or during the first seconds before plateauing (Taylor *et al.*, 2000; Hunter *et al.*, 2006; Hunter *et al.*, 2008). Concomitant normalization of MEP with an index of peripheral transmission (*i.e.* maximal M-wave) is essential because M-wave amplitude and/or area can increase, decrease or remain unchanged during a sustained MVC (Mills & Thomson,

1995; McKay *et al.*, 1996; Taylor *et al.*, 1999; Taylor & Gandevia, 2001). Increasing MEP·Mmax^{-1} during a sustained MVC has been observed in the *biceps brachii* (Taylor *et al.*, 1999) and *first dorsal interosseous* (Szubski *et al.*, 2007). Conversely, CMEP·Mmax^{-1} decreased in the final 30 s of a sustained 2-min elbow-flexor MVC (Butler *et al.*, 2003). This contrasts the increase in MEP·Mmax^{-1} and suggests increased cortical excitability during sustained MVCs. Interestingly, MEP·Mmax^{-1} assessed during brief MVCs interspersed throughout a series of sustained 3-min submaximal isometric contractions of the elbow flexors at 20% MVC remained unchanged from baseline to task failure and immediately after task failure despite increased MEP·Mmax^{-1} at 20% MVC (Yoon *et al.*, 2012; Yoon *et al.*, 2013). It is unknown whether the transient activation of motoneurons not required in the maintenance of the submaximal contraction caused this discrepancy.

Often MEPs have been measured during brief contractions before and after fatiguing (predominantly isometric voluntary contractions) exercise and then compared to evaluate the effects of the intervention. Post-exercise MEPs are usually assessed immediately following the intervention; thus, they must be interpreted in conjunction with the MEP kinetics during the fatiguing intervention. As previously described, MEP and MEP·Mmax^{-1} generally increase during a sustained contraction and are thus larger at task failure than at baseline (Sogaard *et al.*, 2006; Smith *et al.*, 2007; Szubski *et al.*, 2007). MEP·Mmax^{-1} measured immediately after exercise is also elevated compared to pre-exercise (Sogaard *et al.*, 2006; Smith *et al.*, 2007; Szubski *et al.*, 2007; Klass *et al.*, 2008) and returns completely to baseline within several minutes (Sogaard *et al.*, 2006; Smith *et al.*, 2007; Szubski *et al.*, 2007). Any delay between exercise cessation and post-exercise evaluations allows MEP recovery and masks exercise-induced MEP changes as demonstrated by recovery within the initial ~30 s post-exercise (Taylor *et al.*, 1999; Sogaard *et al.*, 2006; Smith *et al.*, 2007; Szubski *et al.*, 2007). The effect of delayed post-exercise evaluations is much more of a concern in studies investigating dynamic or any other exercise that cannot be conducted on the same ergometer as neuromuscular evaluations since a delay would be necessary for subject installation.

There has been little research evaluating the effect a dynamic upper-body exercise bout on MEP amplitude/area. The only published study is one that investigated both submaximal isometric and dynamic concentric elbow flexion at 20% MVC interspersed with brief higher intensity contractions (Yoon *et al.*, 2013). In both conditions MEP amplitude during brief MVCs was unchanged during the fatiguing task and recovery. Additional research remains to be conducted to determine MEP dynamics during dynamic upper-body exercise and subsequent recovery.

Cortical silent period

When single-pulse TMS is delivered during a voluntary contraction, the elicited MEP is generally followed by the CSP, a period of near-silence in the EMG signal. This period of EMG suppression is believed to be mediated by activation of long-lasting $GABA_B$ receptors (McDonnell *et al.*, 2006) although it is acknowledged that spinal mechanisms contribute to the early part (~50 ms) of the CSP (Inghilleri *et al.*, 1993). Since the EMG interruption continues beyond the recovery of motoneuronal excitability, the later part of the CSP is understood to be mediated through intracortical inhibitory mechanisms (Inghilleri *et al.*, 1993). CSP is greatly influenced by stimulus intensity and to a much lesser extent by voluntary contraction intensity (Taylor *et al.*, 1997; Saisanen *et al.*, 2008).

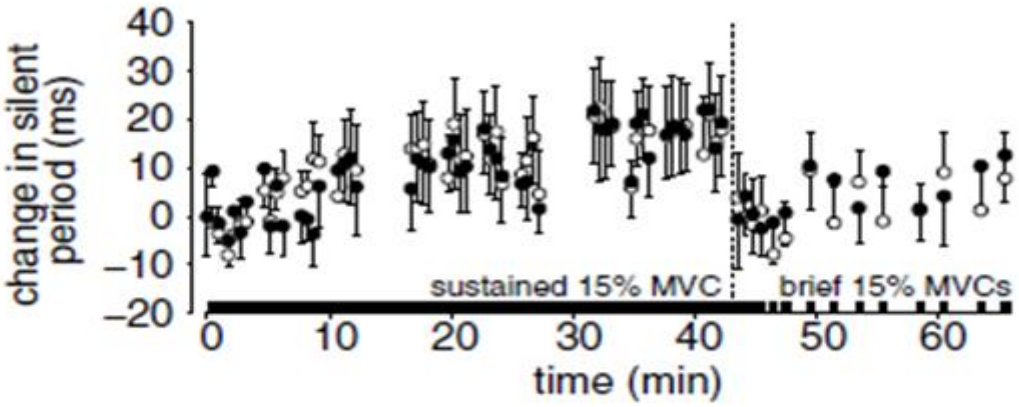

Figure 14. Changes in cortical silent period duration in the *biceps brachii* (○) and *brachioradialis* (●) muscles during a 43-min sustained isometric contraction of the elbow flexors at 15% MVC and during recovery contractions at 15% MVC. Adapted from Sogaard *et al.* (2006).

CSP lengthens during sustained fatiguing isometric contractions and the time to recover increases with increasing task duration (Taylor *et al.*, 2000; Sogaard *et al.*, 2006; Smith *et al.*, 2007) (**Figure 14)**. Because the CSP increase is less after cervicomedullary stimulation-induced CMEPs than after MEPs, at least part of the increased CSP duration following MEPs is believed to result from increased supraspinal inhibition (Taylor *et al.*, 1996; Levenez *et al.*, 2008). The sustained level of force appears to influence CSP kinetics. During prolonged low-to-moderate intensity contractions, CSP gradually increases in length (Taylor *et al.*, 1996; Sogaard *et al.*, 2006; Smith *et al.*, 2007; Levenez *et al.*, 2008) whereas during sustained

MVCs it increases rapidly over the first seconds before plateauing (Taylor *et al.*, 1996; Todd *et al.*, 2005). This suggests that exercise intensity is an important factor in the manifestation of intracortical inhibition. Increased CSP duration has been found in hand muscles (Szubski *et al.*, 2007) and *biceps brachii* (Hunter *et al.*, 2006; Levenez *et al.*, 2008) although it has not been observed in all studies (Ljubisavljevic *et al.*, 1996). These discrepancies may be due to the high intra-subject variability in exercise-induced CSP increase (Cerri *et al.*, 2010). Many factors can induce CSP variability and thus confound results. These include the instruction set given to the subjects (Mathis *et al.*, 1998), the presence of bursts of late EMG activity coinciding with the resumption of voluntary EMG at the end of the CSP (Chin *et al.*, 2012) and low-level EMG present during the CSP from spinal reflex facilitation by muscle spindle afferents (Butler *et al.*, 2012) and the potentially large inter-examiner variability, especially when the CSP is defined to exclude the MEP (Reid *et al.*, 2002).

The assessment of CSPs during brief contractions before and after fatiguing exercise parallels MEP evaluation. Thus, post-exercise CSPs must also be interpreted in conjunction with the CSP kinetics during the fatiguing intervention (Sogaard *et al.*, 2006; Smith *et al.*, 2007; Szubski *et al.*, 2007). Any delay between exercise cessation and post-exercise evaluations allows recovery of exercise-induced CSP changes as demonstrated by recovery within the initial ~30 s post-exercise (Taylor *et al.*, 2000; Sogaard *et al.*, 2006; Szubski *et al.*, 2007).

The ability of TMS at low intensities to influence the most direct motor cortical projections to the spinal motoneurons has also been employed to investigate intracortical inhibition during muscular activity (Butler *et al.*, 2007). At very low TMS intensities (*i.e*. sub-AMT), a number of different responses can be induced in the EMG signal during voluntary muscular contractions. A TMS intensity corresponding to AMT elicits MEPs after the delivery of one half of all stimuli. As the TMS intensity is reduced below AMT, the frequency and amplitude of MEPs diminishes although there may still be facilitation. Eventually, as TMS intensity is decreased, reducing cortical output to descending motoneurons, a suppression of voluntary EMG activity is observed (Davey *et al.*, 1994; Petersen *et al.*, 2001; Butler *et al.*, 2007). Changes in the amount of EMG suppression during an exercise are believed to be indicative of resulting changes in intracortical inhibition. A limitation of this method is that EMG suppression is not clearly observed in all motor units (Butler *et al.*, 2007), at all coil positions (Davey *et al.*, 1994) or in all subjects (Petersen *et al.*, 2001). Seifert and Petersen (2010) employed this method to investigate changes in intracortical inhibition during a submaximal isometric voluntary contraction to task failure at 30% MVC (Seifert &

Petersen, 2010). EMG suppression was greater during the last 2 min than the first 2 min, indicating increased intracortical inhibition immediately before task failure. This finding is consistent with exercise-induced CSP changes and is a technique that needs further investigation due to its potential to evaluate changes to inhibitory cortical mechanisms during dynamic exercise.

Paired pulses

Paired TMS pulses have been proposed to investigate both inhibitory and facilitative mechanisms and complement MEP and CSP findings. $GABA_B$-mediated intracortical inhibition can also be investigated using paired TMS pulses. A conditioning pulse followed by a test pulse at an inter-stimulus interval of 50-200 ms causes LICI, where the conditioned MEP is smaller than a MEP elicited by single-pulse TMS (Valls-Sole *et al.*, 1992). Similarly, $GABA_A$-mediated SICI can be measured by employing shorter inter-stimulus intervals (*i.e.* 2 to 5 ms) (Kujirai *et al.*, 1993). Conversely, increasing the inter-stimulus interval to 8 to 25 ms causes the conditioned MEP to be larger than that elicited by single-pulse TMS. The mechanisms contributing ICF remain to be determined (Reis *et al.*, 2008).

Studies that investigated SICI and ICF changes with fatigue are often difficult to interpret because most employed paired-pulse TMS only when the muscle was in the relaxed state, only before and after exercise or there was a long delay before post-exercise evaluation was conducted. In an exception, McCombe Waller *et al.* (2008) observed increased SICI and unchanged ICF during contractions sufficient to overcome the weight of the arm after a 10-min bout of ipsilateral arm exercise and decreased SICI and increased ICF after similar bouts of bilateral and contralateral arm exercise. This suggests that inhibitory and excitatory mechanisms may not manifest globally during exercise and instead may be specific to the task performed.

McNeil et al. (2009) investigated LICI changes during a 2-min elbow-flexor MVC. To evaluate the role of spinal and supraspinal mechanisms in LICI, the protocol was conducted with both TMS and cervicomedullary test pulses 100 ms after a conditioning pulse. Both conditioned MEPs and CMEPs decreased rapidly and were practically eliminated by the 30-s mark of the 2-min MVC. The parallel of MEP and CMEP dynamics indicates a major spinal, and not cortical, component to LICI during MVCs. Similarly, CSP increased, and unlike for LICI, its rapid recovery after exercise cessation suggests a cortical origin of inhibition. McNeil et al. (2011a) also observed increasing LICI as demonstrated by decreased

conditioned MEP and CMEP areas during a sustained 10-min submaximal elbow-flexor contraction at 25% maximal *biceps brachii* EMG. The similar decrease in conditioned MEP and CMEP areas at both strong and weak stimulus intensities reinforces that impaired spinal mechanisms (*i.e*. the responsiveness of the motoneurons) and not intracortical inhibition account for the fatigue-related changes to conditioned MEPs. The results of these two studies (McNeil *et al.*, 2009; McNeil *et al.*, 2011a) raise a number of questions about inhibitory processes observed in the central nervous system. Despite the initial spinal component of the CSP, increased CSP during submaximal and maximal voluntary contractions have been interpreted to be indicative of changes in intracortical inhibition (see ***Cortical silent period*** section above). McNeil *et al.* (2009) observed the customary increase in CSP over the first minute of a 2-min MVC and very quick recovery after exercise cessation; however, no difference in LICI was observed between stimuli delivered at the cervicomedullary and cortical levels, indicating a spinal component was responsible for the increased inhibition. While McNeil *et al.* (2011a) did not report CSP, the increased CSP generally observed during submaximal isometric contractions is well-established and at odds with conditioned MEP and CMEP changes observed during both submaximal and maximal voluntary contractions. This raises the question of whether CSP is a good indicator of intracortical inhibition. Further investigations are required to determine the mechanisms responsible for observed inhibitory and excitatory responses to TMS and cervicomedullary junction stimulation in order to permit identification of spinal and supraspinal changes.

Lower limbs

With the development of more specialized TMS coils (figure-of-eight and double-cone coils), TMS investigation of the lower limbs became feasible. As in the upper limbs, initial studies focused on isometric voluntary contraction protocols. Investigations employing TMS have rapidly shifted focus to locomotor activities (running and cycling) due to the functional importance of locomotion. To date investigations have predominantly examined pre- to post-exercise changes although more recent studies have begun evaluating changes during cycling. **Table 2** highlights the major findings from lower-limb TMS fatigue investigations, including changes to VAc, MEPs and CSPs.

Cortical voluntary activation

TMS-evoked SIT has been observed to increase during repeated maximal dorsiflexion (Mileva *et al.*, 2012) and from pre- to immediately post- a 2-min sustained MVC of the knee extensors (Goodall *et al.*, 2009). SIT during MVC was also observed to increase in repeated sets of submaximal contractions and an MVC to task failure (Goodall *et al.*, 2010). The increase of SIT during the fatiguing protocols indicates that supraspinal mechanisms contribute to the observed fatigue in the lower limbs. This indicates an increasing deficiency to fully drive the muscle originating from the supraspinal level although deficits at other levels of the corticospinal pathway cannot be excluded.

The validity and reliability of extrapolating the relationship between TMS-evoked SIT and voluntary forces at 50%, 75% and 100% MVC to determine VAc has also been confirmed in the quadriceps (Goodall *et al.*, 2009; Sidhu *et al.*, 2009a) and dorsiflexor (Mileva *et al.*, 2012) muscles. Cortical VA was lower compared to baseline values immediately after intermittent submaximal isometric contractions to task failure (Goodall *et al.*, 2010) and a 2-min MVC (Goodall *et al.*, 2009). In whole-body exercise, VAc of the dorsiflexors was lower immediately after a 42.2-km running bout (Ross *et al.*, 2007) and VAc of the quadriceps was lower after cycling bouts of various durations (Sidhu *et al.*, 2009b; Goodall *et al.*, 2012; Fernandez-del-Olmo *et al.*, 2013), although decreased quadriceps VAc was not observed after 60 min cycling at 55% of maximal aerobic power output (MAP) followed 1-2 min later by a time-trial equivalent to 30 min cycling at 75% MAP (Klass *et al.*, 2012), nor after a series of 6-s sprints (Girard *et al.*, 2013). These results collectively indicate that both single-joint isometric exercise and whole-body dynamic exercise of the lower limbs are capable of inducing supraspinal fatigue. Further investigations must evaluate VAc recovery kinetics because the delay from exercise cessation to post-exercise assessment may account for the equivocal findings.

Goodall *et al.* (2009; 2012) also attempted to quantify the amount of central fatigue originating at the supraspinal level as per Smith *et al.* (2007). They concluded that 38% of the MVC decrease after a 2-min MVC (Goodall *et al.*, 2009) and 41% of the MVC decrease after 8.1 ± 2.9 min cycling at 80% MAP (Goodall *et al.*, 2012) is due to supraspinal fatigue. This is much less than the 66% decrease in MVC calculated by Smith *et al.* (2007) after a 70-min isometric contraction at 5% MVC, suggesting that exercise duration and/or muscle groups are important factors in the development of supraspinal fatigue.

Motor-evoked potentials

During sustained submaximal isometric contractions of the plantar flexors, MEP·Mmax^{-1} increased (Hoffman *et al.*, 2009). This is in accordance with the previously detailed upper-limb studies. Conversely, Hoffman et al. (2009) observed MEP and CMEP kinetic interactions that differed from those observed in the elbow flexors (Levenez *et al.*, 2008). During a sustained 30% MVC plantar-flexor contraction, a constant increase in MEP·Mmax^{-1} in both *soleus* and *medial gastrocnemius* muscles was observed. At task failure, MEP·Mmax^{-1} was similar to that in a brief control MVC. Significantly increased CMEP·Mmax^{-1} was only observed in the *medial gastrocnemius* and CMEP·Mmax^{-1} at task failure was smaller in both muscles than during a brief control MVC. These results suggest that there was only a small spinal contribution to the increased corticospinal responses to submaximal fatiguing contractions. The difference in findings between Levenez *et al.* (2008) and Hoffman *et al.* (2009) may be due to differences in neural control mechanisms to upper- and some lower-limb muscles. Corticospinal projections onto *soleus* are believed to be weaker than those to many other muscles including *biceps brachii*, hand muscles and other lower-limb muscles such as *tibialis anterior* and *rectus femoris* (de Noordhout *et al.*, 1999; Petersen *et al.*, 2003; Martin *et al.*, 2008).

During a sustained MVC, MEP amplitude/area has been reported to remain stable in the *soleus* (Iguchi & Shields, 2012) and *vastus lateralis* (Goodall *et al.*, 2009). This differs from the increased MEP size reported in upper limbs and reinforces the notion that there may be distinct neural control mechanisms for the upper and lower limbs.

Due to the difficulties in performing TMS during whole-body dynamic exercise, most investigations have compared changes pre- and post-intervention. After whole-body dynamic exercise, regardless of exercise duration or intensity, MEP size may be influenced by the delay between the end of the exercise bout and the beginning of post-exercise measurements. This may be important because MEPs recover within ~30 s in upper-limb studies (Taylor *et al.*, 1999; Sogaard *et al.*, 2006; Smith *et al.*, 2007; Szubski *et al.*, 2007). Among lower-limb isometric studies, only Iguchi and Shields (2012) have investigated MEP recovery. Although MEP amplitude at 10% MVC remained increased above baseline 1 min after exercise cessation, it had recovered by the next measurement, 10 min post-exercise.

The two longest duration dynamic whole-body exercise studies employing TMS, including the only previous study investigating corticospinal changes after running, investigated MEP changes in the relaxed muscle and did not normalize MEP amplitude. Ross

et al. (2007) reported decreased MEP amplitude in the relaxed *tibialis anterior* following a marathon. The fact that post-marathon measurements occurred anywhere up to 20 min post-exercise and that decreased MEP amplitude was associated with a non-significant decrease in Mmax does not allow the drawing of clear conclusions on MEP·Mmax^{-1} changes. Similarly, Ross *et al.* (2010b) observed both decreased *vastus lateralis* MEP amplitude and Mmax measured in the relaxed muscle on days 9 and 17 of the 2007 Tour de France and only decreased MEP amplitude 2 days post-Tour. That neuromuscular evaluations were conducted >18 h after the end of the previous stage during the Tour and even longer post-Tour also restrict the useful interpretation of these results in regards to acute exercise-induced fatigue.

Goodall *et al.* (2012) did not observe changes in MEP·Mmax^{-1} area during contractions at 100, 75 and 50% MVC in the *vastus lateralis* after constant load cycling at ~80% MAP to task failure. Similarly, two longer cycling protocols did not observe changes in MEP·Mmax^{-1} (Sidhu *et al.*, 2009b; Klass *et al.*, 2012). Two to three minutes after eight 5-min bouts of cycling at 80% MAP separated by 1 min, Sidhu et al. (2009b) observed unchanged MEP·Mmax^{-1} area in *rectus femoris*. Klass *et al.* (2012) also found unchanged MEP·Mmax^{-1} in *rectus femoris* and *vastus medialis* after ~1.5 h cycling. Two studies specifically examining cycling sprint performance reported contradictory results. Girard *et al.* (2013) observed unchanged *vastus lateralis* MEP·Mmax^{-1} amplitude after a series of fifteen 6-s sprints. Conversely, after 30-s all-out sprints, Fernandez-del-Olmo *et al.* (2013) reported increased MEP·Mmax^{-1} area during contractions at 50 and 75% MVC but not 100% MVC in the *vastus lateralis* evaluated 1 min after each of two Wingate tests. The differences between this study and the others might reflect specific central adaptations to submaximal and very short maximal exercise (Taylor & Gandevia, 2008) or that submaximal isometric contractions were performed at the same absolute force across the experimental session (*i.e.* based on percentages of the baseline MVC). Thus, the increase in MEP amplitude observed in Fernandez-del-Olmo *et al.* (2013) was interpreted as a compensatory mechanism to generate the required motor output and overcome the reduced peripheral force production. Conversely, the unchanged MEP areas observed by Sidhu *et al.* (2009b), Klass *et al.* (2012) and Goodall *et al.* (2012) may be related to evaluations having been conducted at the same relative strength levels (*i.e.* accounting for lower post-exercise MVC). Other factors that may have influenced the differential MEP responses to whole-body lower-limb exercise include TMS intensity (**Table 1**), exercise duration and exercise intensity (**Table 2**).

Changes in corticospinal excitability during submaximal whole-body exercise were first published by Sidhu *et al.* (2012a). Knee extensor (*i.e. vastus lateralis* and *rectus femoris*)

MEP·Mmax^{-1} and CMEP·Mmax^{-1} were assessed every 3 min during 30 min of cycling at 75% of MAP and each minute during a 105% MAP cycling bout to task failure immediately thereafter. Neither MEP·Mmax^{-1} nor CMEP·Mmax^{-1} changed significantly during exercise. MEP and CMEP were also normalized to voluntary EMG during cycling and unlike with conventional normalization methods, CMEP amplitude remained unchanged and MEP amplitude decreased from 10 min to task failure. Together, these results suggest a general inclination towards decreased cortical excitability during exercise and at task failure. There is difficulty in interpreting these results because statistical analysis compared MEPs and CMEPs at 110% MAP (pre-exercise), three points at 75% MAP and 105% MAP (immediately pre-task failure). These findings contradict MEP and CMEP changes during submaximal single-joint isometric contraction protocols (Levenez *et al.*, 2008; Hoffman *et al.*, 2009) although like in Hoffman *et al.* (2009), it suggests that central changes largely occur at the supraspinal level. The higher cardiorespiratory and metabolic demands during whole-body exercise compared to single-joint exercise may increase the role of factors such as core temperature, glycaemia, brain catecholamines and cerebral oxygenation on evoked motor cortical and corticospinal tract responses (Nybo & Nielsen, 2001; Todd *et al.*, 2005; Hasegawa *et al.*, 2008; Secher *et al.*, 2008; Rasmussen *et al.*, 2010; Verges *et al.*, 2012).

Further investigation is required to confirm and elucidate the reported differences in MEP responses elicited during isometric exercise in the upper (*i.e.* increased cortical excitability) and lower (*i.e.* increased cortical excitability in sustained submaximal contractions and unchanged cortical excitability during sustained maximal contractions) limbs. The effects of submaximal whole-body exercise also need to be clarified since measures during exercise differ from those made only before and after exercise and these changes differ from MEP changes observed in isometric contraction protocols. Finally, MEP recovery kinetics, especially as it pertains to the effect of the delay to post-exercise assessment on MEP change, require further study.

Cortical silent period

As previously indicated in upper-limb muscles, CSP generally increases during isometric voluntary contractions. The increased CSP during intermittent plantar flexor (Iguchi & Shields, 2012) and sustained dorsiflexor (McKay *et al.*, 1996) MVCs is consistent with this finding. Conversely, Goodall *et al.* (2010) did not observe any change to CSP duration measured immediately post-task failure after an intermittent submaximal quadriceps

contraction protocol. This suggests that perhaps the combination of both the submaximal contraction intensity and intermittent nature of the protocol prevented the development of intracortical inhibition.

After whole-body exercise, no change in CSP has been observed. Ross *et al.* (2007) observed unchanged *tibialis anterior* CSP during MVCs after a treadmill marathon with TMS delivered at 100% maximal stimulator output. After cycling protocols, irrespective of exercise duration or intensity or TMS intensity, CSP was also unchanged (Sidhu *et al.*, 2009b; Goodall *et al.*, 2012; Klass *et al.*, 2012; Fernandez-del-Olmo *et al.*, 2013; Girard *et al.*, 2013). These findings suggest that previously-evaluated dynamic whole-body exercise protocols may be unable to induce $GABA_B$-related intracortical inhibition or that because of the rapid recovery of CSP after exercise (Taylor *et al.*, 2000), the delay between exercise cessation and post-exercise evaluation masked CSP changes.

Sidhu *et al.* (2013b) assessed the effects of sub-threshold intensity TMS on EMG suppression to evaluate intracortical inhibition. Increased EMG inhibition during the last 5 min of a 30-min cycling bout at 75% MAP compared to the first 5 min was observed. This is in agreement with increased EMG suppression observed during sustained elbow flexion (Seifert & Petersen, 2010). It is also in agreement with most upper- and lower-limb investigations with isometric voluntary contraction protocols that show increased CSP with exercise but contradicts the lack of CSP change with whole-body dynamic exercise. The increased intracortical inhibition as determined by this method and unchanged CSP with dynamic whole-body exercise appears contradictory and must be investigated. Future studies must take into account the delay between the end of the running and cycling bouts and the start of post-exercise evaluation. The fact the method employed by Sidhu *et al.* (2013b) can be performed during cycling (*i.e.* without a delay between exercise cessation and evaluation) may be a key factor in explaining this discrepancy.

Paired pulses

The only study that to date has examined the effect of a lower-limb intervention with paired TMS pulses employed a static squat (Mileva *et al.*, 2009). Neither SICI or ICF, nor pre-TMS EMG changed significantly over the course of the 330-s static squat in the target *tibialis anterior* muscle.

A summary of the changes to VAc and TMS-induced EMG parameters in upper- and lower-limb exercise are resented in **Table 3**.

Table 3. A summary of the principal TMS parameters evaluated during voluntary muscular contraction in response to exercise of the upper and lower limbs.

	Upper-limb exercise		Lower-limb exercise			
	Submaximal isometric	Maximal isometric	Submaximal isometric	Maximal isometric	Whole-body dynamic (measured isometrically)	Whole-body dynamic (measured during activity)
VAc	↓	↓	↓	↓	↓ / ↔	
MEP	↑	↑	↑	↔	↔	↓[1] / ↔[2]
CSP	↑	↑	↑	↑	↔	
EMG suppression[3]	↑					↑
SICI	↑		↔			↔
ICF	↔		↔			↔
LICI	↑	↑				

CSP, cortical silent period; ICF, intracortical facilitation; LICI, long-interval intracortical inhibition; MEP, motor-evoked potential; SICI, short-interval intracortical inhibition; VAc, cortical voluntary activation.

[1] normalized to cycling EMG
[2] normalized to Mmax
[3] indication of intracortical inhibition

TRANSCRANIAL MAGNETIC STIMULATION HIGHLIGHTS

What is known:

- TMS trans-synaptically excites the pyramidal neurons and some direct excitation of pyramidal tract axons is believed to occur depending on numerous factors such as stimulus intensity
- Increasing TMS intensity causes increasing MEP amplitude/area and increasing CSP duration to a maximal response

- TMS allows calculation of VAc and reflects the ability of the upper motoneurons to respond to cortical motor input
- MEP amplitude/area and CSP duration both increase during upper-limb isometric exercise
- Lower-limb whole-body exercise does not appear to induce changes in MEP amplitude/area or CSP duration
- VAc decreases during fatiguing isometric and dynamic whole-body exercise of the upper and lower limbs

What is not known (methodological):

- The effect of employing different methods of determining optimal TMS stimulus intensity on the subsequent evaluation of central parameters
- Whether it is appropriate to use one muscle as a surrogate for a muscle group when there is no dominant muscle
- The effect of different ways of reaching a target force level on MEP and CSP responses

What is not known (applied):

- The effect of TMS stimulus intensity on MEP and CSP responses during the development of fatigue
- The effect of exercise duration and/or modality on supraspinal fatigue development and indices of corticospinal excitation and inhibition
- The effect of extreme duration exercise and confounding factors (*e.g.* sleep deprivation, pacing) on the development of supraspinal fatigue and changes to other TMS-induced parameters

SLEEP DEPRIVATION

Sleep deprivation (SD) is most frequently a condition of insufficient sleep duration. This may present as either complete or partial SD and persons experiencing SD often report subjective feelings of tiredness, clumsiness and fatigue. Whether the experienced fatigue is related to mechanisms causing central fatigue is unknown.

Numerous studies have observed performance deficits during aerobic exercise after SD. In his early study, Holland (1968) observed decreased time to task failure for an incremental cycling test in 24 university students after one night SD. Shorter times to task failure were also found with intense walking after 36-50 h SD (Martin, 1981; Martin & Chen, 1984) and the distance run over 30 min following 30 min of submaximal running was 2.9% less after 30 h SD (Oliver *et al.*, 2009). Conversely, Daanen *et al.* (2013) found no difference

in distance cycled during a 20-min time-trial conducted 30 min after a 30-min cycling bout at the power output eliciting 50% peak oxygen consumption following one night SD. The findings of studies that have investigated the effect of SD on shorter performance durations in running and cycling are equivocal (Chen, 1991; Azboy & Kaygisiz, 2009; Konishi *et al.*, 2012). **Table 4** details studies investigating the effects of SD on aerobic exercise performance and physiological parameters. Maximal strength loss was not observed during either isometric or isokinetic contractions of upper or lower limbs during 60 h SD (Symons *et al.*, 1988a; Symons *et al.*, 1988b), nor was there any effect on grip strength after 41 h SD (Meney *et al.*, 1998). Conversely, 30 h of SD resulted in decreased isokinetic knee extensor torque although isokinetic knee flexor torque was unaffected (Bulbulian *et al.*, 1996). Collectively, these results suggest that the decreased performances sometimes observed in SD may be more likely to occur as the exercise bout duration increases; however, the abundance of conflicting results precludes a definitive explanation.

Neither oxygen consumption (VO_2) nor heart rate (HR) during constant-load efforts of varying intensity up to 1 h (Martin, 1981; Martin & Chen, 1984; Oliver *et al.*, 2009; Daanen *et al.*, 2013) were influenced by SD. This may not be the case in longer duration exercise bouts since Martin *et al.* (1986) reported decreased VO_2 after 3h, but not 1 or 2 h, of light treadmill walking after 36 h SD. Heart rate, however, was similar between SD and control conditions in this study. Meanwhile, Scott and McNaughton (2004) observed lower HR during 30 h SD with 20 min of light exercise (50% peak VO_2) every 4 h but not when exercise frequency was increased to 20 min every 2 h. Results from incremental tests to task failure are equivocal about the effects of at least 24 h SD on HR responses and maximal oxygen uptake (VO_{2max}) (Martin & Gaddis, 1981; Plyley *et al.*, 1987; Goodman *et al.*, 1989; Chen, 1991; Konishi *et al.*, 2012). Similarly, HR during a time-trial of 20-30 min preceded by a steady-state exercise bout was either lower (Oliver *et al.*, 2009) or similar (Daanen *et al.*, 2013) after at least 24 h SD. The results of these investigations do not suggest a clear link between either HR or VO_2 and SD.

Investigation of RPE has been primarily investigated in short exercise bouts under conditions of SD. With a longer exercise bout, Martin *et al.* (1986) observed increased RPE at 3 h of treadmill walking at 5.6 $km \cdot h^{-1}$ and 2% grade; however RPE was unchanged at 1 and 2 h of the protocol. Employing a combination of 30-min treadmill running at 60% VO_{2max} followed immediately by a 30-min time-trial, Oliver *et al.* (2009) reported no difference in RPE in either the fixed intensity or time-trial portions despite subjects running a shorter distance after SD. Although Daanen *et al.* (2013) observed similar 20-min cycling time-trial

Table 4. The effects of complete sleep deprivation on exercise performance, heart rate, oxygen consumption and ratings of perceived exertion

Reference	SD duration	Study design	Exercise protocol	Exercise duration	Effect of SD on physical performance	Effect of SD on exercise VO_2 or VO_{2max}	Effect of SD on exercise HR or HR_{max}	Effect of SD on RPE
(McMurray & Brown, 1984)	24 h	crossover	treadmill running at 80% VO_{2max}	20 min	N/A	no difference in VO_2	no difference in HR	N/A
(Daanen *et al.*, 2013)	one night	crossover	30 min cycling at 50% VO_{2peak}, 30 min rest and 20-min cycling time-trial	50 min	no difference in distance completed during 20-min time-trial	N/A	no difference in HR in either cycling bout	no difference in either cycling bout
(Holland, 1968)	one night	PRE values as control	incremental cycling test to TF	5-9 min	decreased time to TF	N/A	decreased HR at 50 and 150 W; no difference in HR at 100, 200, 250 or 300 W	N/A
(Azboy & Kaygisiz, 2009)	25-30 h	crossover	incremental treadmill running test to TF	mean time to TF of 11-13 min	no difference in time to TF in runners; decreased time to TF in volleyball players	no difference in VO_{2max}	no difference in HR_{max}	N/A
(Chen, 1991)	30 h	crossover	incremental cycling test to TF	mean time to TF of 8-9 min	no difference in Wmax; decreased time to TF	decreased VO_{2max}	decreased HR_{max}	N/A
	30 h	crossover	75% Wmax cycling to TF	mean time to TF of 11-12 min	no difference in time to TF	decreased VO_2 at 6 min	no difference in HR	N/A
(Martin & Gaddis, 1981)	30 h	crossover	25, 50 and 75% VO_{2max} cycling	8 min at each intensity	N/A	no difference in VO_2	no difference in HR	no difference in RPE at 25% VO_{2max}; increased RPE at 50 and 75% % VO_{2max}

	30 h	crossover	incremental cycling test to TF	5-8 min	N/A	no difference in VO_{2max}	N/A	N/A
(Martin & Haney, 1982)	30 h	crossover	treadmill walking at 5.6 km·h^{-1} and grade to elicit RPE of 4 out 5 (~17 on Borg scale)	10 min (grade unchanged from 8 min)	no difference in treadmill grade	no difference in VO_2	decreased HR	N/A
(Oliver *et al.*, 2009)	30 h	crossover	30-min treadmill run at 60% VO_{2max} followed by 30-min run for maximum distance	60 min	decreased distance run	increased VO_2 at 30 min of 60% VO_{2max} running compared to 5 min; N/A during run for distance	decreased HR during 30-min run for distance only	no difference in RPE
(Pickett & Morris, 1975)	30 h	crossover[1]	Bruce treadmill test	≤21 min	no difference in exercise time	N/A	no difference in HR	N/A
(Scott & McNaughton, 2004)	30 h	crossover	20-min cycling bouts at 50% VO_{2max} every 4 h	20 min	N/A	no difference in mean VO_2	decreased mean HR	N/A
	30 h	crossover	20-min cycling bouts at 50% VO_{2max} every 2 h	20 min	N/A	no difference in mean VO_2	no difference in mean HR	N/A
(Skein *et al.*, 2011)	30 h	crossover	incremental treadmill running test at 60, 70 and 80% VO_{2max}	30 min	N/A	N/A	no difference in mean HR	no difference in RPE
	30 h	crossover	self-paced intermittent sprint running exercise (15-m sprint each minute with 1- min break each 10 min)	50 min	decreased mean sprint speed throughout the protocol	N/A	no difference in mean HR[2]	no difference in RPE
(Konishi *et al.*, 2012)	34 h	crossover	incremental treadmill running test to TF	mean time to TF of 12-14 min	no difference in time to TF	no difference in VO_2	decreased HR	N/A

(Martin, 1981)	36 h	crossover	80% VO_{2max} treadmill walking to TF	31-156 min	decreased time to TF	no difference in VO_2 for first 31 min[3]	no difference in HR for first 31 min[3]	increased RPE for first 31 min[3]
(Martin *et al.*, 1986)	36 h	crossover	treadmill walking at 5.6 km·h^{-1} and 2% grade	3 h	N/A	no difference in VO_2 at 1 or 2 h; increased VO_2 at 3 h	no difference in HR	no difference in RPE at 1 or 2 h; increased RPE at 3 h
(Racinais *et al.*, 2004)	38 h	crossover	Leger and Gadoury shuttle test	NR	no difference	no difference in estimated VO_{2max}	N/A	N/A
(Meney *et al.*, 1998)	41 h	crossover	self-selected cycling intensity that subject believed could be maintained for 30 min (every 4 h) [4]	5 min	no difference in self-selected power output	N/A	no difference in HR	decreased RPE on the second day
(Bond *et al.*, 1986)	42 h	crossover	incremental cycling test to TF	mean time to TF 21-23 min	decreased time to TF	decreased VO_{2max}; no difference in VO_2 at 25, 50 and 75% VO_{2max}	decreased HR_{max}; decreased HR at 25, 50 and 75% VO_{2max}	increased RPE at 50 and 75% VO_{2max}
(Rodgers *et al.*, 1995)	48h	PRE values as control	approximately 6 times each (30-min sandbag carrying, walking, stake planting, arm ergometry and wheel barrow loads, 45-min stake planting and 2-min cycling workload test)	various	decreased performance for all tests in second half of intervention except cycling workload test	N/A	N/A	N/A
	48 h	SD only group and SD + work group	PWC_{170}, perceived exertion test and self-paced walking test	various	decreased PWC170 in SD only group; decreased self-selected walking speed and cycling power output at a given RPE in SD + work group	N/A	N/A	increased RPE in SD only group

(Martin & Chen, 1984)	50 h	crossover	treadmill walking at 5.6 km·h^{-1} and grade to elicit HR of 160 beats·min^{-1} after normal sleep	mean time 35-46 min	decreased time to TF	no difference in VO_2	no difference in HR	N/A
(Myles, 1985)	54 h	PRE values as control	incremental cycling test to TF; 10 x 30-s bouts as 2 each at 100, 120, 140, 160 and 180 W	NR	N/A	no difference in VO_{2max}	N/A	no difference in RPE at any power output
(Goodman *et al.*, 1989)	60 h	PRE values as control	incremental cycling test to TF	mean time to TF of 19-20 min	no difference in time to TF	no difference in VO_{2max}	no difference in HR_{max}	N/A
(Symons *et al.*, 1988b)	60 h	crossover	25-min treadmill run including 8 min at 70% VO_{2max} from 6-14 min and 8 min at 80% VO_{2max} from 14-22 min	25 min	N/A	no difference in VO_2	increased HR at 80% VO_{2max}	increased RPE at 80% VO_{2max}
	60 h	crossover	5 min cycling bout at 50% VO_{2max} then alternating 2 min at 80% VO_{2max} and 2 min recovery 8 times	21 min	N/A	N/A	no difference in HR	no difference in RPE
(Plyley *et al.*, 1987)	64 h	PRE values as control	incremental cycling test to TF (every 12 h)	NR	NR	decreased VO_{2max}	no difference in HR_{max}[5]	N/A
	64 h	PRE values as control	incremental cycling test to TF (every 12 h) with intervention of 1 h treadmill walking at ~28% VO_{2max} every 3 h	NR	NR	decreased VO_{2max}	decreased HR_{max}[5]	increased RPE during submaximal exercise
(Horne & Pettitt, 1984)	72 h	separate control and SD groups	10 min cycling bouts at 40, 60 and 80% VO_{2max} and 5 min rest between bouts	40 min	N/A	no difference in VO_2	no difference in HR	N/A

(Brodan *et al.*, 1969)[6,7]	120 h	NR	Harvard Step Test[8]	5 min[9]	decreased score at 24 and 48 h; no change in score at 72, 96 or 120 h	N/A	N/A[10]	N/A

HR, heart rate; HR_{max}, maximal heart rate; MAP, maximal power output; N/A, not applicable (not measured); NR, not reported (measured); PRE, testing before the sleep deprivation intervention; PWC_{170}, estimated peak work capacity at a heart rate of 170 beats·min^{-1}; RPE, ratings of perceived exertion; SD, sleep deprivation; TF, task failure; VO_2, oxygen consumption; VO_{2max}, maximal oxygen consumptionMAP, maximal power output

[1] baseline measures performed one week before intervention
[2] mean HR was lower intervention in both control and SD conditions; however, there was no difference between conditions
[3] only compared over the first 30 min of exercise because TF occurred at 31 min in SD in one subject
[4] no measures performed at 6:00 on the second day for either control or SD conditions
[5] decreased HR_{max} in protocol where subjects walked on a treadmill at ~28% VO_{2max} for 1 h out of every 3 h during SD
[6] subjects were tested numerous times and that duration of SD varied. It is unknown if all subjects presented underwent 120 h of SD
[7] statistical analyses not explained and it is unclear whether statistical analyses were performed
[8] supplementary examinations conducted on a cycle ergometer not explained
[9] duration shortened when individuals performed poorly
[10] Harvard Step Test score calculated from recovery HR, thus indicating slower HR recovery at 24 and 48 h of SD and no difference after 72, 96 or 120 h of SD

distance 30 min after a 30-min fixed-intensity cycling bout, they also found no difference in RPE during either exercise bout. Martin (1981) reported increased iso-time RPE during a treadmill exercise to task failure over the first 31 min; however, this may be heavily influenced by time to task failure decreasing by 15-40% in half the subjects. Plyley *et al.* (1987) also observed increased RPE during 1 h of treadmill walking every 3 h at ~28% VO_{2max} during 64 h of SD. Since RPE was not compared to the SD condition without exercise every 3 h and RPE was compared to pre-intervention values, multiple factors may have influenced RPE change (*e.g.* protocol boredom). Exercise bouts of 30 min or less at various intensities have been equivocal with some studies showing increased (Martin & Gaddis, 1981; Myles, 1985; Bond *et al.*, 1986; Symons *et al.*, 1988b; Rodgers *et al.*, 1995), decreased (Meney *et al.*, 1998) or unchanged (Martin & Gaddis, 1981; Myles, 1985; Symons *et al.*, 1988b; Rodgers *et al.*, 1995; Skein *et al.*, 2011) RPE with SD compared to control conditions.

Early research indicated cognitive deficits during 90 h of SD (Patrick & Gilbert, 1896); however, it was not until several decades later that there was agreement on the negative effect of SD on cognitive performance. There is now consensus that both partial and complete SD have profound effects on cognitive performance. This includes increased performance variability and slowed response speed (*i.e.* reaction time, RT), especially for simple measures of vigilance, attention and alertness that form the basis for higher cognitive functions. Reaction time is a frequently employed measure in the evaluation of simple cognitive functions. It has been observed that RT increases in psychomotor vigilance tasks with SD (Dinges *et al.*, 1997). The failure to respond to a stimulus in a timely manner, referred to as omission, is also characteristic of SD (Dinges *et al.*, 1997; Doran *et al.*, 2001) and this becomes increasingly pronounced as the duration of SD increases. Conclusions about the effect of SD on higher-level cognitive functions, including memory, perception and executive functions (*i.e.* cognitive processes that control and regulate other cognitive processes), are however more equivocal. For example, Sagaspe *et al.* (2006) observed no effect of 36 h of SD on three short Stroop tasks (Color-Word, Emotional, and Specific) that measure selective attention, processing speed and cognitive flexibility. Similarly, 34 to 36 h of complete SD did not impair performance on the Wisconsin Card Sorting Test, a test of the ability to shift focus between single and multiple concepts (Binks *et al.*, 1999). Conversely, Harrison and Horne (1998) observed decrements in word fluency and the capacity to inhibit strong contextual associations in order to create original responses. These equivocal results may indicate that SD does not cause a global impairment in cognitive functioning and that certain aspects of cognition are more greatly affected than others.

A recent meta-analysis has shown that exercise improves cognitive performance across a variety of cognitive tasks and exercise intensities when subjects are not in a state of SD and that these cognitive improvements also transiently persist after exercise cessation (Chang *et al.*, 2012). Most (Chmura *et al.*, 1994; Chmura *et al.*, 1998; Yagi *et al.*, 1999; Davranche *et al.*, 2005; Davranche *et al.*, 2006a; Davranche *et al.*, 2006b) but not all (Delignières *et al.*, 1994; Brisswalter *et al.*, 1997) studies investigating RT have shown that RT decreases during exercise at most intensities. Recent EMG investigations of RT amelioration with exercise indicate that this occurs due to reduced motor time without change in pre-motor time (Davranche *et al.*, 2005, 2006b). Only a few studies have investigated the potential for exercise to act as a countermeasure to SD-induced cognitive deficits. These studies have found exercise to have short-term alerting effects (LeDuc *et al.*, 2000) and to decrease one- and two-choice RTs to a visual stimulus (Scott *et al.*, 2006).

Two studies have previously examined central fatigue with SD and exercise (Skein *et al.*, 2011; Skein *et al.*, 2013). Skein *et al.* (2013) investigated the effects of SD after a rugby league match, *i.e.* the effects of SD on recovery. Meanwhile, Skein *et al.* (2011) investigated the effects of SD on voluntary force, RPE and numerous other parameters, including a 30-min running bout at three intensities and a 50-min intermittent-sprint exercise protocol. Voluntary activation was assessed by ITT from direct stimulation of the muscle. Extremely low levels of voluntary activation (~75% in control conditions before exercise) were reported in team sport athletes at a representative club level, raising serious questions about the method of VA evaluation, and subsequent interpretation of the results.

A number of studies have employed TMS to evaluate corticospinal changes in SD in healthy subjects. All such studies have evaluated the effects of SD in isolation (*i.e.* without exercise or other interventions). The protocols and main findings of all studies employing TMS in the evaluation of SD are detailed in **Table 5**. These studies have rarely investigated measures during voluntary muscular contractions (*i.e.* MEPs (Scalise *et al.*, 2006) and CSPs (Civardi *et al.*, 2001; Manganotti *et al.*, 2001; Manganotti *et al.*, 2006; Scalise *et al.*, 2006; Kreuzer *et al.*, 2011)) and when voluntary contractions were employed, the methodology was extremely vague. This limits the ability to interpret and apply these findings in the context of fatigue. One study observed a change in RMT with SD (De Gennaro *et al.*, 2007), possibly due to SD of 40 h compared to the ~24-h periods employed in the other studies. Single-pulse TMS parameters more commonly investigated in fatigue studies (*e.g.* MEPs and CSPs) were unchanged except for Scalise *et al.* (2006), who observed decreased CSP. Changes to paired-pulse TMS parameters in the relaxed muscle, however, indicated greater likelihood of SD-

Table 5. The effects of complete sleep deprivation (SD) on parameters assessed by transcranial magnetic stimulation in healthy subjects and methodological details from these studies.

Reference	SD duration	Stimulator	Coil	Muscle(s) investigated	Thresholds measured	Threshold change PRE-POST SD	MEP (TMS %)	MEP change PRE-POST	CSP (TMS %)	CSP change PRE-POST	SICI (TMS%)[1]	SICI change PRE-POST	ICF (TMS%)[1]	ICF change PRE-POST
(Badawy *et al.*, 2006)	awake >20 h non-stop in preceding 24 h	Magstim Bistim 200	90-mm circular coil	dominant *abductor pollicis brevis*	RMT	no change	120% RMT	NR	N/A	N/A	80% RMT/120 % RMT	no change	80% RMT/120% RMT	no change
(Civardi *et al.*, 2001)	≥24 h	Magstim Bistim 200	figure-of-eight coil	right *first dorsal interosseous*	AMT (10% MVC), RMT	no change for AMT or RMT	120% RMT	NR	150% RMT at 10% MVC	no change	80% RMT/120 % RMT	decreased	80% RMT/120% RMT	decreased
(Kreuzer *et al.*, 2011)	24 h	NR	NR	right *abductor digiti minimi*	RMT	no change	N/A	N/A	150% RMT	no change	80% RMT/ intensity at rest to elicit MEP of 1 mV	decreased	80% RMT/ intensity at rest to elicit MEP of 1 mV	no change
(Manganotti *et al.*, 2001)	>24 h[2]	Magstim Bistim 200	circular coil	right thenar eminence muscles	RMT	no change[3]	120% RMT	No change	110, 120, 130% RMT[4]	no change[4]	70% RMT/120 % RMT	no change[5]	70% RMT/120% RMT	no change
(Manganotti *et al.*, 2006)	>24 h[6]	Magstim Bistim 200	90-mm circular coil	right thenar eminence muscles	RMT	no change	110, 120, 130% RMT	no change	110, 120, 130% RMT[4]	no change	70% RMT/120 % RMT	no change	70% RMT/120% RMT	no change
(Scalise *et al.*, 2006)	≥24 h	Magstim Bistim 200	double-cone coil	left *opponens pollicis*	RMT	no change	NR[7,8]	no change	NR[8]	decreased	70% RMT/110-120% RMT	decreased	N/A	N/A

(De Gennaro *et al.*, 2007)	40 h[9]	Magstim 200	90-mm figure-of-eight coil	right *abductor digiti minimi*	RMT, lower RMT, upper RMT	increased RMT, increased lower RMT, increased upper RMT	130% RMT	NR	N/A	N/A	70% RMT/130 % RMT	no change	70% RMT/130% RMT	no change; increased in female sub-group post SD only

ICF, intracortical facilitation; N/A, not applicable (the parameter was not evaluated); NR, not reported (includes parameters that were evaluated however the results are not reported in the article); PRE, before sleep deprivation; POST, after sleep deprivation; RMT, resting motor threshold; lower RMT, the highest TMS intensity where no stimuli elicited MEPs; upper RMT, the lowest TMS intensity where all stimuli elicited MEPs; SD, sleep deprivation; SICI, short-interval intracortical inhibition

[1] conditioning stimulus/test stimulus
[2] subjects supervised during 24 h of testing from the time they arrived at the laboratory
[3] significantly lower during the night than PRE or POST
[4] shorter CSP at 10% RMT late at night than PRE or POST
[5] shorter SICI during the night than PRE or POST
[6] testing between 9 and 10 am each day
[7] raw MEP amplitude
[8] voluntary contraction intensity indicated as moderate
[9] testing was always conducted at 10:30 pm meaning that there was 48 h between testing sessions, not 40 h as indicated in methods

induced cortical changes. Several studies observed decreased SICI (Civardi *et al.*, 2001; Scalise *et al.*, 2006; Kreuzer *et al.*, 2011) and the ICF results were equivocal. Badawy *et al.* (2006) also reported SD of 20 h of continued wakefulness over the previous 24 h. It is debatable whether this period constitutes SD or an extended period of wakefulness despite the fact that this study, like almost all the others, failed to observe a difference between SD and control conditions.

After at least one night SD, exercise performance appears to decrease as exercise duration increases. Equivocal effects of SD on exercise HR, VO_2 and RPE have also been observed. Decreased cognitive functioning is a well-established consequence of SD and this is especially true for simple cognitive measures (*e.g*. RT tasks). Meanwhile, there has been a lack of research into the effects of SD on neuromuscular function. The few studies employing TMS have observed few differences between control conditions and SD although most differences are equivocal. The most consistent observation was increased SICI with SD although all measures were conducted at rest and may thus not have any relation to exercise performance and responses in active muscle.

Further confirmation for the reduction of endurance exercise performance is required. Then it remains to be determined whether central fatigue, as quantified by VA, that develops during prolonged exercise is greater with SD. It is possible that decreased exercise performance and increased RPE during exercise with SD occur in conjunction with and possibly contribute to increased central fatigue. The possibility of mechanistic interaction among these parameters should be explored.

SLEEP DEPRIVATION HIGHLIGHTS

What is known:

- SD impairs exercise performance, possibly to a larger extent with increasing exercise duration
- The effects of SD on maximal strength, HR, RPE and VO_2 are equivocal
- SD induces profound negative effects on cognitive performance such as greater performance variability and slower RT, particularly amongst simple measures of alertness, attention and vigilance.
- Cognitive processes are not equally sensitive to SD
- Exercise permits a transient recovery of cognitive functioning during aerobic exercise with SD

What is not known:

- Whether SD causes increased central and/or peripheral fatigue compared to a control condition
- Whether there are other effects of SD on motor cortical excitatory and/or inhibitory mechanisms
- Whether changes in central mechanisms explain reduced exercise and cognitive performance with SD
- The effects of SD duration and type (complete or partial) and exercise duration, intensity and modality on HR and RPE

ULTRA-ENDURANCE EXERCISE

Ultra-endurance exercise can be categorized as exercise of at least 4-5 h in duration. Many studies that examined prolonged exercise investigated whole-body exercise less than 2 h in duration and frequently at a higher intensity than that seen in ultra-endurance events. A characteristic feature of ultra-endurance exercise is a decrease in maximal force production of active muscles as observed in running (Davies & Thompson, 1986; Millet *et al.*, 2002; Place *et al.*, 2004; Easthope *et al.*, 2010; Martin *et al.*, 2010; Millet *et al.*, 2011c; Saugy *et al.*, 2013), cycling (Lepers *et al.*, 2002; Millet *et al.*, 2003c; Ross *et al.*, 2010b) and cross-country skiing (Forsberg *et al.*, 1979; Viitasalo *et al.*, 1982). This maximal strength loss can be attributed to both central and peripheral mechanisms.

The gold standard for determination of central fatigue is VA. As assessed by ITT, VAp decreases with ultra-endurance exercise (Millet *et al.*, 2002; Place *et al.*, 2004; Martin *et al.*, 2010; Ross *et al.*, 2010b; Millet *et al.*, 2011c; Saugy *et al.*, 2013). Other measures of central fatigue have been less frequently assessed. In running, RMS·Mmax^{-1} has been observed to decrease (Place *et al.*, 2004; Martin *et al.*, 2010; Millet *et al.*, 2011c) or have a tendency to decrease (Millet *et al.*, 2002) in the *vastus lateralis* but not *rectus femoris* or *soleus*. Similarly, cycling *vastus lateralis* and *vastus medialis* RMS·Mmax^{-1} decreased over 5 h of cycling (Lepers *et al.*, 2002).

Indices of peripheral deficits after endurance exercise have been more equivocal than central ones. Most studies have observed decreased potentiated twitch amplitude indicative of reduced excitation-contraction coupling (Lepers *et al.*, 2002; Martin *et al.*, 2010; Ross *et al.*, 2010b; Millet *et al.*, 2011c; Saugy *et al.*, 2013) although Easthope *et al.* (2010) reported no

difference and Place *et al.* (2004) observed increased twitch amplitude that became significant only after 5 h of treadmill running. Further elucidation of the type of peripheral fatigue has been limited since evidence of low- or high-frequency fatigue has not been observed except in a single study after 166-km ultra-trail where low-frequency fatigue just reached statistical significance (Millet *et al.*, 2011c). There is also evidence of action potential transmission perturbation although this is not consistent across muscles or even within the same muscle group. Numerous studies showed decreased M-wave amplitude in at least one muscle (Place *et al.*, 2004; Martin *et al.*, 2010; Ross *et al.*, 2010b; Millet *et al.*, 2011c) while Millet *et al.* (2002) reported increased *soleus* M-wave amplitude. Similarly, several studies observed increased M-wave duration in at least one muscle (Lepers *et al.*, 2002; Easthope *et al.*, 2010; Millet *et al.*, 2011c) while another study observed decreased M-wave duration (Place *et al.*, 2004). Collectively, these results suggest that the presence of peripheral fatigue indices may depend on the muscle investigated, sport and intensity and duration of exercise.

Despite the body of literature demonstrating the importance of central fatigue, as determined by peripheral neural stimulation, after an ultra-endurance exercise bout, there are no published studies that have investigated a more precise source of this central fatigue. It remains to be determined whether central fatigue can be observed at the supraspinal level and what roles changes in corticospinal inhibition and excitation have in the development and presentation of central fatigue in ultra-endurance exercise.

ULTRA-ENDURANCE EXERCISE HIGHLIGHTS

What is known:

- Ultra-endurance exercise is characterized by maximal force loss that is caused by a combination of central and peripheral mechanisms
- Large decreases in VAp have been consistently observed
- Peripheral indices of fatigue are not always present and may be dependent on the muscle investigated and intensity, duration and modality of exercise

What is not known:

- The precise location of central fatigue, especially between spinal and supraspinal locations
- Whether supraspinal fatigue develops and contributes to ultra-endurance exercise limitations
- The effects of excitatory and inhibitory corticospinal mechanisms in the context of central fatigue

The methodological hypotheses of this thesis are that:

- The manner in which a target force is approached influences elicited MEP and SIT amplitudes
- Different methods of determining optimal TMS intensity result in the selection of different intensities

The applied hypotheses of this thesis are that:

- SD induces exercise and cognitive performance deficits compared to a control condition
- Exercise to task failure with SD induces greater central fatigue, including supraspinal fatigue, than in a control condition
- Increased central and supraspinal fatigue and RPE contribute to exercise performance impairment
- An ultra-endurance exercise bout induces significant central fatigue and a contribution occurs at the supraspinal level
- The development of supraspinal fatigue occurs with either unchanged or increased MEP amplitude and unchanged CSP duration

REVUE DE LA LITTERATURE

La fatigue se réfère généralement à une diminution de la performance mesurable. La fatigue physique peut se caractériser par une diminution de la force maximale volontaire ou de la puissance maximale. La fatigue est aussi un symptôme primaire dans de nombreuses maladies, en particulier les troubles neuromusculaires, et peut sérieusement affecter la qualité de vie. En même temps que le développement de la fatigue, le coût énergétique de l'activité physique et la perception subjective de l'effort (RPE) augmentent. Cela peut entraîner une diminution de l'intensité de l'exercice ou son arrêt.

FATIGUE CENTRALE

La fatigue peut être classifiée comme centrale ou périphérique. La partie centrale comprend tous les éléments proximaux à la jonction neuromusculaire, y compris le cerveau et les motoneurones supérieurs et inférieurs. Elle inclut également la diminution de la performance cognitive, des changements du contrôle moteur et de la commande motrice et l'augmentation du RPE. La fatigue centrale est évaluée par la détermination de la capacité à activer volontairement au maximum le muscle (activation volontaire, VA). La fatigue centrale a été observée pendant et après des contractions isométriques volontaires sous-maximales et maximales (MVC) intermittentes et maintenues ainsi qu'après des exercices de course à pied et vélo. Le mode, l'intensité et la durée d'exercice sont importants dans le développement de la fatigue centrale.

La fatigue centrale peut être divisée en sections pour mieux comprendre où et comment la fatigue se présente. Des stimulations peuvent être délivrées au niveau du cortex moteur, de la jonction cervicomédullaire et aux racines des nerfs spinaux. D'autres techniques sont souvent utilisées pour étudier les perturbations centrales associées à l'exercice, telles que l'électromyographie (EMG), la spectroscopie proche infrarouge, l'échographie Doppler, l'imagerie par résonance magnétique et l'électroencéphalographie.

STIMULATION MAGNETIQUE

La stimulation magnétique est basée sur le taux de variation du champ magnétique émis par une bobine. Les anodes et des cathodes virtuelles créées induisent un courant électrique dans

le corps. Si le courant électrique induit est suffisamment fort, le tissu est stimulé. Les bobines de la stimulation magnétique sont de forme circulaire, en forme de huit ou de double cône. La stimulation magnétique, et la stimulation magnétique transcrânienne (TMS) en particulier, ont des applications dans de nombreux domaines. Par exemple, la TMS est utilisée pour étudier la psychiatrie, la vision, la langue, l'émotion, la plasticité du cerveau, la fatigue, les fonctions et la cartographie des régions corticales.

STIMULATION MAGNETIQUE POUR LA FATIGUE

La stimulation magnétique est utilisée comme un substitut à la stimulation électrique dans la recherche et l'évaluation clinique de la fatigue. Elle est utilisée pour stimuler les nerfs périphériques, le cortex moteur (TMS) et la jonction cervicomédullaire.

STIMULATION MAGNETIQUE TRANSCRANIENNE

La stimulation magnétique transcrânienne est une technique non invasive et sûre pour étudier le cortex moteur par excitation transynaptique des neurones pyramidaux et en partie par excitation directe des axones pyramidaux. La TMS peut induire des réponses à la fois excitatrices et inhibitrices mesurables par EMG. Le potentiel moteur évoqué (MEP) est la réponse électrique dans le muscle et indique l'excitabilité corticale. La période de silence corticale (CSP) est la période de quasi-silence d'EMG induite par la TMS après un MEP et est proposée comme étant représentative de l'inhibition intracorticale. La production motoneuronale induite par la TMS est très influencée par l'augmentation rapide de l'excitabilité corticospinale lors de contractions musculaires volontaires à intensités faibles et modérées. L'étude des paramètres centraux mesurés pendant une contraction musculaire avant, pendant et après l'exercice permet de mieux comprendre les origines des changements corticospinaux associés à la fatigue. Avec la stimulation de la jonction cervicomédullaire, la TMS peut être utilisée pour différencier les composantes corticales et spinales. Les nombreuses différences de technique et méthodologie entre les protocoles utilisant la TMS rendent l'interprétation et la comparaison de résultats difficiles. Ces différences concernent le type de stimulateurs, de bobines, la position de la bobine sur la tête et l'intensité de la stimulation. Les avantages et les inconvénients de ces méthodes n'ont pas encore été clarifiés et il reste à déterminer si les différentes méthodes utilisées pour déterminer l'intensité de la

TMS donnent la même intensité ou si la sélection de l'intensité de la TMS doit toujours être effectuée de la même manière.

Membres supérieurs

L'augmentation de l'amplitude de la secousse surimposée (SIT) évoquée par la TMS pendant les protocoles fatigants de type sous maximal et maximal indique que des mécanismes supraspinaux contribuent à la fatigue. Bien que cette observation souligne la présence de la fatigue supraspinale, elle n'élimine pas la possibilité de contributions spinales à la fatigue centrale. L'activation volontaire corticale (VAc) évaluée par la TMS diminue pendant les contractions isométriques fatigantes sous maximales et maximales suggérant que la fatigue supraspinale se développe progressivement. Les changements dans la taille de MEP indiquent des changements dans l'excitabilité corticospinale quand le MEP est normalisé à l'onde M maximale (Mmax) pour tenir compte des changements périphériques. Les modifications de MEPs et de potentiels cervicomédullaires évoqués (CMEPs) peuvent aussi être comparées pour identifier des changements aux niveaux corticaux et spinaux. Pendant les contractions isométriques sous-maximales maintenues, MEP·Mmax^{-1} augmente dans les fléchisseurs du coude en raison de l'augmentation de la commande motrice pour maintenir le niveau de force. Le fait que des changements d'excitabilité se produisent au niveau spinal ou cortical, ou les deux, reste à élucider. Une augmentation de MEP·Mmax^{-1} a été observée au cours d'une MVC maintenue alors que CMEP·Mmax^{-1} diminuait. Collectivement, cela indique une augmentation de l'excitabilité corticale pendant une MVC maintenue. MEP·Mmax^{-1} mesuré immédiatement après l'exercice est élevé par rapport à avant l'exercice, récupère en ~30 s après l'exercice et retourne à son amplitude initiale en quelques minutes. La CSP se prolonge pendant des contractions isométriques fatigantes maintenues. Pendant des contractions prolongées d'intensité faible ou modérée, la CSP augmente progressivement bien que pendant une MVC maintenue la CSP augmente rapidement au cours des premières secondes avant de se stabiliser. Ceci suggère que l'intensité de l'exercice est un facteur important qui influe sur l'inhibition intracorticale. Tout délai entre la fin de l'exercice et les évaluations après l'exercice peut masquer des changements dus à la récupération rapide de la CSP.

Membres inférieurs

La SIT évoquée par TMS augmente au cours de contractions isométriques maintenues. Dans les protocoles de type isométrique et à l'exercice dynamique, VAc est généralement plus bas après l'exercice. Ces résultats indiquent que les exercices isométriques et dynamiques peuvent provoquer de la fatigue supraspinale. Au cours de contractions isométriques sous-maximales des fléchisseurs plantaires maintenues, MEP·Mmax^{-1} augmente tandis que MEP·Mmax^{-1} reste stable au cours d'une MVC maintenue. Ceci est différent de l'augmentation de la MEP rapportée dans les membres supérieurs et renforce la suggestion que des mécanismes de contrôle neuronaux distincts existent pour les membres supérieurs et inférieurs. Un MEP·Mmax^{-1} inchangé a été observé après des protocoles de vélo d'intensités modérée et maximale. Au cours d'un récent protocole de vélo, aucun changement de MEP·Mmax^{-1} ni de CMEP·Mmax^{-1} n'a été rapporté bien que les MEPs et CMEPs normalisés à l'EMG volontaire aient diminué ou soient restés inchangés, respectivement. Ces résultats suggèrent une tendance générale à la diminution de l'excitabilité corticale au cours de l'exercice et à l'épuisement. Les demandes cardiorespiratoires et métaboliques plus élevées au cours de l'exercice comme le vélo ou la course à pied peuvent aussi influencer les réponses corticospinales évoquées. La CSP augmente au cours de MVC isométriques intermittentes et maintenues et elle reste inchangée après un protocole intermittent sous-maximal. Après exercice dynamique, aucun changement de CSP n'a été observé, quel que soit le type d'exercice, sa durée ou son intensité. Ceci suggère que ce type d'exercice peut ne pas susciter d'inhibition intracorticale ou que le délai de l'évaluation après l'exercice est trop long pour observer un changement de CSP. Inversement, l'augmentation de la suppression d'EMG pendant le vélo indique une augmentation de l'inhibition intracorticale. Le fait que la suppression d'EMG peut être évaluée au cours de l'exercice et sans délai avant les mesures après l'exercice peut être importante pour expliquer cette différence.

PRIVATION DE SOMMEIL

La privation de sommeil (SD) se rapporte globalement à une condition de durée de sommeil insuffisante. De nombreuses études ont observé des déficits de la performance au cours de l'exercice aérobie après SD même si ce n'est pas toujours le cas. Les résultats d'études réalisées avec des tests de performance plus courtes en vélo et course à pied sont équivoques,

suggérant que la diminution de la performance est plus probable avec l'augmentation de la durée de l'exercice. Les effets de la SD sur la consommation d'oxygène et la fréquence cardiaque ne suggèrent pas de lien évident. Une augmentation du RPE a été observée dans certains protocoles de longue durée mais pas dans tous. L'effet de l'exercice de moins de 30 minutes sur la RPE est aussi équivoque. La SD partielle ainsi que la SD complète ont des effets profonds sur la performance cognitive. Cela inclut une augmentation de la variabilité de la performance, un augmentation du temps de réaction et une augmentation de l'absence de réponse à un stimulus dans les délais impartis. Les effets de la SD sur les fonctions exécutives sont plus équivoques, ce qui suggère que la SD ne provoque pas une déficience globale du fonctionnement cognitif et que certains aspects de la cognition sont plus fortement touchés que d'autres. L'exercice améliore la performance lors de différentes tâches cognitives lorsque les sujets ne sont pas privés de sommeil et ces améliorations cognitives persistent transitoirement après la cessation de l'exercice. L'exercice pourrait ainsi agir comme une contre-mesure à des déficits cognitifs provoqués par la SD. La TMS a rarement été utilisée pour évaluer les changements corticospinaux induits par la SD chez les sujets sains au repos. Les résultats sont difficiles à interpréter dans le contexte de la fatigue parce que très peu d'études ont employé des contractions musculaires volontaires et leur méthodologie est relativement floue. Il reste à déterminer si la fatigue centrale est supérieure pendant l'exercice avec SD et si la diminution de la performance cognitive et à l'exercice et l'augmentation du RPE avec la SD fréquemment observées sont associées à une fatigue centrale élevée.

EXERCICE D'ULTRA-ENDURANCE

L'exercice d'ultra-endurance est un effort qui dure au minimum 4 à 5 h et jusqu'à plusieurs jours. Une caractéristique de l'exercice d'ultra-endurance est une diminution de la production de force maximale volontaire qui peut être attribuée aux mécanismes centraux et périphériques. Les indices de la fatigue centrale (par exemple, VA) baissent pendant le vélo et la course à pied bien que les déficits périphériques après un exercice d'ultra-endurance soient plus équivoques. La plupart des études ont observé une diminution de l'amplitude de la secousse potentiée ce qui suggère une réduction de couplage excitation-contraction. Il existe aussi des preuves inconsistantes de la perturbation de la transmission du potentiel d'action qui sont démontrés par des altérations de l'onde M. Le développement de la fatigue

périphérique peut dépendre du muscle, du type de sport et de l'intensité et durée de l'exercice. Malgré le développement de la fatigue centrale pendant l'exercice d'ultra-endurance, aucune étude n'a examiné si la fatigue se produit au niveau supraspinal et si les changements de l'inhibition et de l'excitation corticospinale ont un rôle dans le développement de la fatigue centrale au cours d'un exercice d'ultra-endurance.

Les hypothèses méthodologiques de cette thèse sont les suivantes:

• La manière dont une force de cible est approchée influence les amplitudes des MEPs et SITs induits par TMS

• L'intensité de TMS optimale est différente selon les méthodes de détermination choisies

Les hypothèses appliquées de cette thèse sont les suivantes:

• La SD provoque des déficits de performance cognitive par comparaison à une condition contrôle

• L'exercice conduit jusqu'à épuisement dans un état de SD provoque une plus grande fatigue centrale que dans une condition contrôle, et ceci est dû à une augmentation de la fatigue supraspinale

• L'augmentation de fatigue centrale, de fatigue supraspinale et du RPE contribuent à la diminution de la performance à l'exercice avec SD

• Un exercice d'ultra-endurance induit une fatigue centrale importante et une partie de cette fatigue centrale se situe au niveau supraspinal

• Le développement de la fatigue supraspinale se produit avec une amplitude de MEP inchangée ou augmentée et avec une durée de CSP inchangée

INTRODUCTION TO STUDIES 1 AND 2

The two studies forming the first part of this thesis examine two of the methodological issues presented in the literature review (Transcranial Magnetic Stimulation – Methodological Issues). Study 1 examined the effect of stimulating at the specified force on TMS-induced responses while force increased or decreased to the target force or remained stable at the target force. Many studies require subjects to perform brief isometric voluntary contractions and once the subject is at a pre-determined force, TMS is delivered. It has also been observed that corticospinal excitability increases rapidly as voluntary contraction intensity increases from rest to ~50% MVC in numerous muscles (Ugawa *et al.*, 1995; Taylor *et al.*, 1997). Thus, a change in contraction intensity may result in a transient change in corticospinal excitability, a change that may require time for stabilization once the contraction intensity itself has stabilized (*i.e*. a temporal delay between contraction intensity and corticospinal excitability). Thus, the corticospinal excitability present during a voluntary contraction may not be representative of the true corticospinal activity at the target force level if the latter was not maintained for a sufficient duration. Study 1 investigated whether the contraction intensity alone influences evoked responses or whether the manner in which a target force is approached, and any possible changes to corticospinal excitability arising therefrom, may also influence evoked responses.

Study 2 examined three commonly employed methods used to determine TMS intensity in order to compare their effects on selection of stimulus intensity. This has enormous implications in both research and clinical settings because selection of a stimulus intensity that permits evaluation of the desired parameters may preclude repeat visits (*i.e*. follow-up visits might be necessary if the selected stimulus intensity was too high or too low to properly interpret the desired parameters), allow selection of the lowest required stimulus intensity to reduce subject discomfort, including undesired stimulation of non-targeted muscles, and save time. Different research groups use different methods of determining stimulus intensity. These methods include RMT, AMT and selection of a stimulus intensity to evoke MEP responses of a certain size. Groppa *et al.* (2012) indicate that optimal stimulus intensity occurs at the transition from the rising slope to the flat portion of the sigmoid stimulus-response (stimulus intensity-MEP amplitude/area) curve and suggest that this corresponds approximately to 140% RMT or 170% AMT. Instead of estimating optimal stimulus intensity, it may be more valid to select the intensity from an appropriate stimulus-

response curve (*i.e.* with the voluntary optimal contraction intensity and curve analysis). It also remains to be evaluated whether the percentages of RMT and AMT frequently used to determine optimal stimulus intensity correspond to the optimal intensity as proposed by Groppa *et al.* (2012).

In the study of fatigue, many protocols have employed brief voluntary contractions, especially in the evaluation of VAc. These brief voluntary contractions are of intensities ≥50% MVC (Todd *et al.*, 2003; Goodall *et al.*, 2009; Sidhu *et al.*, 2009a). As reported by Rupp *et al.* (2012), a series of 32 brief contractions at 50% MVC with at least 10 s rest between each followed by baseline evaluation of VAc is sufficient to produce residual fatigue that can remain in evidence 1 h later. Thus, a stimulus-response curve performed at 50% MVC would not be ideal despite corresponding to contraction intensities to be evaluated. It has not been evaluated whether stimulus-response curves at lower contraction intensities (e.g. 10 or 20% MVC) may be used to determine a comparable stimulus intensity.

STUDY 1

Effect of different approaches of target force on transcranial magnetic stimulation responses

Effet de différentes approches de la force cible sur les réponses induites par stimulation magnétique transcrânienne

Mathieu Gruet,[1]* John Temesi,[2]* Thomas Rupp,[1] Guillaume Millet,[2] Samuel Verges,[1]

[1] HP2 Laboratory (U1042 INSERM), Joseph Fourier University, Grenoble, France

[2] Université de Lyon, F-42023, Saint-Etienne, France

* Both authors contributed equally to this work

Published as:

Gruet M, Temesi J, Rupp T, Millet GY & Verges S. (2013). Effect of different approaches to target force on transcranial magnetic stimulation responses. *Muscle Nerve* doi: 10.1002/mus.23786.

ABSTRACT

The aim of this study was to determine whether the manner in which a target force is approached can influence the EMG parameters evoked by transcranial magnetic stimulation (TMS) during brief muscular contractions. The amplitude of motor-evoked potentials (MEPs) and duration of the silent period were recorded in 8 healthy participants in response to TMS delivered during brief isometric voluntary contractions of the quadriceps maintaining a target force (10 and 50% of maximal voluntary force) or gradually increasing or decreasing to it. This study demonstrates that MEPs, unlike silent periods, are influenced by the manner of reaching a low (10% of maximal voluntary force) but not moderate (50% of maximal voluntary force) force level. Clear instructions must be provided to research participants and patients. Rapidly increasing to a target force without exceeding it and maintaining the force before the delivery of TMS results in stable and representative MEP amplitudes.

Keywords: transcranial magnetic stimulation; muscle contraction; motor evoked potential; cortical silent period; variability.

RÉSUMÉ

Le but de cette étude était de déterminer si la manière avec laquelle une force cible est atteinte peut influencer l'EMG et les paramètres mécaniques évoqués par la stimulation magnétique transcrânienne (TMS) lors de courtes contractions musculaires. L'amplitude des potentiels moteurs évoqués (MEP) et des secousses surimposées ainsi que la durée des périodes de silence ont été enregistrés chez 8 sujets sains en réponse à une TMS délivrée au cours de courtes contractions volontaires isométriques du quadriceps. Les stimulations ont été effectuées en maintenant une force cible (10 et 50% de la force maximale volontaire) ou en augmentant ou en diminuant progressivement la force produite jusqu'à cette cible. Contrairement à la période de silence, les MEPs et secousses surimposées sont influencés par la manière d'atteindre une force cible d'intensité faible (10% de la force maximale volontaire) mais pas modérée (50% de la force maximale volontaire). Cette étude démontre que des instructions claires doivent être fournies aux sujets participants à des protocoles de recherche ainsi qu'aux patients lors d'investigation clinique. Une montée rapide à la force

cible sans la dépasser et un maintien de cette force avant de délivrer la TMS permet d'obtenir des amplitudes de MEPs stables et représentatives.

Mots clés : *stimulation magnétique transcrânienne, contraction musculaire, potentiel moteur évoqué, période de silence corticale, variabilité*

INTRODUCTION

Transcranial magnetic stimulation (TMS) over the motor cortex during muscle contraction produces a motor-evoked potential (MEP) followed by interruption of ongoing electromyographic (EMG) activity, termed silent period (CSP). MEP and CSP are useful measures of corticospinal excitability and intracortical inhibition but can be highly variable in response to the same stimulus (Darling *et al.*, 2006; Saisanen *et al.*, 2008). As this variability may also be affected by different experimental conditions, it appears critical to identify optimal experimental protocols in order to accurately detect adaptive changes in motor cortical pathways. As participants contract to a visual target force, they may sometimes gradually increase to it or exceed it before decreasing to the target level. It is unknown whether the means of approaching a target force influences EMG responses to TMS. The main objective of this study was to investigate the effects of three means of reaching a desired force on CSP and MEP amplitude.

METHODS

Eight healthy males were studied (age: 29.5 ± 7.8 years) after providing written informed consent. The study was conducted according to the Declaration of Helsinki and approved by the local ethics committee.

Participants sat upright in a custom-built chair with both hips and right knee at 90° of flexion. The distal part of the right shank was connected with a non-compliant strap to a force transducer just proximal to the lateral malleolus. Surface EMG signals were recorded from the right *vastus lateralis*, *vastus medialis*, *rectus femoris* and *biceps femoris* muscles in bipolar configuration as previously described (Rupp *et al.*, 2012).

TMS (Magstim 200², Magstim Co., Whitland, UK) by double cone coil (110 mm diameter, 1.4 T) positioned over the left motor cortex was used to elicit MEPs and SPs in the contralateral knee extensors. Optimal coil position and stimulus intensity corresponded to the site and intensity that elicited the largest MEP amplitudes in quadriceps muscles and small MEPs in *biceps femoris* (Rupp *et al.*, 2012).

Participants performed 24 contractions of the right knee extensors (4 brief contractions for each of six conditions, the first three at 10% of maximum voluntary contraction (MVC) then three at 50% MVC in random order with visual feedback). The conditions were i) increasing force at a constant rate to 10% MVC over ~2 s (increasing, $INC_{10\%}$), ii) contracting

rapidly to 10% MVC and maintaining the force for ~2 s (plateau, $PLA_{10\%}$), iii) contracting rapidly to 20% MVC and maintaining the force for ~1 s then decreasing force at a constant rate to 10% MVC over 1-2 s (decreasing, $DEC_{10\%}$), iv) contracting rapidly to 40% MVC and maintaining the force for ~1 s then increasing force at a constant rate to 50% MVC over 1-2 s ($INC_{50\%}$), v) contracting rapidly to 50% MVC and maintaining the force for ~2 s ($PLA_{50\%}$) and vi) contracting rapidly to 60% MVC and maintaining the force for ~1 s then decreasing force at a constant rate to 50% MVC over 1-2 s ($DEC_{50\%}$). PLA stimuli were manually delivered after maintenance of force for ~2 s. INC and DEC stimuli were delivered automatically once the force increased (INC) or decreased (DEC) to the threshold. After each stimulus, participants were asked to return as fast as possible to the target force level. The inter-contraction interval was 20 s for conditions i-iii and 30 s for conditions iv-vi.

MEP amplitude and EMG root mean square (RMS) for 200 ms prior to the stimulus were measured offline. Contraction time (CT) and CSP were calculated as the intervals from voluntary force initiation to the stimulus and from the stimulus to the resumption of continuous EMG (Hunter *et al.*, 2008), respectively. MEP, CSP, RMS and CT are the means of four contractions for each of the six conditions. Within-participant coefficients of variation (CVs) for MEP and CSP were also determined for each condition.

Statistical significance was set at $P < 0.05$. Data are presented as mean ± standard deviation. To assess condition differences, one-way analyses of variance for repeated measures were conducted on mean values for each muscle at both contraction levels. MEP and CSP CVs were also analyzed to determine whether parameter variability was affected by condition. Tukey's post-hoc tests were used when necessary.

RESULTS

MEP amplitude was lower in $DEC_{10\%}$ than both $INC_{10\%}$ and $PLA_{10\%}$ for all muscles ($P < 0.01$, **Figure 15A**). MEP amplitude was similar for all conditions at 50% MVC. There was no difference in CSP at either 10% (**Figure 15B**) or 50% MVC. MEP CVs were similar between the three conditions for all muscles and both force levels. CSP CVs were higher in DEC at both force levels for the *vastus lateralis*, in $DEC_{10\%}$ for *the rectus femoris* and in $DEC_{50\%}$ for the *vastus medialis* (all $P < 0.05$). CT was longer in $DEC_{10\%}$ (3.69 ± 0.38 s) than both $PLA_{10\%}$ (2.75 ± 0.33 s) and $INC_{10\%}$ (1.86 ± 0.42 s) ($P < 0.01$) and shorter in $DEC_{50\%}$ (2.62 ± 0.26 s)

than in $INC_{50\%}$ (3.13 ± 0.63 s) ($P < 0.05$). EMG RMS was similar between the three conditions for all muscles at both force levels.

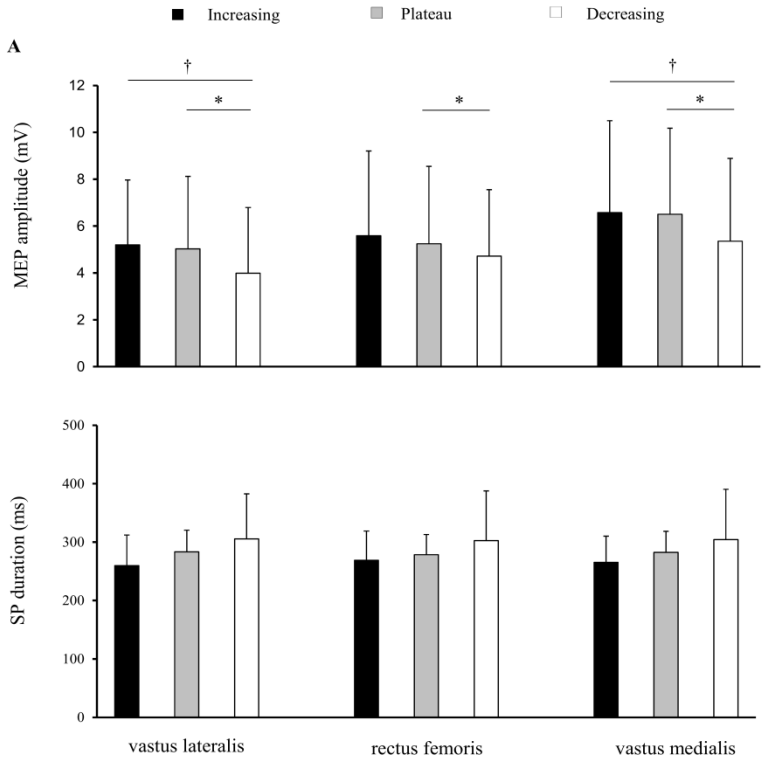

Figure 15. Effects of different means of approaching a target force on MEP amplitude (Panel **A**) and CSP duration (Panel **B**) for the *vastus lateralis*, *rectus femoris* and *vastus medialis* at 10% MVC. * Significant difference between the decreasing and plateau conditions ($P < 0.01$). † Significant difference between the decreasing and increasing conditions ($P < 0.01$). Due to background EMG noise, it was only possible to determine CSPs in 6 participants.

DISCUSSION

The main result of this study is that, unlike the CSP, the MEP was influenced in all quadriceps muscles by the manner of approaching a low force level; the $DEC_{10\%}$ condition produced smaller MEP amplitudes.

This condition effect might be caused by differences in volitional EMG before the stimulus (Sidhu *et al.*, 2012a) and/or CT between conditions. However, the similar volitional EMG for conditions at both 10 and 50% MVC and the absence of MEP changes between

conditions presenting different CT such as at 50% MVC and between $INC_{10\%}$ and $PLA_{10\%}$ suggest otherwise.

In $DEC_{10\%}$, the force reduction from 20% to 10% MVC may have transiently decreased the spinal excitability at stimulus application leading to reduced MEP amplitude without affecting the intracortical inhibitory mechanisms responsible for the CSP. A similar transient depression in spinal excitability in $DEC_{50\%}$ may have been masked by the substantially higher corticospinal excitability required to exert a contraction at 50% MVC. The highly variable CSP in both $DEC_{10\%}$ and $DEC_{50\%}$ suggest that the instructions given to the participants (*i.e.* returning as fast as possible to the desired force level) were more difficult to follow in these conditions (Mathis *et al.*, 1998).

Particular attention is needed regarding the manner a low target force is reached before delivering a TMS pulse in order to obtain stable and representative MEP and CSP measurements. These results highlight the importance of not surpassing the target force before delivering the TMS.

STUDY 2

Resting and active motor thresholds versus stimulus-response curves to determine transcranial magnetic stimulation intensity in *quadriceps femoris*

Comparaison entre le seuil moteur de repos, le seuil moteur actif et les courbes stimulus-réponse pour déterminer l'intensité de stimulation par stimulation magnétique transcrânienne du quadriceps femoris

John Temesi,[1] Mathieu Gruet,[2,3] Thomas Rupp,[2,3] Samuel Verges,[2,3] Guillaume Millet[1,3]

[1] Laboratoire de Physiologie de l'Exercice, Université de Lyon, F-42023, Saint-Etienne, France
[2] Laboratoire HP2, Université Joseph Fourier & CHU Grenoble, F-38000, Grenoble, France
[3] U1042, INSERM, F-38000, Grenoble, France

ABSTRACT

Transcranial magnetic stimulation (TMS) is a widely-used investigative technique in motor cortical evaluation. Recently, there has been a surge in TMS studies evaluating lower-limb fatigue. TMS intensity of 120-130% resting motor threshold (RMT) and 120% active motor threshold (AMT) and TMS intensity determined using stimulus-response curves during muscular contraction have been used in these studies. With the expansion of fatigue research in locomotion, the quadriceps femoris is increasingly of interest. It is important to select a stimulus intensity appropriate to evaluate the variables, including voluntary activation, being measured in this functionally important muscle group. This study assessed whether selected quadriceps TMS stimulus intensity determined by frequently employed methods is similar between methods and muscles. Stimulus intensity in vastus lateralis, rectus femoris and vastus medialis muscles was determined by RMT, AMT (i.e. during brief voluntary contractions at 10% maximal voluntary force, MVC) and maximal motor-evoked potential (MEP) amplitude from stimulus-response curves during brief voluntary contractions at 10, 20 and 50% MVC at different stimulus intensities. Stimulus intensity determined from a 10% MVC stimulus-response curve and at 120 and 130% RMT was higher than stimulus intensity at 120% AMT (lowest) and from a 50% MVC stimulus-response curve ($P < 0.05$). Stimulus intensity from a 20% MVC stimulus-response curve was similar to 120% RMT and 50% MVC stimulus-response curve. Mean stimulus intensity for stimulus-response curves at 10, 20 and 50% MVC corresponded to approximately 135, 115 and 100% RMT and 180, 155 and 130% AMT, respectively. Selected stimulus intensity was similar between muscles for all methods ($P > 0.05$). The higher stimulus intensity at 120-130% RMT with the potential to cause increased coactivation and discomfort and the lower stimulus intensity at 120% AMT that may underestimate evoked responses preclude their use to accurately determine maximal MEP amplitude. Similar optimal stimulus intensity and maximal MEP amplitudes at 20 and 50% MVC and the minimal risk of residual fatigue at 20% MVC suggest that a 20% MVC stimulus-response curve is appropriate for determining TMS stimulus intensity. One muscle may also act as a surrogate in determining optimal quadriceps femoris stimulation intensity.

Keywords: stimulus intensity determination, fatigue, methodological considerations.

RÉSUMÉ

La stimulation magnétique transcrânienne (TMS) est une technique d'investigation souvent utilisée dans l'évaluation du cortex moteur. Récemment, il y a eu une augmentation exponentielle du nombre de recherches évaluant la fatigue des membres inférieurs par TMS. Les intensités de TMS généralement utilisées dans ces études correspondent à 120-130% du seuil moteur de repos (RMT), 120% du seuil moteur actif (AMT) ou ont été déterminées à partir des courbes stimulus-réponse obtenues au cours de contractions musculaires sous-maximales. Dans le cadre de la recherche sur la fatigue et la locomotion, le quadriceps est un muscle particulièrement important. Il est essentiel de choisir une intensité de stimulation pertinente pour évaluer les variables mesurée dans ce groupe musculaire fonctionnellement clef, par exemple pour l'évaluation de l'activation volontaire. La présente étude a évalué si les intensités de stimulation du quadriceps déterminées par les méthodes fréquemment employées sont similaires entre elles et ceci au niveau des différents chefs du muscle quadriceps. L'intensité de la stimulation du vastus lateralis, rectus femoris et vastus medialis a été déterminée par RMT, AMT (i.e. pendant de brèves contractions volontaires à 10% de la force maximale volontaire, MVC) et à partir de l'amplitude maximale du potentiel moteur évoqué (MEP) obtenue sur des courbes de stimulus-réponse pendant de brèves contractions volontaires à 10, 20 et 50% MVC. L'intensité de la stimulation déterminée à partir d'une courbe stimulus-réponse à 10% MVC et à 120 et 130% RMT était plus haute que l'intensité de la stimulation à 120% AMT (la plus basse) et d'une courbe stimulus-réponse à 50% MVC ($P < 0,05$). L'intensité de la stimulation déterminée par une courbe stimulus-réponse à 20% MVC était similaire à celle obtenue à 120% RMT et par courbe stimulus-réponse à 50% MVC. Les intensités de stimulation moyennes pour les courbes stimulus-réponse à 10, 20 et 50% MVC correspondaient à environ 135, 115 et 100% RMT, respectivement, et 180, 155 et 130% AMT, respectivement. Les intensités de stimulation choisies étaient similaires entre les muscles pour toutes les méthodes ($P > 0,05$). Les intensités de stimulation plus élevées correspondant à 120-130% RMT avec l'occurrence d'une augmentation de co-activation et de l'inconfort ainsi que l'intensité de stimulation plus basse à 120% AMT pouvant sous-estimer les réponses évoquées empêchent leur utilisation dans la détermination de l'amplitude de MEP maximale. Les intensités optimales et amplitudes de MEP maximales similaires obtenues avec les courbes stimulus-réponse à 20 et 50% MVC ainsi qu'un risque inférieur de fatigue résiduelle à 20% MVC par rapport à 50% MVC suggèrent qu'une courbe stimulus-réponse à 20% MVC est recommandée pour déterminer l'intensité de stimulation optimale

par TMS dans le contexte de l'étude des réponses à l'effort et à la fatigue. Un seul chef du quadriceps peut également être représentatif du quadriceps pour déterminer l'intensité optimale de TMS de ce groupe musculaire dans son ensemble.

Mots clés : *Détermination de l'intensité du stimulus, fatigue, considération méthodologique*

INTRODUCTION

Transcranial magnetic stimulation (TMS) is a safe non-invasive technique employed to investigate the motor cortex. A rapidly changing magnetic field is produced by a coil placed over the target area of the brain and this causes electromagnetic induction to generate an electrical current in the brain. When sufficiently strong, this electrical current causes direct and trans-synaptic depolarization, and stimulation, of the pyramidal tract axons.

Selection of suitable TMS intensity is an important concern for researchers and clinicians. While being non-invasive, stimulation of the brain may be uncomfortable, particularly at high stimulus intensities. Thus, reducing the number of stimuli necessary to determine stimulus intensity and selecting the minimum intensity necessary to appropriately measure the desired parameters is beneficial to both investigators and subjects. The latter point has been largely absent in the literature despite several studies finding either similar or contradictory results when two different stimulus intensities were employed (McNeil *et al.*, 2011a; Temesi *et al.*, 2013). The majority of recent research has been conducted on clinical populations, and thus, recommendations are generally directed towards investigations in clinical populations or for clinical purposes (Lefaucheur *et al.*, 2011; Groppa *et al.*, 2012). International Federation of Clinical Neurophysiology (IFCN) practical guidelines (Groppa *et al.*, 2012) discuss different methods of determining cortical motor threshold in relaxed muscle (RMT, resting motor threshold) and subsequent implications for stimulus intensity. These practical guidelines state that optimal intensity for TMS should correspond to the transition from the rising slope to the flat portion of the sigmoid stimulus-response (stimulator intensity-elicited motor-evoked potential (MEP) amplitude) curve and that this optimal intensity corresponds approximately to 140% RMT or 170% cortical motor threshold determined during voluntary muscular contraction (AMT, active motor threshold) (Groppa *et al.*, 2012). Stimulus-response curves are not currently used for diagnostic purposes despite providing a direct means to determine stimulus intensity to elicit maximal MEP responses. This type of method has recently been employed by several research groups in the applied exercise sciences (Sidhu *et al.*, 2009b; Klass *et al.*, 2012; Rupp *et al.*, 2012; Temesi *et al.*, 2013) while several other studies have determined stimulus intensity from RMT or AMT (Sammut *et al.*, 1995; Goodall *et al.*, 2012; Iguchi & Shields, 2012; Weier *et al.*, 2012). It remains to be determined if commonly employed selection of TMS intensity as determined by RMT, AMT and stimulus-response curves in this applied field result in selection of similar TMS intensities. Furthermore, practical guidelines for TMS intensity determination are normally

based on investigations in upper-limb muscles. Data from lower-limb muscles are limited despite the functional importance of the lower limbs, specifically in regards to locomotion.

Studies utilizing TMS to investigate fatigue or acute exercise interventions in lower-limb muscles have used various methods to determine stimulus intensity. The most common of these has been RMT (the lowest intensity necessary to elicit MEPs, usually of at least 0.05 mV in amplitude, in at least one half of a given number of stimuli in the relaxed muscle) (Sammut *et al.*, 1995; Lentz & Nielsen, 2002; Mileva *et al.*, 2009; Goodall *et al.*, 2012; Tallent *et al.*, 2012). Another common method is AMT (the lowest intensity necessary to elicit detectable MEPs or MEPs of a pre-determined amplitude in at least one half of a given number of stimuli during weak voluntary contraction) (Kalmar & Cafarelli, 2006; Hilty *et al.*, 2011; Iguchi & Shields, 2012; Krishnan & Dhaher, 2012; Weier *et al.*, 2012). More recently, numerous studies have selected a stimulus intensity to evoke MEP responses of a certain size in the target muscle during voluntary contraction (Sidhu *et al.*, 2009a, b; Klass *et al.*, 2012; Rupp *et al.*, 2012; Fernandez-del-Olmo *et al.*, 2013; Temesi *et al.*, 2013). Some studies are unclear about the intensity chosen for TMS (McKay *et al.*, 1995) or whether intensity determination was performed with the muscle in the relaxed or contracted state (Hollge *et al.*, 1997). Other studies based stimulus intensity on the intensity chosen to stimulate another muscle group (Verin *et al.*, 2004) or simply selected maximal stimulator output (Gibbons *et al.*, 2010).

Each of these methods produces a unique set of concerns. Cortical excitability is intrinsically linked to voluntary contraction intensity. While cortical excitability is low at rest, it increases rapidly as contraction intensity increases from rest (Ugawa *et al.*, 1995; Taylor *et al.*, 1997). Whether determination of stimulus intensity in relaxed muscle (as with RMT) is appropriate for conducting measures in contracting muscle is unknown. Similarly, it remains to be determined whether determining stimulus intensity at a different contraction level than that employed during evaluation is appropriate.

An additional complexity when evaluating leg muscles (*e.g.* knee extensors, knee flexors, plantar flexors) is that, unlike the elbow flexors, there is not a single dominant muscle. Whether it is appropriate to use a single muscle as a surrogate for all muscles within a muscle group (*e.g. rectus femoris* (RF) for the *quadriceps femoris*) when determining stimulus intensity remains to be investigated, especially since muscles and muscle groups may respond differently to TMS. This is a pertinent issue given both the functional importance of the *quadriceps femoris* and its increasing prevalence in studies utilizing TMS in the

evaluation of fatigue (Sidhu *et al.*, 2009b; Goodall *et al.*, 2010; Goodall *et al.*, 2012; Klass *et al.*, 2012; Temesi *et al.*, 2013).

Fatigue of the quadriceps is increasingly being evaluated in both healthy and clinical populations. An important measure in fatigue evaluation is voluntary activation (VA) (Gandevia, 2001; Gruet *et al.*, 2013a). Evaluation of cortical VA utilizes superimposed twitches (SIT) evoked by TMS delivered during moderate- to high-intensity voluntary contractions (*i.e.* ≥50% maximal voluntary force, MVC) (Todd *et al.*, 2003; Goodall *et al.*, 2009; Sidhu *et al.*, 2009a). Evoked MEP responses at ~50% MVC are theoretically maximal due to the firing of almost all motoneurons and maximal corticospinal excitability (Taylor *et al.*, 1997; Todd *et al.*, 2003; Sidhu *et al.*, 2009a). Since a key component of VA is the requirement that the muscle is driven maximally, maximal MEP amplitude is believed to be essential to ensure that SIT, and by extension VA, is not underestimated. Recently, *quadriceps femoris* studies have begun using TMS-induced antagonist coactivation as a criterion in the selection of TMS intensity (Sidhu *et al.*, 2009b; Goodall *et al.*, 2012; Klass *et al.*, 2012; Rupp *et al.*, 2012) since this may cause SIT underestimation, and thus underestimate the development of central fatigue.

A comparison of selected stimulus intensity between published studies is impossible due to the use of different methods and equipment and different study aims. Thus, the primary objective of this study was to compare different methods of determining TMS intensity for the purposes of fatigue evaluation in the *quadriceps femoris* on selected stimulus intensity. Because of the use of voluntary contractions ≥50% MVC to determine VA and because maximal MEP responses have been observed to occur during contractions of approximately 50% MVC, a stimulus-response curve at 50% MVC was used as a baseline for comparison with other methods (*i.e.* this method most closely resembles fatigue evaluation). By using the same stimulator, coil and stimulation site, this protocol permits the isolation of differences between methods of stimulus intensity determination. The secondary objective was to determine whether selected stimulus intensity is similar for each of the three superficial quadriceps muscles.

METHODS

Subjects

Eight healthy active men participated in this study (means ± standard deviation: age, 30 ± 8 years; height, 181 ± 5 cm; body mass, 73 ± 4 kg). Subjects were informed of the experimental protocol and all associated risks prior to giving written informed consent as part of a medical inclusion. All procedures conformed to the Declaration of Helsinki and were approved by the local ethics committee.

Experimental design

Each subject completed one familiarization session and one experimental session. During the familiarization session, subjects were introduced to all procedures conducted in the experimental session and repeated trials until they performed all tests consistently and as directed. The largest MVC from the familiarization session was used to calculate contraction intensities and the reproducibility of MVC between sessions was verified.

Force and electromyographic recordings

Knee extensor force was measured during voluntary and evoked contractions by a calibrated force transducer (Meiri F2732 200 daN, Celians, Montauban, France) with amplifier that was attached by a non-compliant strap to the right leg immediately proximal to the malleoli of the ankle joint. Subjects were seated upright in a custom-built chair with both hips and right knee at 90° of flexion. The force transducer was fixed to the chair such that force was measured in direct line to the applied force. Electromyographic (EMG) activity of the right knee extensors (RF, *vastus lateralis* (VL) and *vastus medialis* (VM)) and flexors (*biceps femoris*, BF) was recorded.

EMG activity was recorded with a pair of self-adhesive surface electrodes (Meditrace 100, Covidien, Mansfield, USA) in bipolar configuration with a 30-mm interelectrode distance and the reference on the patella. Low impedance (<5 kΩ) between electrodes was obtained by shaving, gently abrading the skin with sandpaper and then cleaning it with isopropyl alcohol. Signals were analogue-to-digitally converted at a sampling rate of 2000 Hz by PowerLab system (16/30—ML880/P, ADInstruments, Bella Vista, Australia) and octal bio-amplifier (ML138, ADInstruments) with bandpass filter (5-500 Hz) and analyzed offline using Labchart 7 software (ADInstruments).

Femoral nerve stimulation

Single electrical stimuli of 1-ms duration were delivered via constant-current stimulator (DS7A, Digitimer, Welwyn Garden City, Hertfordshire, UK) to the right femoral nerve via a 30-mm diameter surface cathode in the femoral triangle (Meditrace 100, Covidien, Mansfield, USA) and 50 x 90 mm rectangular anode (Durastick Plus, DJO Global, Vista, USA) on the *gluteus maximus*. Single stimuli were delivered incrementally until plateaus in maximal M-wave (Mmax) and twitch amplitude were reached. Three supramaximal stimuli at 130% of the intensity to produce maximal Mmax and twitch responses (52 ± 9 mA) were delivered at rest.

Transcranial magnetic stimulation

Single-pulses (0.1-ms rise time; 1-ms duration) were manually delivered by TMS to elicit MEPs and twitches in the right knee extensors. The contralateral motor cortex was stimulated by a magnetic stimulator (Magstim 200², The Magstim Company Ltd, Whitland, UK) with 110-mm double-cone coil (maximum output of 1.4 T) to induce a postero-anterior current. The coil was manually controlled by an experienced investigator throughout the protocol. Subjects wore a cervical collar during all TMS measures to stabilize the head and neck.

Determination of coil position

Subjects wore a latex swim cap on which lines were drawn between the preauricular points and from nasion to inion to identify the vertex. Every centimeter from 1 cm anterior to 3 cm posterior to the vertex was demarcated along the nasal-inion line and also to 2 cm over the left motor cortex. At each point a stimulus was delivered at 70% maximal stimulator output during brief voluntary contraction of the knee extensors at 10% MVC. Target force was displayed on a screen and subjects were provided with real-time visual feedback during all voluntary contractions throughout the protocol. The coil was positioned at the site evoking the largest VL (39.5 ± 19.2% Mmax), RF (75.9 ± 26.7% Mmax) and VM (45.0 ± 21.3% Mmax) MEP amplitudes and SIT with minimal BF MEP amplitude. This coil position was drawn directly onto the swim cap and used throughout the protocol. Coil position was also verified before the delivery of each stimulus.

Determination of stimulus intensity

Four methods of determining stimulus intensity were investigated in the following order: 1) RMT: Beginning at 30% of maximal stimulator output and increasing by 5% increments to 80%, subjects received 10 stimuli at each stimulus intensity with the knee extensors completely relaxed. Stimuli were delivered at 10-s intervals. 2) AMT/stimulus-response curve at 10% MVC: Subjects performed brief voluntary contractions (~2-3 s) of the knee extensors with TMS delivered 10 times at 20, 25, 30, 35 and then 40% of maximal stimulator output. Subjects then performed brief contractions with TMS delivered 4 consecutive times at each of the following randomly-ordered stimulus intensities: 50, 60, 70 and 80% of maximal stimulator output. All stimuli were delivered at 15-s intervals. 3) Stimulus-response curve at 20% MVC: Subjects performed brief contractions (~2-3 s) of the knee extensors with TMS delivered 4 consecutive times at each of the following randomly-ordered stimulus intensities: 20, 30, 40, 50, 60, 70 and 80% maximal stimulator output. Stimuli were delivered at 15-s intervals. 4) Stimulus-response curve at 50% MVC: Similar to the stimulus-response curve at 20% MVC except that stimuli were delivered at 20-s intervals. During voluntary contractions, TMS was always delivered once the subject had contracted to the appropriate force level and the force had stabilized (Gruet *et al.*, 2013b) and 10 min rest was provided between each of the four methods.

Data analysis

Peak-to-peak MEP and Mmax amplitudes were measured offline for each individual response. Individual MEP and Mmax amplitudes were then averaged and MEP amplitudes were normalized to Mmax amplitudes evoked in relaxed muscle. Data collected from a similar group of subjects in our laboratory indicated Mmax amplitudes were similar at rest and at the contraction intensities employed in this study (*i.e*. up to 50% MVC, unpublished observations, 2012). RMT was determined as the lowest stimulus intensity producing at least 5 MEPs of at least 0.05 mV from 10 stimuli. RMT was also determined from 6 and 8 stimuli (minimum of 3 and 4 MEPs, respectively). Stimulus intensities of 120 and 130% RMT were determined for comparison with methods used in other lower-limb studies (Sammut *et al.*, 1995; Lentz & Nielsen, 2002; Ross *et al.*, 2007; Goodall *et al.*, 2009; Goodall *et al.*, 2010; Goodall *et al.*, 2012; Tallent *et al.*, 2012). AMT was determined by visual identification of MEPs above background EMG from contractions at 10% MVC (Sacco *et al.*, 1999) and corresponded to the lowest stimulus intensity producing MEPs in at least half the contractions. Classically, fixed thresholds are used to determine the presence of a MEP (*i.e*. 0.2 mV at 10% MVC

(Weier *et al.*, 2012)); however, the large variability in background EMG activity for the three measured quadriceps muscles rendered this method impractical. AMT was also determined from 6 and 8 stimuli (minimum of 3 and 4 MEPs, respectively). The stimulus intensity of 120% AMT was determined for comparison because of its use in other lower-limb studies (Iguchi & Shields, 2012; Weier *et al.*, 2012). Stimulus-response curves at 10, 20 and 50% MVC were used to determine stimulus intensity by identifying the minimum stimulus intensity to evoke maximal MEP amplitude (*i.e.* the lowest intensity resulting in an increase of less than 5% MEP amplitude at higher stimulus intensities). Individual MEPs from a typical stimulus-response curve at 20% MVC for one subject are presented in **Figure 16**. Antagonist MEP amplitude was examined to verify that this stimulus intensity did not elicit increased TMS-induced coactivation. For the 10% MVC stimulus-response curve, only the first 4 stimuli at 20, 30 and 40% maximal stimulator output were considered. Where a plateau was not reached, MEP amplitude at 80% maximal stimulator output was compared to the estimated maximal MEP amplitude from Boltzmann modeling (see next paragraph). If mean MEP amplitude was greater or equal to the maximal modeled MEP amplitude, 80% was accepted as being part of the plateau and selected as the appropriate stimulus intensity. Otherwise, a plateau was determined to not have occurred and the data was excluded from analyses.

MEP amplitude from stimulus-response curves were modeled with a Boltzmann sigmoidal function (Devanne *et al.*, 1997) using the equation:

$$\mathrm{MEPmax}(S) = \frac{\mathrm{MEPmax}}{1 + \exp\left[\frac{S50 - S}{k}\right]}$$

where MEPmax is the estimated maximal MEP amplitude, S is the stimulus intensity, $S50$ is the stimulus intensity required to produce a response equal to half MEPmax and k is the slope parameter (inversely proportional to maximal function steepness). To eliminate the effects of background EMG in the modeling process, an amplitude of 0 mV was assigned to all responses in which there was no discernible MEP.

Statistics

Statistical analyses were performed with Statistica (version 8, Tulsa, USA). The Shapiro-Wilk test was used to verify data normality. One-way repeated measures analyses of variance

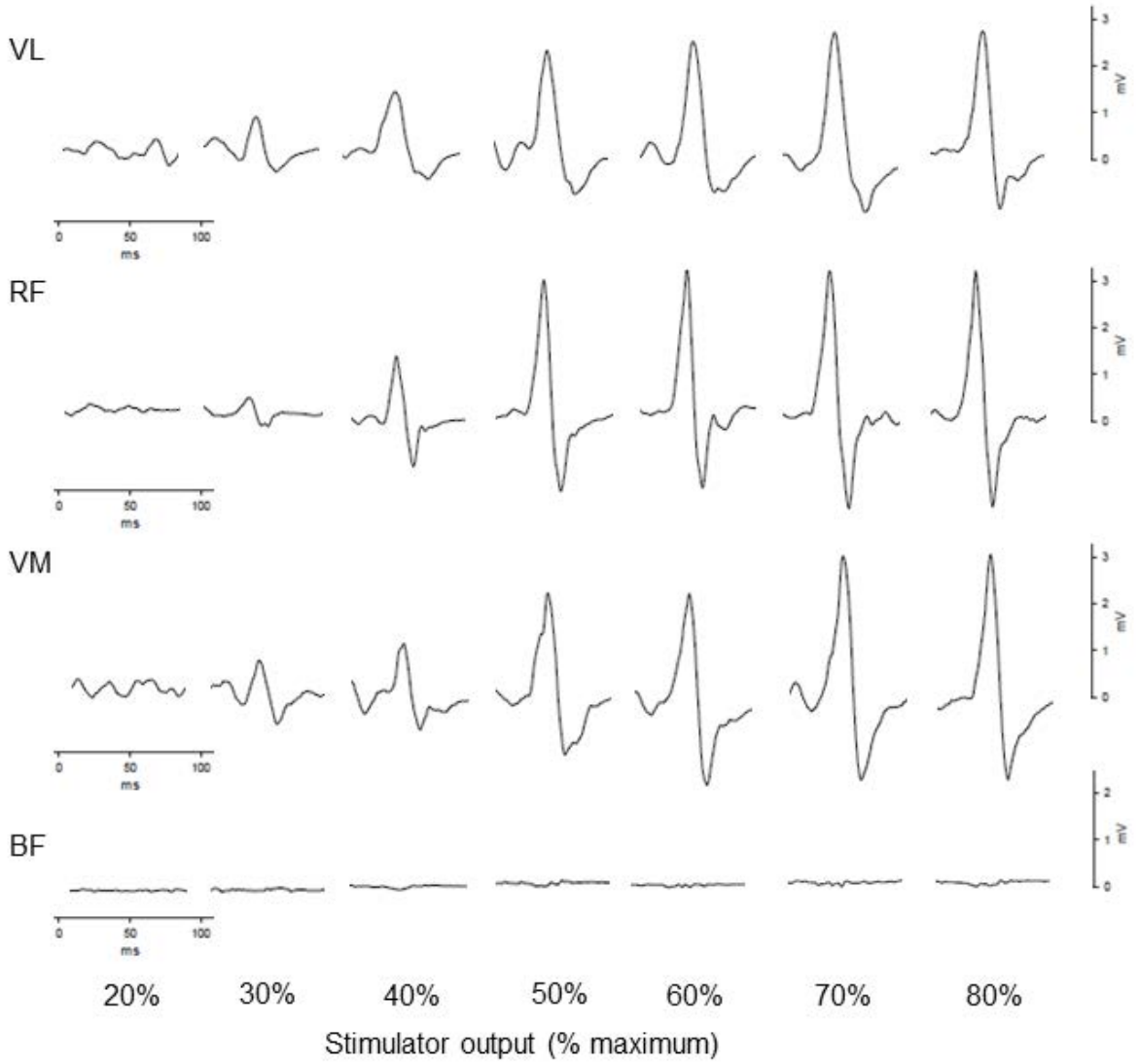

Figure 16. Representative individual motor-evoked potentials from a stimulus-response curve. Representative individual motor-evoked potentials elicited in the vastus lateralis (VL), rectus femoris (RF), vastus medialis (VM) and and biceps femoris (BF) for one subject from a stimulus-response curve at 20% maximal voluntary force.

(ANOVA) were used to evaluate the method of stimulus determination (120 and 130% RMT, 120% AMT and stimulus-response curves), any difference between muscles and the effect of contraction intensity on Boltzmann parameters. One-way repeated measures ANOVA were also used to compare AMT and RMT determined from 6, 8 and 10 stimuli. When ANOVA revealed significant interactions, the Newman-Keuls *post-hoc* test was used to identify differences. Dependent t-tests were used to compare Boltzmann and linear relationships for the coefficient of determination of MEP amplitude. Statistical significance was set at $p < 0.05$. All data are expressed as means ± standard deviation except **Figure 2** where values are expressed as means ± standard error of the mean.

RESULTS

Selected stimulus intensity

One subject did not reach a plateau in MEP amplitude in RF with the 10% MVC stimulus-response curve and was thus excluded from all relevant analyses.

Neither AMT nor RMT were different whether determination occurred with the first 6, 8 or 10 responses at each stimulus intensity for any muscle ($P > 0.05$). Therefore all subsequent analyses were conducted based upon AMT and RMT determined from 10 stimuli at each stimulus intensity. Selected TMS intensity determined by RMT, AMT and stimulus-response curves are presented in **Figure 17**. Stimulus intensities determined from RMT (120 and 130%) and stimulus-response curves at 10% MVC were higher than the intensity determined by stimulus-response curve at 50% MVC (VL: $F(5,35) = 7.00$, $P < 0.001$; RF: $F(5,30) = 8.13$, $P < 0.001$; VM: $F(5,35) = 8.71$, $P < 0.001$). Stimulus intensity at 120% AMT was lower than stimulus intensity determined from stimulus-response curves at both 10 and 20% MVC ($P < 0.05$). **Table 6** presents the selected stimulus intensities from the stimulus-response curves as a percentage of both RMT and AMT to contextualize the differences between these methods. There was also no difference in selected intensity between muscles for any method (RMT: $F(2,14) = 2.62$, $P = 0.11$; AMT: $F(2,14) = 1.21$, $P = 0.33$; 10% MVC: $F(2,12) = 1.00$, $P = 0.40$; 20% MVC: $F(2,14) = 1.15$, $P = 0.35$; 50% MVC: $F(2,14) = 0.778$, $P = 0.48$) nor difference in normalized MEP amplitude at the selected stimulus intensity between 10, 20 and 50% MVC stimulus-response curves (VL: $F(2,14) = 3.23$, $P = 0.07$; RF: $F(2,12) = 2.48$, $P = 0.13$; VM: $F(2,14) = 2.81$, $P = 0.09$) (**Table 7**). A single stimulus-response curve at 50% MVC is presented in **Figure 18**.

In VL, RF and VM, Mmax amplitudes were 16.2 ± 4.1 mV, 7.4 ± 1.8 mV and 17.0 ± 6.7 mV, respectively. Central drive as indicated by RMS·$Mmax^{-1}$ for VL (0.0046 ± 0.0014), RF (0.0039 ± 0.0007) and VM (0.0053 ± 0.0025) at 10% MVC and VL (0.0088 ± 0.0024), RF (0.0086 ± 0.0019) and VM (0.0100 ± 0.0039) at 20% MVC were similar ($F(2,14) = 1.32$, $P = 0.30$ and $F(2,14) = 0.660$, $P = 0.53$, respectively). At 50% MVC, RMS·$Mmax^{-1}$ for RF (0.0376 ± 0.0160) was greater than for both VL (0.0237 ± 0.0094) and VM (0.0264 ± 0.0115) ($F(2,14) = 8.36$, $P = 0.004$).

Boltzmann sigmoidal curves

Boltzmann curves from a typical subject are presented in **Figure 19**. Boltzmann curves provided a significantly better fit for the relationship between MEP amplitude and stimulator intensity than a linear relationship for stimulus-response curves at 10, 20 and 50% MVC for all muscles ($P < 0.05$). As contraction intensity increased, $S50$ decreased in all muscles (VL: $F(2,14) = 33.1$, $P < 0.001$; RF: $F(2,14) = 55.6$, $P < 0.001$; VM: $F(2,14) = 32.5$, $P < 0.001$). Few differences were observed in MEPmax/Mmax (only RF lower at 10% MVC; VL: $F(2,14) = 1.88$, $P = 0.19$; RF: $F(2,14) = 3.88$, $P = 0.046$; VM: $F(2,14) = 2.40$, $P = 0.13$) and k (only VL lower at 10% MVC; VL: $F(2,14) = 7.50$, $P = 0.006$; RF: $F(2,14) = 1.62$, $P = 0.23$; VM: $F(2,14) = 0.911$, $P = 0.42$). Results from modeling the stimulus-response curve data with the Boltzmann equation are presented in **Table 8**.

Table 6. Selected stimulus intensity from stimulus-response curves presented as a percentage of stimulator intensity to elicit active and resting motor thresholds.

		Vastus lateralis	*Rectus femoris*	*Vastus medialis*
10% MVC	RMT	135 ± 26	138 ± 26	129 ± 20
		(109 – 175)	(109 – 175)	(107 – 160)
	AMT	179 ± 48	187 ± 46	177 ± 46
		(120 – 250)	(120 – 250)	(117 – 250)
20% MVC	RMT	117 ± 27	113 ± 15	114 ± 16
		(86 – 175)	(86 – 133)	(92 – 140)
	AMT	154 ± 40	151 ± 32	156 ± 36
		(100 – 200)	(120 – 200)	(100 – 200)
50% MVC	RMT	96 ± 21	100 ± 23	98 ± 21
		(71 – 127)	(75 – 140)	(67 – 120)
	AMT	124 ± 22	131 ± 26	132 ± 27
		(100 – 150)	(100 – 175)	(86 – 160)

AMT : active motor threshold; MVC: maximal voluntary force; RMT: resting motor threshold. For *rectus femoris*, values from the stimulus-response curve at 10% MVC are n = 7. Values are expressed as means ± standard deviation and (range).

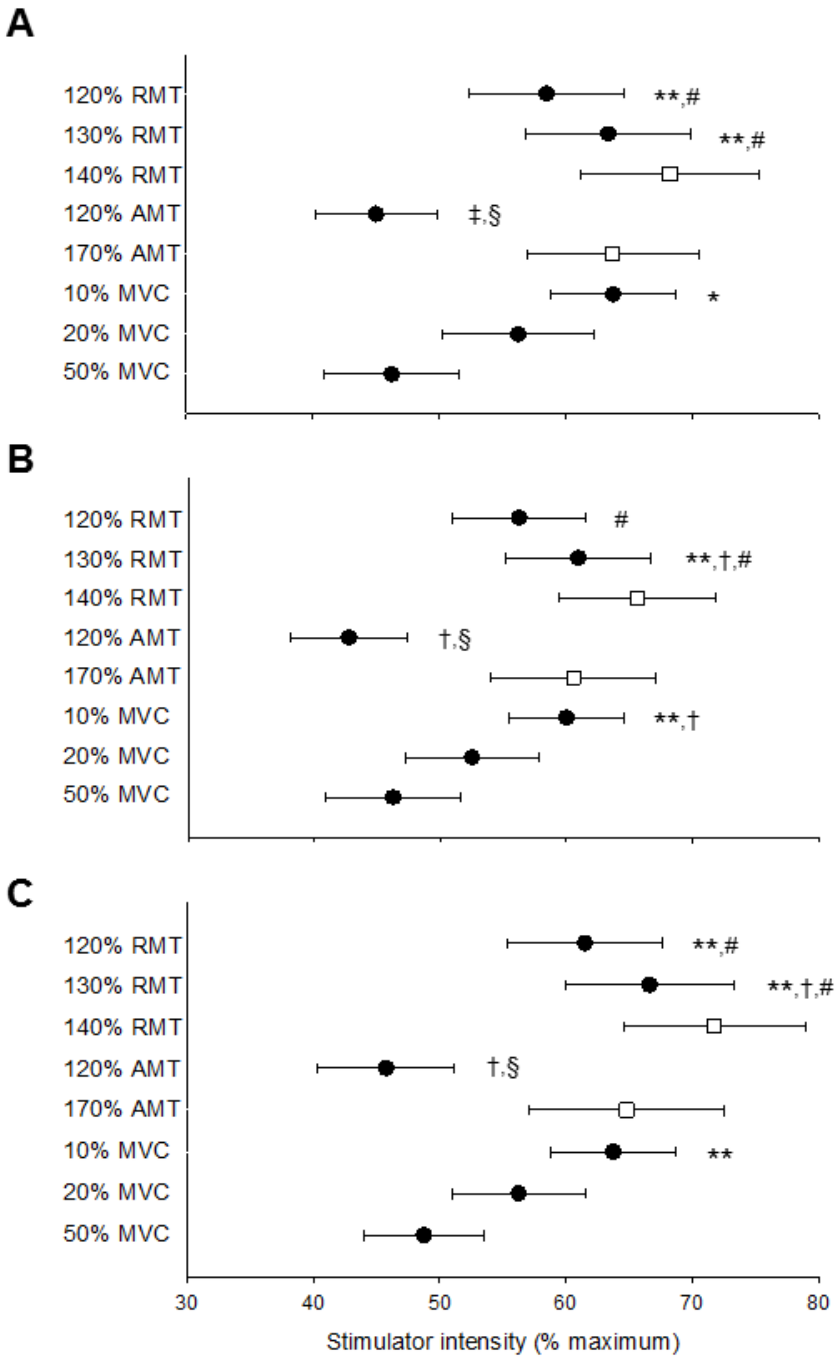

Figure 17. Comparison of methods for determination of TMS stimulus intensity. Comparison of different methods of determining TMS stimulus intensity for vastus lateralis in Panel A, rectus femoris (n=7) in Panel B and vastus medialis in Panel C. The methods compared are resting motor threshold (RMT), active motor threshold (AMT) during contractions at 10% maximal voluntary force (MVC) and stimulus-response curves at 10, 20 and 50% MVC. Stimulus intensity is presented as means ± standard error of the mean for stimulus-response curves and commonly utilized intensities derived from thresholds (●) and estimated optimal intensity (□) [4]. Significantly different from 50% MVC, * ($p < 0.05$) and ** ($p < 0.01$); significantly different from 20% MVC, † ($p < 0.05$) and ‡ ($p < 0.01$); significantly different from 10% MVC, § ($p < 0.01$); significantly different from 120% AMT, # ($p < 0.01$).

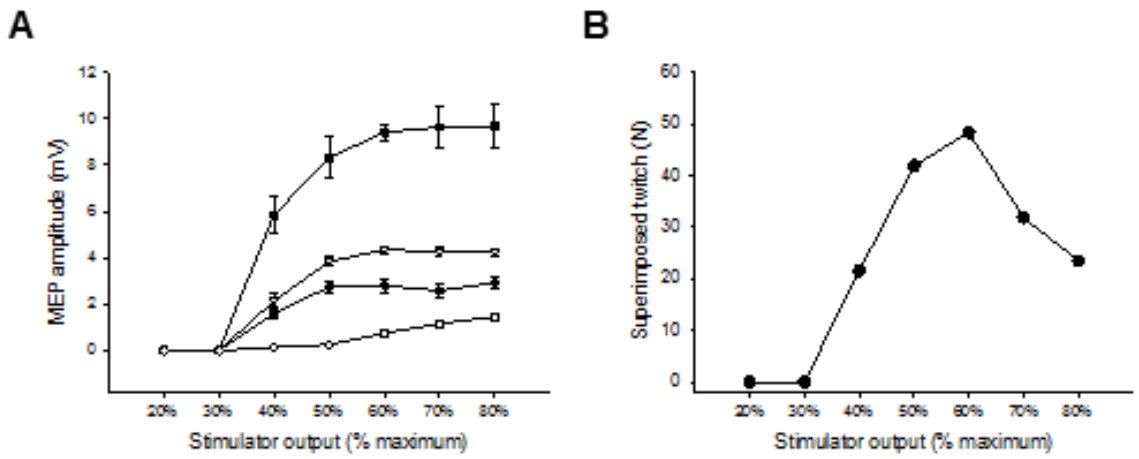

Figure 18. Sample stimulus-response curves. Stimulus-response curves at 50% maximal voluntary force for one subject for Panel **A)** *vastus lateralis* (●), *rectus femoris* (▽), *vastus medialis* (■) and *biceps femoris* (◊) in and Panel **B)** superimposed twitch. All values are presented as means ± standard deviation of four evoked responses at each stimulus intensity.

Table 7. Normalized motor-evoked potential amplitudes at selected stimulus intensity from stimulus-response curves for all quadriceps muscles.

	Vastus lateralis	*Rectus femoris*	*Vastus medialis*
10% MVC	34.5 ± 20.5	75.8 ± 16.1	43.4 ± 21.6
	(13.2 – 75.9)	(53.3 – 98.7)	(14.3 – 82.1)
20% MVC	42.9 ± 16.7	82.8 ± 18.7	52.8 ± 18.9
	(22.9 – 69.2)	(58.6 – 111.0)	(16.9 – 82.6)
50% MVC	45.3 ± 11.1	85.9 ± 22.2	49.7 ± 10.8
	(28.1 – 63.3)	(62.4 – 130.5)	(30.2 – 69.5)

MVC: maximal voluntary force. For *rectus femoris*, values from the stimulus-response curve at 10% MVC are n = 7. Normalized motor-evoked potential amplitudes are expressed as means ± standard deviation and (range).

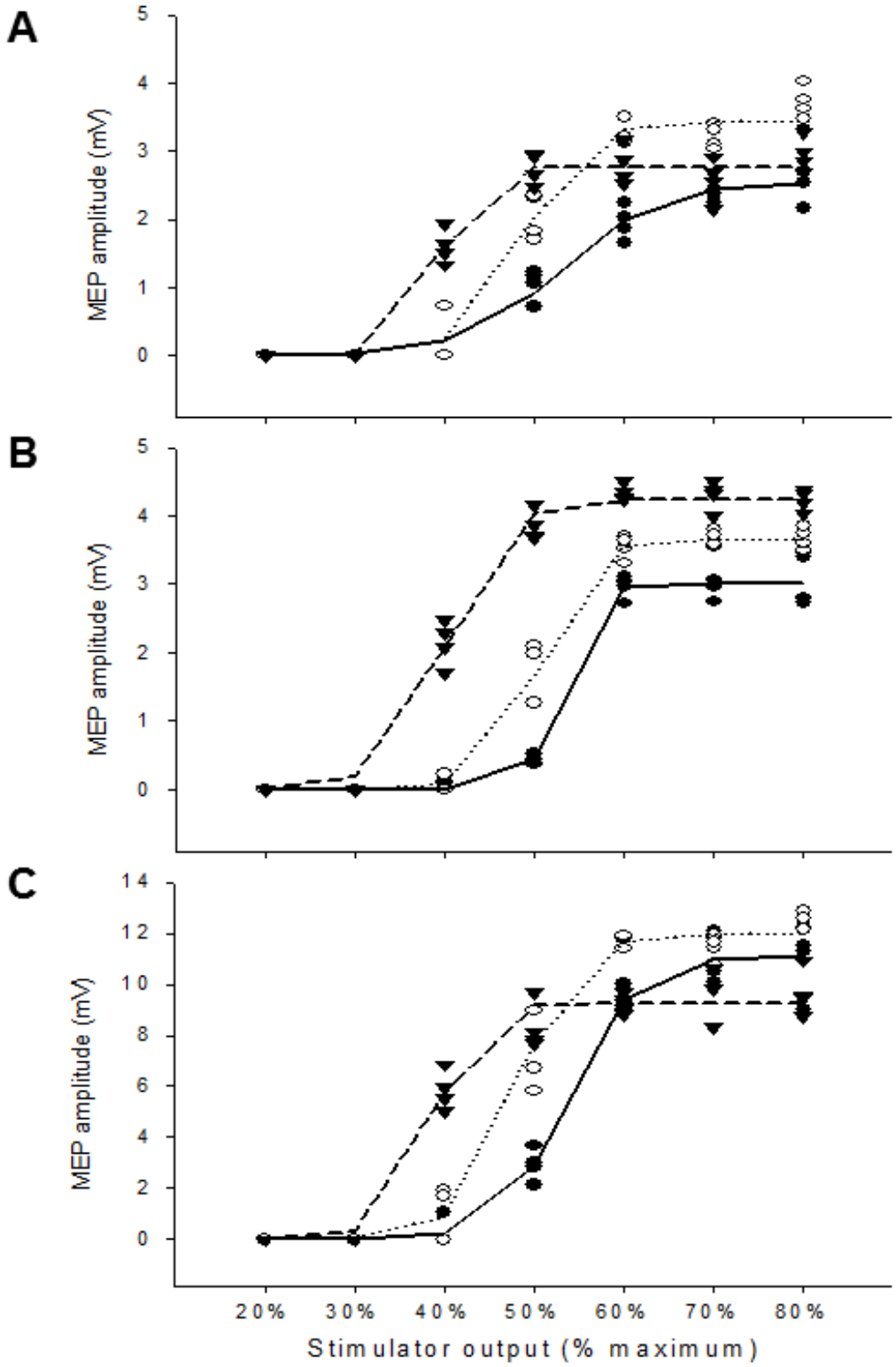

Figure 19. Sample Boltzmann curves. Boltzmann sigmoidal function plotted versus stimulator intensity for one subject for Panel **A)** *vastus lateralis*, Panel **B)** *rectus femoris* and Panel **C)** *vastus medialis*. All motor-evoked potentials used in the modeling and the Boltzmann curves are presented for stimulus-response curves at 10 (●,▬), 20 (○,▪▪▪▪▪) and 50% (▼, ▬ ▬ ▬) of maximal voluntary force.

Table 8. Modeled Boltzmann parameters for *vastus lateralis*, *rectus femoris* and *vastus medialis* muscles.

	Vastus lateralis	*Rectus femoris*	*Vastus medialis*
MEPmax/ Mmax			
10% MVC	34.7 ± 21.6	68.8 ± 19.6$^{*,\#}$	42.7 ± 22.3
20% MVC	42.9 ± 17.1	83.3 ± 19.3	51.2 ± 18.7
50% MVC	42.6 ± 11.1	83.0 ± 23.0	47.7 ± 11.9
*S*50			
10% MVC	43 ± 10$^{**,\#\#}$	45 ± 11$^{**,\#\#}$	44 ± 10$^{**,\#\#}$
20% MVC	38 ± 11**	40 ± 10**	39 ± 10**
50% MVC	32 ± 9	30 ± 7	34 ± 7
k			
10% MVC	0.051 ± 0.018$^{**,\#}$	0.036 ± 0.029	0.037 ± 0.018
20% MVC	0.032 ± 0.020	0.027 ± 0.015	0.031 ± 0.026
50% MVC	0.020 ± 0.018	0.019 ± 0.014	0.027 ± 0.012
r^2			
10% MVC			
Model	0.928 ± 0.045†	0.964 ± 0.051‡	0.937 ± 0.045‡
Linear regression	0.804 ± 0.095	0.770 ± 0.118	0.779 ± 0.112
20% MVC			
Model	0.943 ± 0.048†	0.982 ± 0.012‡	0.933 ± 0.050‡
Linear regression	0.724 ± 0.173	0.716 ± 0.180	0.688 ± 0.196
50% MVC			
Model	0.919 ± 0.052‡	0.900 ± 0.092‡	0.882 ± 0.115‡
Linear regression	0.563 ± 0.214	0.537 ± 0.207	0.598 ± 0.190

MEPmax/Mmax: maximal motor-evoked potential amplitude (MEPmax) normalized to maximal M-wave amplitude, MVC: maximal voluntary force, *S*50: stimulus intensity to evoke motor-evoked potentials half the amplitude of MEPmax (as % maximal stimulator output), *k*: slope parameter (inversely proportional to maximal function steepness), r^2: coefficient of determination. Values are expressed as means ± standard deviation. Significantly different from 50% maximal voluntary force (MVC), * ($p < 0.05$) and ** ($p < 0.01$); significantly different from 20% MVC, # ($p < 0.05$) and ## ($p < 0.01$); significantly different from linear relationship, † ($p < 0.05$) and ‡ ($p < 0.01$).

DISCUSSION

The main findings of this study are that (i) commonly-used stimulus intensities based upon RMT and a stimulus-response curve at 10% MVC are higher than those when determined using stimulus-response curves at 20 and 50% MVC and AMT and (ii) selected stimulus intensity, as determined by all methods, is similar between the three quadriceps muscles investigated. Because a stimulus-response curve performed at 20% MVC resulted in selection of a similar stimulus intensity to a stimulus-response curve at 50% MVC and because a stimulus-response curve at 20% MVC has a lower risk of inducing fatigue with repeated submaximal contractions, the present study indicates that this method is suitable for determining optimal stimulus intensity.

Comparison of methods

Resting motor threshold

In evaluation of the lower limbs to investigate fatigue or the effect of an exercise intervention, RMT has often been used to determine stimulus intensity. Most frequently this has been at 120 (Sammut *et al.*, 1995; Lentz & Nielsen, 2002; Ross *et al.*, 2007; Tallent *et al.*, 2012) and 130% RMT (Goodall *et al.*, 2009; Goodall *et al.*, 2010; Goodall *et al.*, 2012). The present study found that the use of these RMT intensities results in selection of higher stimulus intensities than a stimulus-response curve at 50% MVC and that stimulus intensity at 130% RMT is significantly greater than that from a stimulus-response curve at 20% MVC. No studies in the lower limbs were found to employ the suggested IFCN equivalent of 140% RMT (Groppa *et al.*, 2012), an intensity higher than the intensity at the transition from the rising slope to the plateau of the stimulus-response curves in the present study (**Table 6**).

There are several concerns about using RMT to determine optimal stimulus intensity in fatigue studies. The most important is whether it is appropriate to determine stimulus intensity in the relaxed muscle when evaluation of TMS-related parameters is conducted during muscular contraction. The rapid increase in cortical excitability from rest to even very weak contraction (Ugawa *et al.*, 1995; Taylor *et al.*, 1997) and the differential results in MEP evolution evaluated after fatiguing contractions when assessed in relaxed (*i.e.* decreased MEP amplitude/area (Gandevia *et al.*, 1999; Khedr *et al.*, 2007; Milanovic *et al.*, 2011)) and contracting (*i.e.* no change or increased MEP amplitude/area (Sogaard *et al.*, 2006; Klass *et*

al., 2008; Iguchi & Shields, 2012)) muscle present conceptual difficulties. More practically, increased stimulus intensity is associated with greater subject discomfort and this is important when recruiting healthy subjects and critical when evaluating clinical populations. If RMT is used to select stimulus intensity, no more than 6 stimuli should be delivered at each stimulus intensity since more stimuli do not better identify RMT contrary to the accepted standard of 10 stimuli at each intensity (Rossini *et al.*, 1994; Groppa *et al.*, 2012). It has also been reported that extremely high stimulus intensities are often required to determine RMT due to low cortical excitability at rest and that in some subjects RMT cannot be determined (Kalmar & Cafarelli, 2006). This difficulty has also occurred in our laboratory. For example, MEPs were not consistently elicited in one particular subject in VL or VM at 80% maximal stimulator output. Further difficulties in employing RMT may result during the determination of coil position. Given that high stimulus intensities may be required to evoke a MEP and the variable nature of MEP responses (Kiers *et al.*, 1993), particularly in the relaxed muscle (Darling *et al.*, 2006), it may be difficult to identify an appropriate coil position.

Magnetic stimulation of the motor cortex with a double-cone coil permits more precise localization of specific brain areas than with a circular coil. It does not, however, permit localization with pin-point accuracy. Barker (1999) detailed the induced electrical field and its rate of change with different coil types. Given that the motor cortex is not divided into discrete sections corresponding to individual muscles (Nudo *et al.*, 1996) and the imprecise area of stimulation with TMS, other muscle groups will inevitably be stimulated. Awiszus *et al.* (1997) discussed the problem of high-intensity electrical muscle stimulation stimulating both agonist and antagonist muscles of the upper limb and these findings can likely be applied to transcranial motor cortical stimulation. To our knowledge, Todd *et al.* (2003) were the first to specifically address this with a criterion in the determination of stimulus intensity (*i.e.* "a small MEP" in the antagonist). **Figure 18** illustrates the 50% MVC stimulus-response curve of one subject. A plateau in quadriceps MEP amplitude corresponds to increased BF MEP amplitude and decreased SIT. In this subject, 120% RMT equated to 72, 66 and 78% maximal stimulator output in VL, RF and VM, respectively. This indicates that in some subjects, at 120% RMT, coactivation becomes apparent. Coactivation is problematic in the study of fatigue since quantification of cortical VA is essential. By determining stimulus intensity in the relaxed muscle, SIT during voluntary contraction may be underestimated because the selected stimulus intensity increases the contribution of antagonistic muscles without corresponding augmentation of *quadriceps femoris* MEP amplitude.

Active motor threshold

Selected stimulus intensity at 120% AMT is significantly lower than stimulus-response curves at 10 and 20% MVC. All lower-limb studies employing AMT as a basis for TMS intensity determination utilized intensities much lower than the IFCN comparison equivalent of 170% AMT (Groppa *et al.*, 2012), recommendations much closer to a 10% MVC stimulus-response curve in this study (**Table 6**). As with RMT, the use of 6, 8 or 10 stimuli at each stimulus intensity when determining AMT did not affect the stimulus intensity selected.

Background EMG activity varies between individuals and also between muscles at a given contraction intensity; in some cases normal peak-to-peak amplitudes vary by >500% between subjects in the same muscle. Thus, the appropriateness of the common use of a fixed MEP amplitude to determine the presence of a MEP in evaluating AMT at different contraction intensities and in different subjects and/or muscles must be investigated. Boltzmann modeling indicates high inter-subject variability in evolution from no evoked MEP response to a maximal one (*i.e*. *k*; see **Table 8**). Some subjects demonstrated what could be characterized as a threshold from which no response immediately became a maximal one while in other subjects MEP amplitude gradually increased to maximum as stimulus intensity increased. Comparison with stimulus-response curves indicates that using AMT to determine stimulus intensity may result in submaximal MEP responses that are situated on the rising part of the Boltzmann curve. Unlike the use of maximal muscular responses to neural stimulation allowing serial or between-subject comparisons, comparison of submaximal evoked responses may introduce additional confounding factors. It remains to be established whether submaximal and maximal MEP responses and their evolution (*e.g*. with fatigue) are similar, particularly since preliminary indications from upper- (Temesi *et al.*, 2013) and lower-limb (McNeil *et al.*, 2011a) studies suggest this may not always be the case. The evaluation of cortical VA may also be affected by the use of stimulus intensities derived from AMT (*e.g*. 120%). Stimulus intensity at 120% AMT was non-significantly lower than that determined from a 50% MVC stimulus-response curve and this might result in delivery of TMS at a submaximal intensity during contractions between 50 and 100% MVC and result in underestimated SIT. The effect on estimated resting twitch, calculated from the linear regression of three SITs from three different contraction intensities in this range and acceptable if $r > 0.9$ (Hunter *et al.*, 2006; Hunter *et al.*, 2008), and subsequent estimation of cortical VA are unknown.

Generally, AMT is evaluated in voluntary contractions at 5 or 10% MVC in the upper limbs (Todd *et al.*, 2006; Sale *et al.*, 2008; Cirillo *et al.*, 2009; Kidgell & Pearce, 2010). In

lower limbs, Kalmar and Cafarelli (2006) and Hilty *et al.* (2011) used 3% MVC and found higher AMT than in Weier *et al.* (2012) and the present study, the latter two having employed contractions at 10% MVC. This is consistent with Boltzmann modeling showing decreased stimulus intensity to evoke a MEP of half maximal amplitude (*i.e*. *S*50; see **Table 8**) as contraction intensity increases.

Stimulus-response curves

All stimulus-response curves demonstrated a Boltzmann sigmoidal relationship, thus permitting the use of a stimulus-response curve to identify maximal MEP amplitudes and directly determine optimal diagnostic TMS stimulus intensity (Groppa *et al.*, 2012). Modeling of data indicated that estimated maximal MEP amplitude was lower at 10% MVC than at other contraction intensities although this was only significant in RF. Stimulus intensity to evoke a MEP of half maximal amplitude also decreased as contraction intensity increased. Determining stimulus intensity during contractions at 50% MVC would appear to be appropriate since evoked MEP responses at this contraction intensity are theoretically maximal (Taylor *et al.*, 1997; Todd *et al.*, 2003; Sidhu *et al.*, 2009a) and both this and higher contraction intensities are used to determine cortical VA. A concern, however, is that an extended series of such contractions may produce measurable effects of fatigue, and consequently, that residual effects of fatigue may be present during a subsequent protocol as reported in a recent study (Rupp *et al.*, 2012). The lack of difference between stimulus intensity as determined by stimulus-response curves at 20 and 50% MVC and the similar maximal MEP amplitudes as determined by Boltzmann modeling suggest that in the *quadriceps femoris*, a stimulus-response curve at 20% MVC is appropriate to determine TMS intensity when the aim is to evaluate fatigue-related parameters such as VA.

Comparison of muscles

Studies determining stimulus intensity during contractions have often used normalized MEP amplitude or area of a given size as criteria (Sidhu *et al.*, 2009a, b; Klass *et al.*, 2012; Fernandez-del-Olmo *et al.*, 2013). For example, Sidhu *et al.* (2009a) selected an intensity that produced the largest RF MEP with the stipulations that this must be at least 50% Mmax and that antagonist BF MEP amplitude be less than 10% raw RF MEP amplitude. In VL and VM in the present study, only 2 of 8 and 3 of 8 subjects, respectively, satisfied the requirement that MEP amplitude be ≥50% Mmax. In the case where several quadriceps muscles are examined, the latter criterion is ambiguous. BF may often be greater than 10% raw MEP

amplitude in at least one muscle and for one subject in the present study this was the case in all muscles at almost all coil positions and stimulus intensities evaluated.

There was no difference in selected stimulus intensity between muscles as determined by any method. This suggests that one muscle could be used as a surrogate for other quadriceps muscles. RF alone has frequently been used to determine quadriceps stimulus intensity (Sidhu *et al.*, 2009a, b; Rupp *et al.*, 2012; Fernandez-del-Olmo *et al.*, 2013). When RF is normalized, MEP amplitude is larger than for either VL or VM due to consistently smaller Mmax in the RF and little differences in raw MEP amplitude. The presentation of normalized RF MEP amplitudes instead of VL and VM may give the impression of eliciting greater corticospinal drive to the quadriceps muscles. In the present study, this was not due to a greater RF contribution since $RMS \cdot Mmax^{-1}$ was only greater than that of VL and VM at 50% MVC and normalized RF MEPs are larger than VL and VM at all contraction intensities. RF may not be an ideal surrogate because of the difficulty in recording clear M waves in this muscle. Furthermore, RF is the sole biarticular muscle of the *quadriceps femoris*, and thus, may not be representative of the muscle group.

An important limitation to the protocol is that it was not conducted on a second day to investigate the day-to-day variability of the methods employed in this study. Further investigations are required to establish whether the different methods employed to evaluate TMS parameters with fatigue are reproducible on different days. The present study also used a maximal response in contracting muscle as a reference point to evaluate multiple fatigue-related TMS parameters since this provides important insights into the manifestation and development of fatigue; however, recent studies suggest that TMS responses elicited by a submaximal stimulus intensity may also further understanding of fatigue mechanisms (McNeil *et al.*, 2011a; Temesi *et al.*, 2013). This reinforces the necessity of selecting an appropriate method to determine TMS intensity directly related to the parameters being investigated. In the context of the evaluation of cortical voluntary activation and corticospinal excitability with fatigue, maximal responses as investigated in this study are pertinent. In other research and diagnostic areas employing TMS, this may not be the case, and methods such as RMT and AMT may be the methods of choice for determining optimal stimulus intensity. Further studies must also determine the specific relevance of TMS-induced maximal and submaximal responses in both healthy and clinical populations in the context of fatigue, including the manner in which this affects measures of cortical voluntary activation and both excitatory and inhibitory mechanisms.

Conclusion

Percentages of AMT and RMT have often been employed to determine TMS intensity in studies evaluating fatigue; however these methods do not accurately identify the minimum stimulus intensity to elicit MEPs of maximal amplitude in the *quadriceps femoris*. Thus, they may be inappropriate for cortical excitability and voluntary activation assessment. The potential for increased coactivation and discomfort at 120 and 130% RMT and possible underestimation of evoked responses at 120% AMT preclude their use. There are minor differences between selected stimulus intensity (lower at 50% MVC for VL only) from stimulus-response curves at 20 and 50% MVC. Both MEP amplitude at selected stimulus intensity and estimated maximal MEP amplitude determined from these stimulus-response curves are similar. This indicates that a stimulus-response curve performed at 20% MVC is a suitable method of determining TMS stimulus intensity while reducing the risk of inducing fatigue compared to methods at a higher percentage of MVC. From the present study, it is also concluded that determining stimulus intensity from a single muscle is acceptable in the *quadriceps femoris*.

INTRODUCTION TO STUDIES 3 AND 4

The two preceding studies addressed two important methodological questions. Study 1 demonstrated that during brief voluntary contractions, TMS must be delivered once the force has stabilized at the target force without exceeding it to accurately determine corticospinal excitability at this force level. Meanwhile, Study 2 determined that the use of a stimulus-response curve at 20% MVC to select optimal stimulus intensity is both feasible and appropriate. These findings were put into practice in two studies employing TMS to investigate fatigue under extreme conditions.

Our research group has extensively studied the effects of endurance and ultra-endurance exercise bouts on a number of physiological (Millet *et al.*, 2011a; Gimenez *et al.*, 2013) and biomechanical (Millet *et al.*, 2009; Morin *et al.*, 2011) parameters, including the effects of ultra-endurance sports on neuromuscular fatigue (Martin *et al.*, 2010; Millet *et al.*, 2011c). Ultra-endurance sporting events have been proposed as a model from which to understand fatigue in addition to sport-induced pathologies, cerebral adaptations from endurance exercise and the ability of the human body to respond and adapt to extreme conditions (Millet & Millet, 2012). Previous neuromuscular studies have demonstrated central fatigue, assessed by VA via peripheral nerve stimulation, after endurance exercise. Only a couple studies however have employed TMS in any manner with ultra-endurance exercise (Ross *et al.* (2010b) investigating a Tour de France course in endurance-trained cyclists) or exercise in extreme conditions (high-intensity exercise by Goodall *et al.* (2012) in hypoxic conditions).

As detailed in literature review, SD is a condition predominantly of inadequate sleep duration. This may be either complete SD such as that often found during ultra-endurance sporting events and military exercises or partial SD such as in persons suffering from sleep disorders, shift workers, persons flying across time zones and athletes in ultra-endurance sporting events lasting beyond a couple days. SD is associated with subjective feelings of tiredness, clumsiness and fatigue. Thus, it has the potential to cause negative emotions and/or physiological consequences, especially at the cerebral level, that may affect physical performance or directly cause performance decrements in sporting events, especially as the duration of SD increases. Similarly, SD has the potential to negatively affect quality of life and the daily functioning of persons with sleep disorders and impaired sleep.

At the extreme end of the ultra-endurance event spectrum are Race Across America and Tor des Geants. Race Across America is a west-to-east coast cycling race across the United States with a maximum time limit of 12 days for the 4500-5000 km route. The race record is 8 days 3 h 11 min to complete 4685 km in 1992. The official race website states that top racers sleep approximately 90 min each day and suggests it would be difficult to sleep more than 4 h per day to complete the course within the time limit (RAAM, 2013). Similarly, top competitors in the Tor des Geants, a 330-km ultra-trail with 24 000 m of positive elevation change, sleep only a few hours over the >70 h required to complete the course. It is unknown if or how SD may contribute to the previously observed central fatigue in ultra-endurance exercise bouts

Study 3 investigated the possibility that SD causes increased central fatigue and other central perturbations during exercise compared to a control condition. In this study, the effect of one night complete SD was investigated on cycling performance to task failure in conjunction with both peripheral electrical stimulation and TMS measures of neuromuscular functioning. Reaction time to a Simon task and RPE were also assessed to explore the possible interactions of neuromuscular, and specifically central, fatigue, cognitive functioning and RPE with SD on endurance cycling performance.

Study 4 took TMS from the laboratory to a real-world environment. The purpose of this study was to build upon previous investigations of neuromuscular fatigue, principally central fatigue, caused by a 166-km ultra-trail (Millet *et al.*, 2011c) and long-duration (24 h) treadmill running (Martin *et al.*, 2010). The addition of TMS to the investigative protocol was to better understand how central fatigue manifests itself and what portion, if any, stems from supraspinal fatigue. Furthermore, TMS allows for investigation of both corticospinal excitability and inhibition, factors that may have important roles in ultra-endurance performance.

STUDY 3

Does central fatigue explain reduced cycling after complete sleep deprivation?

La fatigue centrale peut-elle expliquer la diminution de performance en cyclisme après une nuit de privation complète de sommeil ?

John Temesi,[1] Pierrick J. Arnal,[1] Karen Davranche,[4] Régis Bonnefoy,[1] Patrick Levy,[2,3] Samuel Verges[2,3] & Guillaume Y. Millet[1,3]

[1] Université de Lyon, F-42023, Saint-Etienne, France

[2] HP2 Laboratory, Joseph Fourier University, F-38000, Grenoble, France

[3] U1042, INSERM, F-38000, Grenoble, France

[4] Cognitive Psychology Laboratory and 3C Research Federation, Aix-Marseille University & CNRS, F-13331, Marseille, France.

Published as:

Temesi J, Arnal PJ, Davranche K, Bonnefoy R, Levy P, Verges S & Millet GY. (2013). Does Central Fatigue Explain Reduced Cycling after Complete Sleep Deprivation? *Med Sci Sports Exerc*. doi: 10.1249/MSS.0b013e31829ce379.

ABSTRACT

Sleep deprivation (SD) is characterized by reduced cognitive capabilities and endurance exercise performance and increased perceived exertion (RPE) during exercise. The combined effects of SD and exercise-induced changes on neuromuscular function and cognition are unknown. This study aimed to determine if central fatigue is greater with SD, and if so, whether this corresponds to diminished cognitive and physical responses. Twelve active males performed two 2-day conditions (SD and control, CO). On day 1, subjects performed baseline cognitive and neuromuscular testing. After one night SD or normal sleep, subjects repeated day 1 testing and then performed 40 min submaximal cycling and a cycling test to task failure. Neuromuscular and cognitive functions were evaluated during the cycling protocol and at task failure. After SD, exercise time to task failure was shorter (1137 ± 253 s vs. 1236 ± 282 s, $P = 0.013$) and RPE during 40 min submaximal cycling was greater ($P = 0.009$) than in CO. Maximal peripheral voluntary activation decreased by 7% ($P = 0.003$) and cortical voluntary activation tended to decrease by 5% ($P = 0.059$) with exercise. No other differences in neuromuscular function or cognitive control were observed between conditions. After SD, mean reaction time was 8% longer ($P = 0.011$) and cognitive response omission rate before cycling was higher ($P < 0.05$) than in CO. Acute submaximal exercise counteracted cognitive performance deterioration in SD. One night of complete SD resulted in decreased time to task failure and cognitive performance and higher RPE compared to a control condition. The lack of difference in neuromuscular function between CO and SD indicate decreased SD exercise performance was probably not caused by increased muscular or central fatigue.

Key words: transcranial magnetic stimulation, endurance, neuromuscular fatigue, cognition

RÉSUMÉ

La privation de sommeil (SD) est caractérisée par une réduction des capacités cognitives et de performance en endurance et une augmentation de l'effort perçu (RPE) au cours de l'exercice. Les effets combinés de la SD et des changements induits par l'exercice sur la fonction neuromusculaire et la cognition sont inconnus. Cette étude visait à déterminer si la fatigue centrale est supérieure avec SD, et dans ce cas, si cela est associé à des réponses cognitives et physiques altérées. Douze hommes actifs ont réalisé deux conditions

expérimentales de 2 jours (SD et contrôle, CO). Le premier jour, les sujets ont effectué des tests cognitif et neuromusculaire de référence. Après une nuit de SD ou de sommeil normal, les sujets ont répété les tests du premier jour. Ensuite, ils ont effectué 40 min de vélo à intensité sous-maximale puis une épreuve de vélo jusqu'à épuisement (incapacité à maintenir la puissance cible). Les fonctions cognitives et neuromusculaires ont été évaluées au cours du protocole de vélo et après arrêt de l'exercice. Après SD, le temps d'effort était plus court (1 137 ± 253 s contre 1 236 ± 282 s, P = 0,013) et RPE pendant les 40 min de vélo à intensité sous-maximale était plus élevé (P = 0,009) que dans CO. L'activation maximale volontaire périphérique a diminué de 7% (P = 0,003) et l'activation volontaire cortical a eu tendance à diminuer de 5% (P = 0,059) après l'exercice. Aucune autre différence de fonction neuromusculaire ou de contrôle cognitif entre CO et SD n'a été observée. Après SD, le temps de réaction moyen a augmenté de 8% (P = 0,011) et le taux d'omission de réponse cognitive au repos était plus élevé (P < 0,05) que pour CO. L'exercice sous-maximal normalisait la détérioration de la performance cognitive observée au repos en condition SD. Après une nuit complète de SD, une diminution de la durée maximale d'effort et de la performance cognitive ainsi qu'un RPE plus élevé sont observés par rapport à CO. L'absence de différence au niveau de la fonction neuromusculaire entre CO et SD indique que la diminution de la performance physique avec SD n'est pas causée par une augmentation de la fatigue musculaire ou centrale.

Mots clés : *stimulation magnétique transcrânienne, endurance, fatigue neuromusculaire, cognition*

INTRODUCTION

Sleep deprivation (SD) is usually a condition of inadequate sleep duration. This may be complete SD such as in ultra-endurance sporting events and military exercises or partial SD as with persons suffering from sleep disorders, shift workers and individuals flying across time zones. In both complete and partial SD, affected individuals self-report feelings of tiredness, clumsiness and fatigue.

Numerous studies have also demonstrated performance deficits during prolonged exercise under conditions of SD. Intense walking to task failure was significantly shorter following 36-50 h sleep deprivation (Martin, 1981; Martin & Chen, 1984) and distance run over 30 min following 30 min submaximal running was decreased by 2.9% after 30 h sleep deprivation (Oliver *et al.*, 2009). Results from studies examining the effect of SD on performance in shorter running or cycling exercise bouts however are contradictory (Chen, 1991; Azboy & Kaygisiz, 2009; Konishi *et al.*, 2012), suggesting that SD-induced performance decrements may be more likely to occur in longer exercise bouts. Maximal strength loss was not observed during either isometric or isokinetic contractions of upper or lower limbs during 60 h SD (Symons *et al.*, 1988a; Symons *et al.*, 1988b). Attempts to explain decreased exercise performance measures have failed due to the abundance of conflicting results. Oxygen consumption and heart rate (HR) during constant-load efforts of varying intensity up to 1 h (Martin, 1981; Martin & Chen, 1984; Oliver *et al.*, 2009) were unaffected by SD although this may not be true in longer duration exercise as decreased oxygen consumption was observed after 3h, but not 1 or 2 h, of light treadmill walking after 36 h SD (Martin *et al.*, 1986). Conversely, Scott and McNaughton (2004) observed lower HR during 30 h SD with 20 min of light exercise every 4 h, but not when exercise frequency was doubled. Results from incremental tests to task failure are equivocal about the effects of SD of at least 24 h on HR responses and maximal oxygen uptake (VO_2max) (Martin & Gaddis, 1981; Plyley *et al.*, 1987; Chen, 1991; Konishi *et al.*, 2012).

Ratings of perceived exertion (RPE), a subjective measure of exertion, have been shown to be increased with SD in prolonged exercise at a given speed or intensity. This occurred in protocols involving light to intense walking and SD of at least 30 h (Martin, 1981; Myles, 1985; Plyley *et al.*, 1987). Oliver *et al.* (2009) showed no difference in RPE during a 30-min time trial despite a reduction in distance run with SD. This suggests that at identical running speeds, SD RPE would have been greater.

Total and partial SD are associated with a general slowing of response speed and decreased alertness and attentional capacities. Disagreement remains over the effect of SD on higher-level cognitive functions such as learning, memory and executive functioning (Balkin *et al.*, 2008; Killgore, 2010; Lo *et al.*, 2012). The few studies investigating exercise-induced cognitive changes with SD have found exercise to have short-term alerting effects (LeDuc *et al.*, 2000) and decrease reaction time (RT) to a stimulus (Scott *et al.*, 2006). The positive effects of exercise on RT are well-established in non-SD conditions (for review see (McMorris & Graydon, 2000)), especially when evaluated after at least 20 min of exercise (Chang *et al.*, 2012). This has been suggested to result from greater nervous system activation (McMorris & Graydon, 2000) or peripheral motor processes efficiency (Davranche *et al.*, 2005, 2006b) during exercise than at rest.

While central changes (*e.g.* augmented RPE during exercise and decrements in cognitive performance) have been observed after extended periods of SD and decreased central activation detected after endurance exercise (Millet *et al.*, 2003c), no study has examined the potential implications of increased central fatigue, *i.e.* decreased maximal voluntary activation, in performance decrements with SD. To our knowledge, the effects of complete SD on neuromuscular parameters have been limited to transcranial magnetic stimulation (TMS) measures in the upper limbs without exercise. In healthy subjects, De Gennaro *et al.* (2007) observed increased resting motor threshold after 40 h SD. This was not observed in other studies after 24 h SD (Civardi *et al.*, 2001; Scalise *et al.*, 2006; Kreuzer *et al.*, 2011), possibly due to circadian effects since the 40-h period ended at midnight. The single study reporting motor-evoked potential (MEP) amplitude during muscular contractions did not observe a change with SD of at least 24 h (Scalise *et al.*, 2006). This study also reported decreased cortical silent period (CSP) (Scalise *et al.*, 2006) while others observed no change after 24 h of SD (Civardi *et al.*, 2001; Kreuzer *et al.*, 2011). Intra-cortical inhibition tended to decrease (Civardi *et al.*, 2001; Scalise *et al.*, 2006) while changes in intra-cortical facilitation in these studies were equivocal (Civardi *et al.*, 2001; De Gennaro *et al.*, 2007). Difficulty in interpreting these studies is compounded by the lack of both a control condition and pre- and post-SD testing to account for normal inter-day variability and that all studies included both men and women.

The present study aimed to quantify the effects of SD on central fatigue, neuromuscular responses, cognitive control and RPE in response to whole-body exercise and to determine if SD results in decreased endurance cycling performance. Secondary objectives were to link the cognitive, physical and neuromuscular responses to SD together, including

the assessment of whether response inhibition, a crucial aspect of human cognitive control (*i.e.* cognitive processes that ensure adaptive goal-directed behavior), is affected by SD. It was hypothesized that one night of SD would result in decreased neuromuscular functioning evaluated during isometric contractions after exercise and in changes in RPE, HR and performance during cycling. Furthermore, it was anticipated that submaximal exercise would negate deterioration of information processing efficiency under SD.

METHODS

Subjects

Twelve healthy active men (mean ± SD: age, 28 ± 9 years; height, 1.80 ± 0.06 m; body mass, 71 ± 8 kg; MAP, 324 ± 31 W; VO_2max, 60 ± 7 ml·kg^{-1}·min^{-1}) participated in a study with randomized counterbalanced crossover design. Subjects were non-smokers, non-epileptic and free of cardiovascular disease and contraindications to TMS. They had 11 ± 9 years (range: 5-35) of endurance sport experience and trained 5 ± 3 sessions (range: 3-12) per week. Inclusion criteria included verification of normal sleep patterns using the French versions of the Pittsburgh Sleep Quality Index (exclusion if score ≥ 5), Horne-Ostberg Morningness-Eveningness questionnaire (exclusion if score < 30 or > 70), and Epworth Sleepiness Scale (exclusion if score ≥10). Written informed consent was obtained from all subjects prior to their participation and this study conformed to the standards from latest revision of the Declaration of Helsinki. All procedures were approved by Comité de Protection des Personnes Sud-Est 1, France. Subjects were instructed to maintain normal sleep/wake patterns the week before each condition. They were also instructed to avoid strenuous exercise for the 2 days preceding each trial and to abstain from alcohol and caffeine from a minimum of 24 h before the start of each trial until its completion. Sleep and physical activity were recorded by subjects for the three days prior to each condition and verified upon arrival at the laboratory.

Experimental design

The subjects were required to visit the laboratory for 3 sessions totaling 5 days. The preliminary visit was performed 1 to 2 weeks before the first experimental session and consisted of a medical inclusion, maximal incremental cycling test to task failure and familiarization with all testing procedures. The experimental conditions were performed between 2 and 4 weeks apart. These were a SD condition and a control (CO) condition. Due to the nature of complete SD, neither subjects nor investigators could be blinded. Subjects

were not informed of experimental hypotheses. Each condition comprised 2 days with the first day providing baseline cognitive and neuromuscular measures from which day-to-day effects of SD and CO conditions were evaluated. On the second day a submaximal cycling bout was followed by an incremental cycling test to task failure. Cognitive and neuromuscular measures were evaluated before, during and after the exercise performance test (**Figure 20**).

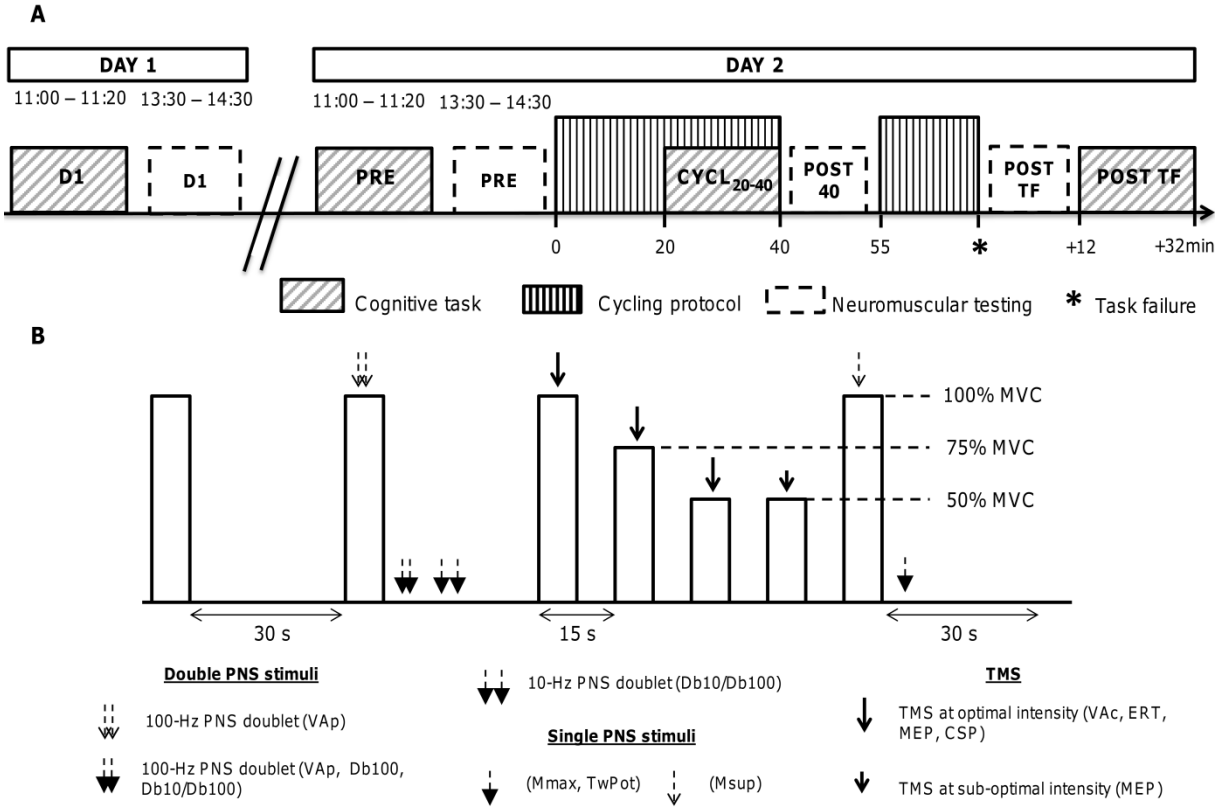

Figure 20. Panel **A**) Experimental condition test order with time indicated in minutes from the start of the exercise protocol to task failure and Panel **B**) neuromuscular testing protocol. The neuromuscular testing protocol began 2 min 30 s after exercise cessation at POST40 and POST TF.

Preliminary visit

Subjects performed a maximal cycling test to task failure on a cycle ergometer (Monark 839E, Monark Exercise AB, Vansbro, Sweden). The test commenced with 3 min of warm-up at 90 W. Power output was then increased by 15 $W \cdot min^{-1}$ until task failure. Respiratory measures were assessed breath-by-breath by an online system (Ergocard, Medisoft, Sorinnes, Belgium) and averaged every 30 s. VO_2max was considered as the highest 30-s mean value prior to task failure and MAP, the power output at the last completed stage. The familiarization portion of the preliminary visit included maximal and submaximal contractions of the knee extensors with and without electrical femoral nerve and trancranial magnetic stimuli (see

Neuromuscular testing section). This included training subjects to return to the pre-stimulus force level as soon as possible after TMS to permit consistent measurement of the CSP. Subjects repeated trials until they were able to perform all tests consistently and as directed. Subjects also completed a session of the Simon task (see *Cognitive task* section) consisting of 4 blocks of 96 trials at 5-min intervals. Each block lasted approximately 3 min 40 s.

Experimental conditions

Sleep, activity and condition control

Subjects were instructed to maintain their normal sleep/wake and activity patterns before and during the protocol (except during the night of SD). They recorded their sleep/wake patterns and physical activity (duration and intensity) for three days prior to both experimental conditions. An Actiheart (Version 2.2, CamNTech Ltd., Cambridge, UK) was used to measure HR, sleep time and physical activity, the latter by internal accelerometer that sensed the intensity and frequency of torso movements, from 8:00 the first morning of the experimental condition to the end of the protocol. During the night between days 1 and 2, subjects were permitted to return home to sleep in CO. In SD, subjects remained at the laboratory under the supervision of the investigators where they were only permitted to perform sedentary activities such as watching films and listening to music between 23:00 and 7:00 to limit differences in physical activity and mental stress between conditions. Only the consumption of water *ad libitum* was permitted after lunch on day 2 (12:00). Subjects rated their perception of sleepiness on the Stanford Sleepiness Scale before each cognitive test and before and after the 40-min submaximal exercise.

Force and Electromyography

Knee extensor force was measured during voluntary and evoked contractions with a calibrated force transducer (Meiri F2732 200 daN, Celians, Montauban, France) with amplifier attached by non-compliant strap to the right leg immediately proximal to the malleoli of the ankle joint. Subjects were seated upright in a custom-built chair with both hips and right knee at 90° of flexion. The load cell was fixed to the chair and in a position that force was measured in direct line to the applied force. Electromyographic signals of the right knee extensors (*vastus lateralis* (VL), *rectus femoris* (RF) and *vastus medialis* (VM)) and flexors (*biceps femoris*) was recorded.

Electromyographic signals were recorded with pairs of self-adhesive electrodes (Meditrace 100, Covidien, Mansfield, USA) in bipolar configuration with 30-mm interelectrode distance and the reference on the patella. Low impedance (<5 kΩ) between electrodes was obtained by shaving, gently abrading the skin with sandpaper and then cleaning it with isopropyl alcohol. Electromyographic data were analogue-to-digitally converted at a sampling rate of 2000 Hz by a PowerLab system (16/30—ML880/P, ADInstruments, Bella Vista, Australia) and octal bio-amplifier (ML138, ADInstruments) with bandpass filter (5-500 Hz) and analyzed offline using Labchart 7 software (ADInstruments).

Femoral nerve stimulation

Single electrical stimuli of 1-ms duration were delivered via constant-current stimulator (DS7A, Digitimer, Welwyn Garden City, Hertfordshire, UK) to the right femoral nerve (PNS, peripheral nerve stimulation) via a 30-mm diameter surface cathode in the femoral triangle (Meditrace 100, Covidien) and 50 x 90 mm rectangular anode (Durastick Plus, DJO Global, Vista, USA) on the gluteus maximus. Single stimuli were delivered in the relaxed muscle incrementally until plateaus in maximal M-wave (Mmax) and peak evoked force were reached. Stimulus intensity throughout the protocol was maintained at 130% of the intensity to produce maximal Mmax and twitch responses to ensure supramaximality. Stimulus intensity was determined each day (51 ± 9 and 52 ± 9 mA for CO and 49 ± 10 and 48 ± 11 mA for SD for days 1 and 2, respectively).

Transcranial magnetic stimulation

Single-pulse TMS was used to evoke MEPs in the right quadriceps muscles. The motor cortex was stimulated by a magnetic stimulator (Magstim 200², The Magstim Company Ltd, Whitland, UK) with a 110-mm double-cone coil (maximum output of 1.4 T). Single stimuli were applied to the contralateral motor cortex producing an induced postero-anterior current. Subjects wore a cervical collar during all TMS measures to stabilize the head and neck. Every centimeter from 1 cm anterior to 3 cm posterior of the vertex was demarcated along the nasal-inion line and to 2 cm over the left cortex. Optimal coil position was determined by assessing MEP responses evoked during brief isometric knee extension at 10% MVC and 50% maximal stimulator output. The optimal coil position corresponded to the site producing the largest MEP amplitudes in VL, RF and VM with minimal biceps femoris MEP amplitude. Optimal coil position was marked on a cloth cap secured to the scalp and it was determined each day since the wearing of an immovable head covering over the course of two days was

impractical. Stimulus intensity was determined by stimulus-response curve from responses during brief isometric knee extension at 20% MVC. Four consecutive contractions were performed at 15-s intervals at each of the following randomly-ordered stimulus intensities: 20, 30, 40, 50, 60, 70 and 80% maximal stimulator output. Optimal stimulus intensity was defined as the minimum stimulus intensity evoking maximal MEP amplitude in all measured quadriceps muscles. A sub-optimal stimulus intensity was also determined from the stimulus-response curve at 20% MVC. This intensity corresponded to a stimulus intensity evoking MEP amplitudes approximately half their maximum for VL, RF and VM.

Neuromuscular testing

Neuromuscular measures (force and electromyography) were assessed at four time points during each condition (day 1 (D1), day 2 pre-cycling (PRE), post-40 min submaximal cycling (POST40) and post-cycling task failure (POST TF)) (**Figure 20A**). After determining the optimal site and intensity for TMS and PNS each day, maximal force was determined from four MVCs separated by 30 s. In the latter two MVCs, PNS (100-Hz doublet) was delivered at peak force and immediately after in the relaxed state (100- and 10-Hz doublets). Three series of five contractions were performed with real-time visual feedback, consisting of four during which TMS was delivered (100, 75 and 50% MVC at optimal stimulus intensity (Todd *et al.*, 2003) and 50% MVC at sub-optimal stimulus intensity) and another MVC with PNS (single stimulus delivered at peak force and again in the relaxed muscle in the potentiated state). Contractions began at 15-s intervals and sets were separated by 30 s. Subjects were instructed to maintain or return to the pre-stimulus force level after TMS. At POST 40 and POST TF, measures began exactly 2 min 30 s after the cessation of cycling. Only two MVCs, the latter with PNS doublets, and two series of five contractions (100, 75 and 50% MVC at optimal stimulus intensity, 50% MVC at sub-optimal stimulus intensity and MVC with single PNS stimuli) were performed due to the time-sensitive nature of the measurement with fatigue (**Figure 20B**).

Cognitive task

Subjects were required to complete 4 blocks of the Simon task (*i.e.* a classical paradigm used to assess the ability to focus attention while ignoring irrelevant information; for a review, see (Simon, 1990)) at four time points during each condition (day 1 (D1), day 2 pre-cycling (PRE), from 20 to 40 min of the 40-min submaximal cycling bout ($CYCL_{20\text{-}40}$) and post-cycling task failure (POST TF)) (**Figure 20A**). Each block consisted of 96 trials and blocks

were performed at precisely 5-min intervals, giving subjects between 60 and 90 s of "cognitive rest." The cognitive task was performed while seated on the cycle ergometer facing a computer screen at a distance of 1.0 m. A response button was fixed to each of the handlebars (right and left) of the ergometer. A fixation point (white circle) was located in the center of the screen and remained present throughout the trials. Subjects were instructed to respond as quickly and accurately as possible by pressing the appropriate response button according to the color of circle presented either to the left or right of the fixation point at a visual angle of 8.6 degrees. Subjects were instructed to respond according to the color of the stimulus while ignoring its spatial location. The mapping of stimulus color to response button was counterbalanced across subjects. The task was comprised of two equally probable trial types: congruent trials where the spatial location of the stimulus corresponded to the task-relevant aspect of the stimulus (*e.g.*, left stimulus/left response) and incongruent trials where the spatial location of the stimulus corresponded to the opposite spatial location of the response (*e.g.*, left stimulus/right response). As soon as a response button was pressed, or after 1500 ms in the absence of a response, the stimulus was removed and the next trial presented.

Exercise protocol

On Day 2, subjects performed a two-part cycling test at self-selected pedal frequency. The first part consisted of 40 min of submaximal exercise as 5 min warm-up at 50% MAP and 35 min at 65% MAP (*i.e.* 210 ± 20 W). Ratings of perceived exertion were assessed by 100-mm visual analogue scale (Neely *et al.*, 1992) every 5 min from 10 min and HR was recorded throughout. Beginning at 20 min of part 1, subjects performed the cognitive task while cycling. The second part, *i.e.* the timed exercise to task failure (TTF), commenced with 5 min at 65% MAP, increasing step-wise by 5% MAP every 5 min until task failure. Ratings of perceived exertion were assessed every 5 min and at task failure and HR was recorded throughout. Subjects were required to remain seated throughout the cycling test and an investigator blinded to exercise time provided standardized encouragement in both conditions.

Data analysis

Activity

Mean activity in arbitrary units per min was determined from 8:00 on day 1 to 14:30 on day 2. Sub-analyses on the normal sleep period from (23:00 to 8:00) and the non-sleep period (Day 1 from 8:00-23:00 and Day 2 from 8:00-14:30) were also conducted.

Peripheral nerve stimulation

Voluntary activation was assessed peripherally (VAp) by twitch interpolation using the superimposed and potentiated twitch amplitudes elicited by PNS 100-Hz doublets during and after MVCs and calculated from the equation: [1 – (PNS 100-Hz superimposed twitch / Db100)] × 100. The evolution of low- and high-frequency fatigue was evaluated from the change in the ratio of low-frequency (Db10, 10-Hz) doublet to high-frequency (Db100, 100-Hz) doublet (Verges *et al.*, 2009).

Transcranial magnetic stimulation

Peak-to-peak amplitude of MEPs and M waves were measured and MEP amplitude was normalized to maximal M-wave amplitude during MVC (Msup) and Mmax measured at the same time point. In one subject MEP normalization by Msup was not performed due to difficulties in eliciting Msup. All analyses involving Msup or values normalized with Msup were thus performed on 11 subjects. Cortical voluntary activation (VAc) during maximal effort was measured by modified twitch interpolation. Corticospinal excitability increases substantially during the transition from relaxed to contracted muscle states (Ugawa *et al.*, 1995), thus underestimating TMS in the relaxed muscle. Instead the potentiated twitch amplitude elicited by TMS in relaxed muscle was estimated. At each time point, a linear regression was performed on the relation between SITs evoked when TMS was delivered at 100, 75 and 50% MVC and voluntary force (Todd *et al.*, 2003). This relation was extrapolated and the y-intercept was interpreted as the estimated resting twitch amplitude. VAc was assessed with the equation: [1 – (TMS superimposed twitch / estimated resting twitch)] × 100. The reliability of this method has recently been validated in the knee extensors (Goodall *et al.*, 2009). The duration of the CSP was determined visually and defined as the duration from the cortical stimulus to the return of continuous voluntary electromyography (Sidhu *et al.*, 2009b).

Cognitive task

Reaction times less than 100 ms were considered anticipated responses and were thus excluded from further analyses. The rates of errors and omissions (RT greater than 1500 ms) were both calculated as a percentage of the total number of trials. Mean RT for correct trials was calculated for each of condition (SD, CO) time (D1, PRE, $CYCL_{20\text{-}40}$, POST TF), block (1, 2, 3, 4) and congruency (congruent, incongruent).

Statistics

Exercise and neuromuscular responses

All data was assessed for normality before statistical analysis was performed. Two-way repeated-measures ANOVA (condition × time) were used to test evaluate differences between D1 and PRE in CO and SD. Then two-way repeated-measures ANOVA (condition × time) were used to assess changes on day 2 for all neuromuscular measures. Two-way repeated-measures ANOVA (condition × time) were conducted on RPE and HR in parts 1 and 2 of the cycling protocol. Comparison of CSP between days was not conducted because optimal stimulus intensity was determined each day and changes to stimulus intensity influence CSP duration independent of other factors. When ANOVA revealed significant interactions, the Newman-Keuls *post hoc* test was used to identify differences. Cortical voluntary activation was assessed by two-way non-parametric repeated-measures ANOVA because this data was not normally distributed. Students paired *t*-tests were used to evaluate differences in TTF performance, activity and sleep patterns. Data are presented as mean ± standard deviation.

Cognitive task

The arcsine transformations of mean RT and error rate were both evaluated by ANOVA with condition (SD, CO), time point (D1, PRE, $CYCL_{20\text{-}40}$, POST TF), block (1, 2, 3, 4) and congruency as within-subject factors. To correct for violation of sphericity assumptions, a Greenhouse–Geisser degree of freedom correction was applied. *Post hoc* Newman-Keuls analyses were conducted on all significant interactions. Arcsine transformations of omission rate were assessed by non-parametric Wilcoxon Signed-Rank test. Data are presented as mean ± standard error of the mean.

Statistical significance was set at $P < 0.05$ for all statistical analyses.

RESULTS

Sleep patterns and sleepiness

Normal sleep patterns were characterized by scores of 3 ± 1 on the Pittsburgh Sleep Quality Index, 56 ± 8 on the Horne-Ostberg Morningness-Eveningness questionnaire and 6 ± 2 on the Epworth Sleepiness Scale. There were no differences between conditions in the time subjects slept (CO, 11:35 pm vs. SD, 11:35 pm; $P = 1.00$) or woke up (CO, 8:04 am vs. SD, 8:02 am;

$P = 0.88$) or in the number of hours they slept (CO, 8 h 29 min ± 53 min vs. SD, 8 h 27 min ± 48 min; $P = 0.88$) the three nights before the experimental protocols. Subjects were more active in SD than CO (CO, 71 ± 15 arbitrary units·min^{-1} vs. SD, 89 ± 25 arbitrary units·min^{-1}; $P = 0.028$). This was exclusively due to a difference in activity during the normal sleep period (CO, 19 ± 27 arbitrary units·min^{-1} vs. SD, 45 ± 15 arbitrary units·min^{-1}; $P = 0.002$).

There was no difference between conditions on day 1 on the Stanford Sleepiness Scale ($P = 1.00$). Sleepiness increased from day 1 to day 2 in SD only (CO, 1.7 ± 0.5 and 1.8 ± 0.6 vs. SD, 1.7 ± 0.7 and 4.0 ± 1.2 for days 1 and 2, respectively; $P < 0.001$). Subjective sleepiness was greater at all time points on day 2 in SD than CO ($P < 0.001$).

Performance, RPE and HR during exercise

Cycling time to task failure was significantly shorter in SD than CO (**Figure 21A**). RPE was significantly greater in SD than CO and increased ($P < 0.001$) during 40 min of submaximal exercise. There was no difference in RPE during TTF between conditions ($P = 0.15$) as RPE increased to task failure ($P < 0.001$) (**Figure 21B**). There was also no difference in HR between SD and CO during 40-min submaximal cycling (mean HR: CO, 159 ± 14 beats·min^{-1} vs. SD, 157 ± 15 beats·min^{-1}; $P = 0.12$). During TTF, HR was higher in CO than SD at all time points (HR at task failure: CO, 180 ± 12 beats·min^{-1} vs. SD, 173 ± 14 beats·min^{-1}; $P < 0.001$).

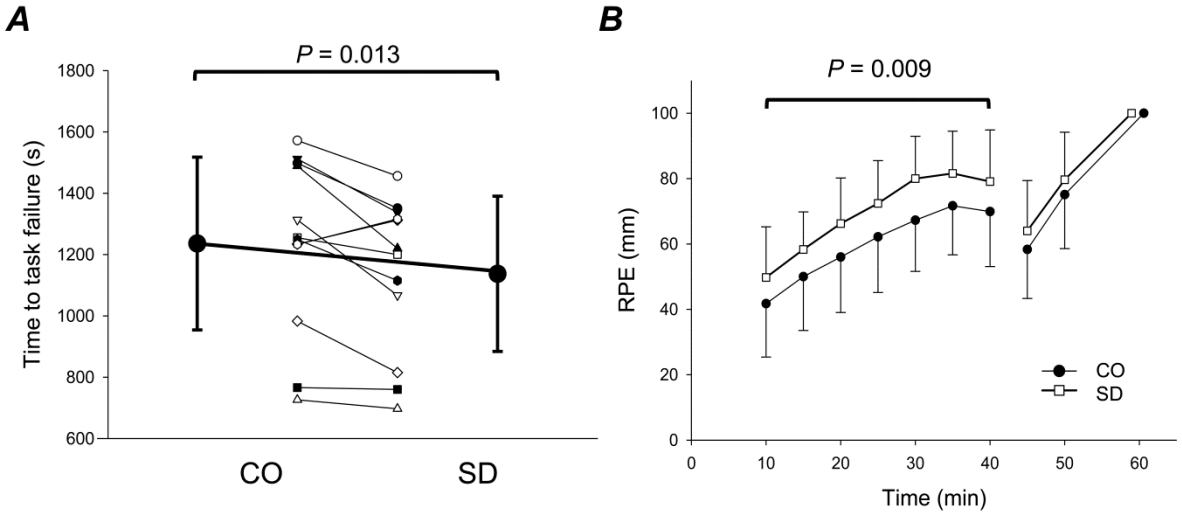

Figure 21. Effect of SD and CO conditions on (Panel **A**) mean and individual cycling time to task failure and (Panel **B**) RPE during the cycling protocol. There was higher RPE in SD than CO ($P = 0.009$) during the first 40 min. Values are presented as mean ± standard deviation.

Neuromuscular responses

Maximal voluntary and evoked forces

There were no differences in MVC between conditions or days ($P > 0.05$). MVC decreased with exercise from PRE to POST40 ($P = 0.011$) and then no further to POST TF ($P = 0.09$). Similarly, Db100, Db10/Db100 and potentiated twitch and estimated resting twitch amplitudes were similar between D1 and PRE and between conditions ($P > 0.05$) and all decreased with exercise (**Table 9**).

M-waves

Decreased VL and RF Mmax and RF Msup were observed from D1 to PRE ($P < 0.01$). No differences in VM Mmax nor VL or VM Msup were observed between days ($P > 0.05$). Both Mmax and Msup decreased with exercise in both conditions and all muscles ($P < 0.01$) (**Table 9**).

TMS stimulus intensity

There was no difference between conditions ($P = 0.71$) or days ($P = 0.68$) for optimal stimulus intensity. Mean optimal stimulus intensity was 65 ± 8 and 62 ± 9% for CO and 62 ± 9 and 63 ± 12% for SD for days 1 and 2, respectively. There was also no difference between conditions ($P = 0.46$) or days ($P = 0.59$) for submaximal stimulus intensity. Mean submaximal stimulus intensity was 35 ± 7 and 35 ± 8% for CO and 36 ± 8 and 36 ± 8% for SD for days 1 and 2, respectively.

Voluntary activation

There were no differences between conditions for either VAc ($P = 0.34$) or VAp ($P = 0.31$). There was a trend for VAc to decrease with exercise; however, this did not achieve statistical significance ($P = 0.059$) (**Figure 22A**). Peripheral voluntary activation decreased with exercise ($P = 0.003$) and was lower at POST TF than both PRE ($P = 0.003$) and POST40 ($P = 0.014$) (**Figure 22B**).

Motor-evoked potentials (at optimal stimulus intensity)

No differences in $MEP \cdot Mmax^{-1}$ or $MEP \cdot Msup^{-1}$ were observed between days or conditions for any muscle or contraction intensity ($P > 0.05$). Increased VL $MEP \cdot Mmax^{-1}$ and $MEP \cdot Msup^{-1}$ with exercise at all contraction intensities were observed ($P < 0.05$). *Vastus*

Table 9. Neuromuscular parameter evolution with time in SD and CO conditions at D1, PRE, POST40 and POST 40 (n=12 unless otherwise indicated).

			D1	PRE	POST40	POST TF
MVC (N)		CO	599 ± 121	610 ± 100	544 ± 97‡‡	515 ± 85‡‡
		SD	589 ± 95	577 ± 94	510 ± 92‡‡	494 ± 71‡‡
Potentiated		CO	159 ± 34	160 ± 35	123 ± 30‡‡	115 ± 26‡‡,*
twitch (N)		SD	158 ± 30	160 ± 30	125 ± 26‡‡	117 ± 27‡‡,*
Db100 (N)		CO	268 ± 50	271 ± 45	218 ± 47‡‡	206 ± 48‡‡
		SD	268 ± 44	266 ± 46	222 ± 51‡‡	211 ± 49‡‡
Db10/Db100		CO	1.01 ± 0.08	1.01 ± 0.06	0.75 ± 0.12‡‡	0.74 ± 0.11‡‡
		SD	1.04 ± 0.06	1.01 ± 0.08	0.75 ± 0.09‡‡	0.73 ± 0.09‡‡
Estimated resting		CO	101 ± 45	102 ± 45	69 ± 42‡‡	52 ± 27‡‡,**
twitch (N)		SD	103 ± 35	98 ± 34	78 ± 41‡‡	59 ± 33‡‡,**
Mmax (mV)						
	VL	CO	17.1 ± 3.3‡	15.7 ± 3.5	14.4 ± 4.3†	12.5 ± 4.6‡‡,***
		SD	16.4 ± 3.1‡	15.5 ± 2.5	14.6 ± 2.7†	12.4 ± 4.5‡‡,***
	RF	CO	7.7 ± 2.5‡‡	6.9 ± 2.3	6.4 ± 2.1†	4.9 ± 1.8‡‡,***
		SD	8.5 ± 2.6‡‡	8.0 ± 2.5	7.1 ± 2.5†	5.8 ± 2.8‡‡,***
	VM	CO	13.5 ± 4.4	13.1 ± 4.4	12.5 ± 5.3	10.1 ± 4.5‡,*
		SD	12.1 ± 4.2	11.5 ± 3.8	9.9 ± 3.0	7.7 ± 3.9‡,*
Msup (mV) (n=11)						
	VL	CO	15.2 ± 3.9	14.4 ± 3.9	12.9 ± 4.3†	11.1 ± 4.1‡‡,**
		SD	14.3 ± 3.6	14.2 ± 4.0	13.3 ± 3.1†	12.0 ± 5.4‡‡,**
	RF	CO	8.2 ± 3.2‡	7.2 ± 2.7	6.7 ± 2.5	5.5 ± 2.2‡‡,**
		SD	9.0 ± 3.3‡	8.6 ± 3.0	7.6 ± 2.5	6.2 ± 3.0‡‡,**
	VM	CO	10.3 ± 3.8	9.6 ± 3.8	9.3 ± 3.7	8.0 ± 4.7†,*
		SD	10.3 ± 2.9	10.1 ± 2.3	9.5 ± 2.1	7.6 ± 3.2†,*

There were no differences between CO and SD ($P > 0.05$). Time point significantly different from PRE † ($P < 0.05$), ‡ ($P < 0.01$) or ‡‡ ($P < 0.001$). Time point significantly different from POST40 * ($P < 0.05$), ** ($P < 0.01$) or *** ($P < 0.001$).

medialis MEP·Mmax^{-1} at 100% and 75% MVC and MEP·Msup^{-1} at 100% MVC increased with exercise ($P < 0.05$). The increase in VM MEP·Mmax^{-1} at 50% MVC approached statistical significance ($P = 0.050$). There were no changes in RF MEP·Mmax^{-1} or MEP·Msup^{-1} ($P > 0.05$) with exercise (**Figure 23**).

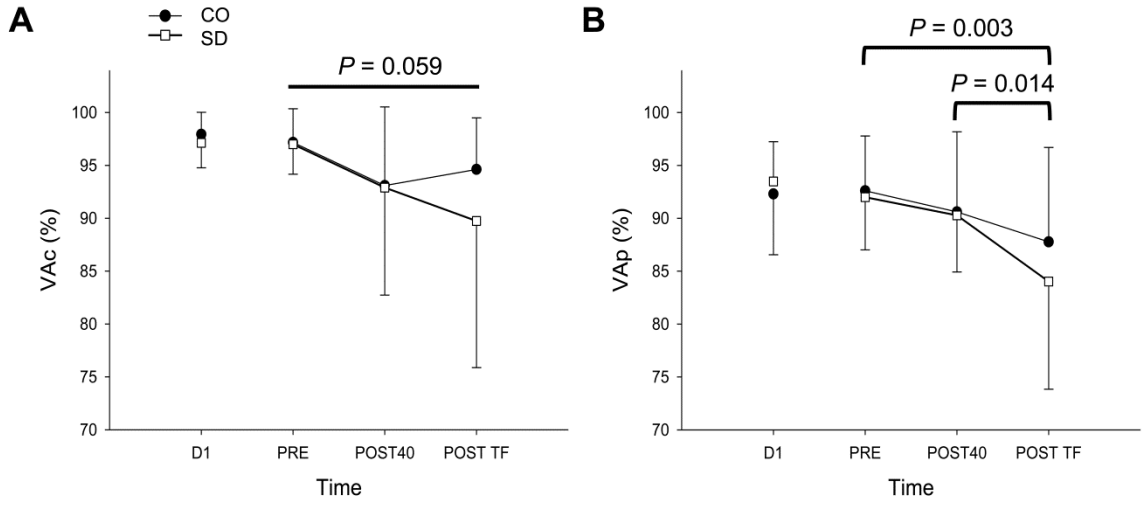

Figure 22. Effect of SD and CO conditions and exercise on (Panel **A**) VAc and (Panel **B**) VAp. Values are presented as mean ± standard deviation.

Motor-evoked potentials (at sub-optimal stimulus intensity)

Both VL MEP·Mmax^{-1} ($P = 0.011$) and MEP·Msup^{-1} ($P = 0.026$) increased with exercise. There were no changes in RF or VM MEP·Mmax^{-1} or MEP·Msup^{-1} ($P > 0.05$) with exercise and no differences between conditions or days for any muscle ($P > 0.05$) (**Figure 23**).

Cortical silent period

Analysis of CSP was performed on 11 subjects because one subject did not return to pre-contraction force levels after the delivery of TMS, thus making CSP determination impossible. There were no differences in CSP between conditions for any muscle or contraction intensity ($P > 0.05$). Cortical silent periods were shorter at both POST40 and POST TF than at PRE for all muscles and contraction intensities ($P < 0.01$) (**Figure 24**).

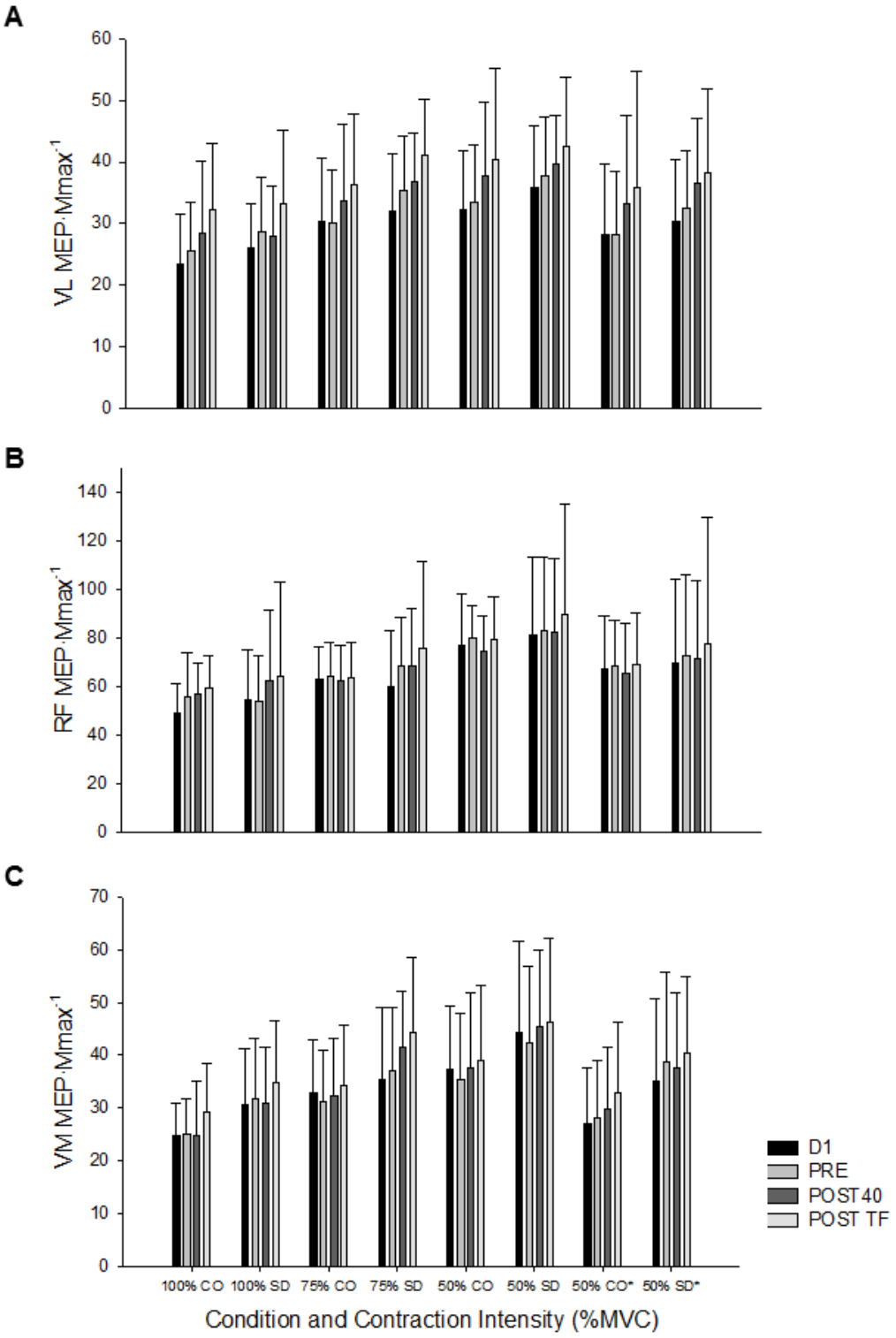

Figure 23. Effect of SD and CO conditions and exercise on MEP·Max^{-1} in (Panel **A**) *vastus lateralis* (VL), (Panel **B**) *rectus femoris* (RF) and (Panel **C**) *vastus medialis* (VM) during contractions at 50, 75 and 100% MVC with TMS delivered at optimal stimulus intensity and 50% MVC (50S) with TMS delivered at sub-optimal stimulus intensity. Values are presented as mean ± standard deviation. Significant changes are presented in the text only.

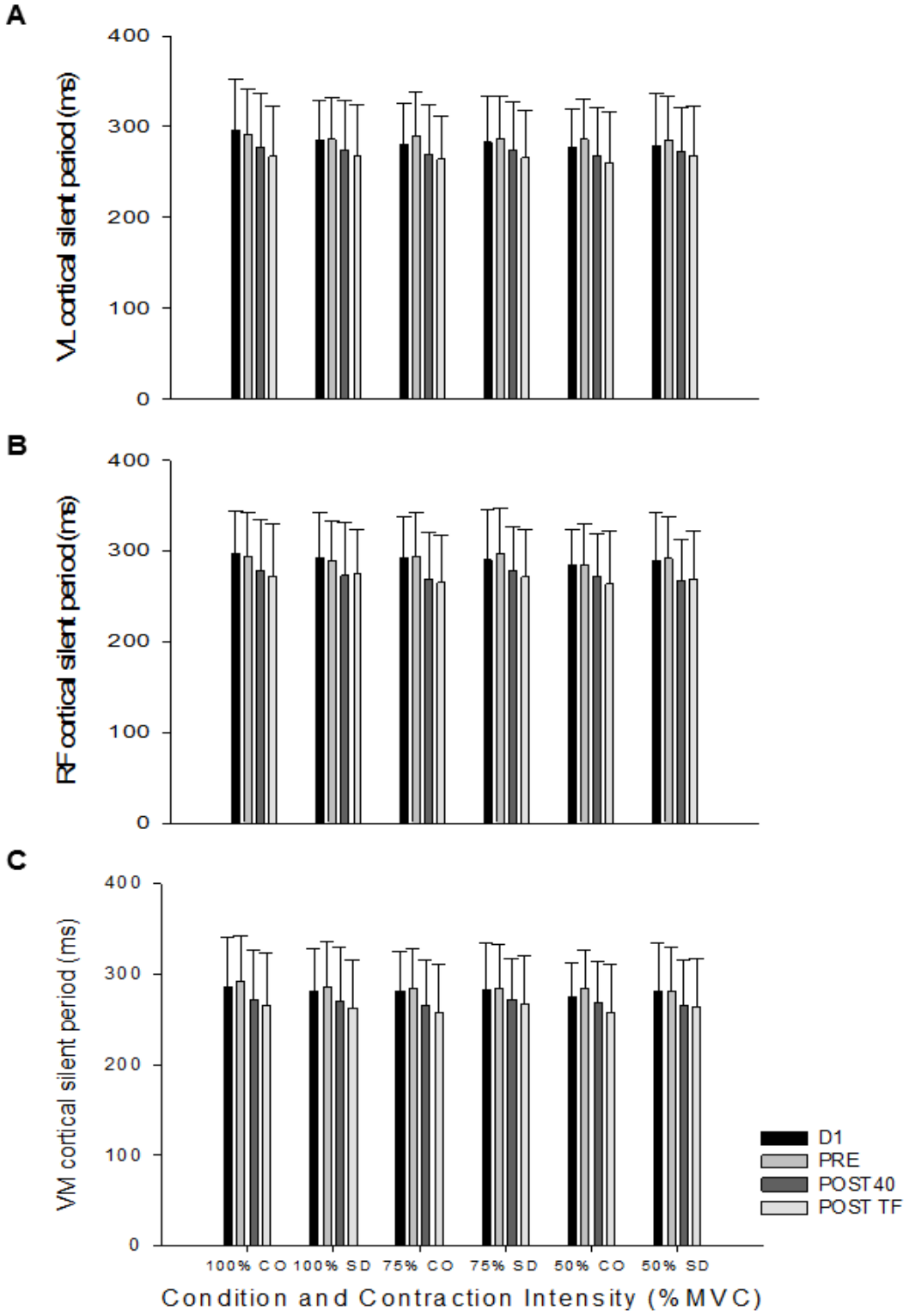

Figure 24. Effect of SD and CO conditions and exercise on CSPs in (Panel **A**) *vastus lateralis* (VL), (Panel **B**) *rectus femoris* (RF) and (Panel **C**) *vastus medialis* (VM) during contractions at 50, 75 and 100% MVC with TMS delivered at optimal stimulus intensity and 50% MVC (50S) with TMS delivered at sub-optimal stimulus intensity. Values are presented as mean ± standard deviation. Significant changes are presented in the text only.

Cognitive task

Reaction time

Results showed main effects of condition ($P = 0.011$), trial congruency ($P = 0.019$), time ($P = 0.023$), block ($P = 0.024$) and an interaction between condition and time ($P = 0.035$). Reaction times were longer for incongruent trials (406 ± 11 ms) than congruent trials (377 ± 10 ms). The interaction between condition and time indicated that RT lengthened in SD in PRE (375 ± 9 ms, $P = 0.007$) and POST TF (371 ± 16 ms, $P = 0.002$) compared to CO (349 ± 8 ms and 337 ± 10 ms for PRE and POST TF, respectively). Conversely, during $CYCL_{20\text{-}40}$ RT in SD (347 ± 11 ms) did not differ from RT observed in CO (CO, 333 ± 9 ms vs. 347 ± 11 ms; $P = 0.20$) (**Figure 25A**). No other interactions were observed.

Decision errors and omissions

A classic congruency effect was observed with the prevalence of errors in incongruent trials (6.19 ± 0.7%) greater than in congruent trials (3.04 ± 0.4%; $P < 0.001$). There were no other main effects or interactions. Wilcoxon Signed-Rank test showed that the omission rate was greater in SD during PRE (0.82%, $P = 0.012$) and POST TF (1.68%, $P = 0.002$) than CO (0.02 and 0% for PRE and POST TF, respectively). Conversely, no omissions were observed in either SD or CO during $CYCL_{20\text{-}40}$ (**Figure 25B**).

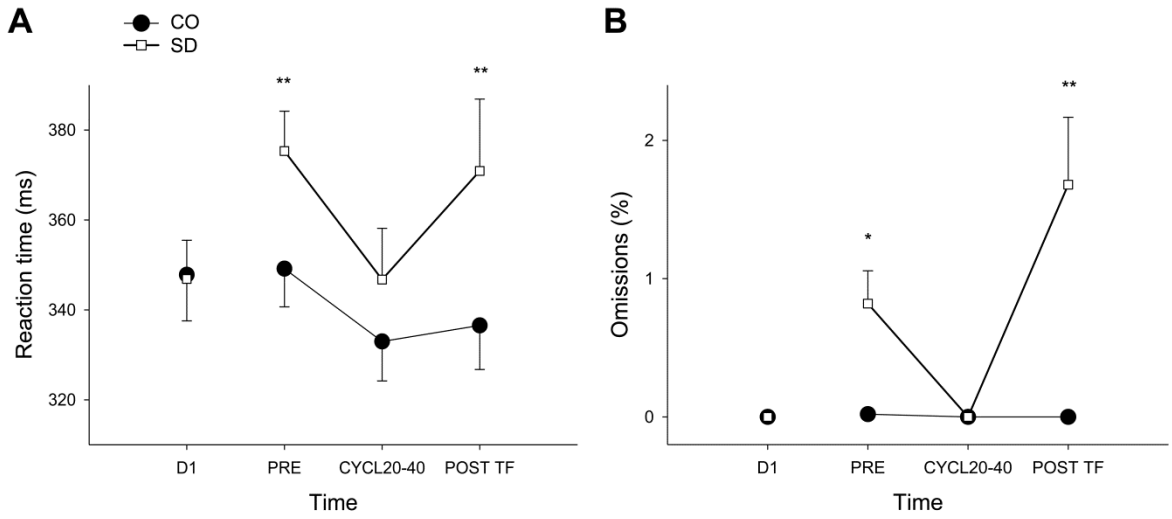

Figure 25. Effect of SD and CO conditions and exercise on (Panel **A**) RT and (Panel **B**) omission rate. Values are presented as mean ± standard error of the mean. Results in SD significantly different than CO, * ($P < 0.05$) and ** ($P < 0.01$).

DISCUSSION

The principal findings of this study are that one night SD resulted in decreased cycling time to task failure, increased RPE during cycling and both longer RT and higher omitted response rates at rest without evidence of decreased cognitive control efficiency compared to CO. Despite increased RPE in SD, submaximal cycling exercise restored information processing efficiency to baseline levels. Furthermore, changes within the muscle or to voluntary activation measured after task failure cannot explain the decrement in exercise performance with SD. The hypothesis that increased central fatigue might elucidate performance deterioration was refuted since neuromuscular function was not affected by SD.

Cycling performance

The diminished cycling performance in SD may be explained by differences in RPE and sleepiness. Motivation and the decision to stop exercise involve complex cognitive functions. Sleepiness, as assessed by the Stanford Sleepiness Scale, was greater in SD than CO, also during exercise when sleepiness increased in CO and was unchanged by SD. These coupled with prior research indicating that combined intermittent exercise and SD causes individuals to be more susceptible to negative mood states than SD alone (Scott *et al.*, 2006) suggest that increased sleepiness during exercise and mood disturbances may have contributed to reduced exercise performance in SD.

RPE and HR

During the 40 min of submaximal cycling, RPE was significantly greater with SD. Despite this difference, there was no difference in RPE during TTF between conditions. All subjects however had maximal RPE at task failure although this occurred 59 s later in CO (mean performance time decrement of 7.5% in SD). This result concurs with the findings of Marcora *et al.* (2009), who compared TTF after both a 90-min mentally fatiguing task and a 90-min mentally neutral task. In this study, RPE was higher in the mentally fatiguing condition except at task failure which occurred earlier after the mentally fatiguing task. Sleep loss has previously been shown to have dramatic effects on emotional processing, judgment and self-esteem and subjects were more likely to report increased feelings of worthlessness, inadequacy, powerlessness and failure (Killgore, 2010). Emotional modifications may explain the difference in a self-reported measure like RPE and require further investigation. Sleep deprivation also reduced exercise HR only during TTF in the present study. The finding that

SD results in decreased exercise HR is equivocal (Martin, 1981; Martin & Gaddis, 1981; Martin & Chen, 1984; Scott & McNaughton, 2004; Oliver *et al.*, 2009), suggesting that exercise duration and/or intensity may be important factors influencing the impact of SD on HR. Scott and McNaughton (2004) discussed several proposed mechanisms to explain lower HR during exercise in SD, including plasma volume expansion and decreased respiratory controller sensitivity, and their potential problems or the data required to support them. Interestingly, in conjunction with the increased RPE during TTF after a mentally fatiguing task, Marcora *et al.* (2009) observed lower HR only at task failure and attributed this difference to task failure occurring earlier. Further investigation is required in order to identify the mechanisms and conditions underlying decreased exercise HR with SD.

Neuromuscular function

Our hypothesis that a greater reduction in the neural recruitment of motor units, central fatigue, might partially explain diminished cycling performance with SD was refuted. Maximal voluntary force and electrically evoked M-wave and force decreased with exercise, agreeing with previous studies of aerobic exercise (Millet *et al.*, 2003c). There was evidence of decreased VA, including VAc showing a strong tendency to decrease with exercise (P = 0.059). Isometric MVC has been shown to begin to recover immediately after a fatiguing task (Froyd *et al.*, 2013). Peripheral voluntary activation was evaluated before VAc at each evaluation and the additional recovery time may have been sufficient to create this discrepancy and render VAc evaluation insufficiently sensitive to real changes in some subjects. However, measures of central fatigue recover more slowly than peripheral responses (unpublished data and (Froyd *et al.*, 2013)), suggesting that the effect of PNS and TMS testing order was likely minimal. Previous studies evaluating TMS measures in SD generally observed results in SD and CO to be similar (Civardi *et al.*, 2001; Scalise *et al.*, 2006; De Gennaro *et al.*, 2007; Kreuzer *et al.*, 2011). Only MEP amplitude during muscular contraction was a common measure with any of these studies. Scalise *et al.* (2006) observed no change in absolute MEP amplitude after at least 24 h SD in opponens pollicis, mirroring our observation that MEP amplitude is unaffected by SD. *Vastus lateralis* MEP amplitude and VM MEP amplitude at some contraction intensities increased with exercise, consistent with findings in fatiguing submaximal and maximal isometric-contraction protocols (Gruet *et al.*, 2013a). Conversely, RF MEP amplitude and VM MEP amplitude at some contraction intensities did not change with exercise, consistent with other cycling protocols (Sidhu *et al.*, 2009b; Goodall *et al.*, 2012; Klass *et al.*, 2012), including two of comparable duration. The

discrepancy between these studies (Sidhu *et al.*, 2009b; Klass *et al.*, 2012) and the present study may be due to their use of lower TMS intensities (30-60% maximal stimulator output vs. mean stimulus intensity > 60% maximal stimulator output in all sessions in the present study). These results also suggest that different muscles of the quadriceps may not demonstrate a homogeneous response to exercise although the rapid recovery of MEPs to baseline levels post-exercise (Taylor *et al.*, 1996) may mask exercise-induced changes in RF and VM. Changes in MEP amplitude during exercise did not differ between SD and CO, indicating that corticospinal excitability was unaffected by SD, both at rest and following fatiguing exercise.

The amplitude of MEPs at 50% MVC was evaluated by TMS delivered at two stimulus intensities, one to evoke maximal MEP amplitudes and the other half-maximal MEP amplitudes, both determined from the stimulus-response curve at 20% MVC. For all muscles, the same changes were observed at both TMS stimulus intensities. The changes in MEP amplitude observed in this study were independent of TMS stimulus intensity. If submaximal MEP responses are not measured, real changes in cortical excitability may be overlooked if the stimulus-response curve shifts to the left or right and maximal MEP amplitude remains unaffected. This however was not the case in the present study.

The finding that CSP decreased with exercise is novel. This contrasts the increased CSP observed in sustained submaximal and maximal isometric contractions (Gruet *et al.*, 2013a) and its lack of change after other cycling protocols (Sidhu *et al.*, 2009b; Goodall *et al.*, 2012; Klass *et al.*, 2012). The difference between cycling protocols of similar duration (Sidhu *et al.*, 2009b; Klass *et al.*, 2012) may be due, at least in part, to the aforementioned difference in TMS intensities employed. After exercise cessation, CSPs have been observed to rapidly return to baseline values (Taylor *et al.*, 2000), suggesting that the magnitude of decrease may be underestimated. The primary inhibitory cerebral neurotransmitter is GABA, which is derived from glutamate. Cortical silent periods are predominantly mediated by $GABA_B$ receptors (McDonnell *et al.*, 2006); thus, decreased $GABA_B$ concentration would reduce cortical inhibition and CSP duration. After 3 h of cycling at 60% VO_2max, cerebral ammonia uptake and its accumulation in cerebral spinal fluid was observed (Nybo *et al.*, 2005). Previously, maximal incremental cycling to task failure (~12 min) showed cerebral ammonia uptake without cerebral spinal fluid accumulation (Dalsgaard *et al.*, 2004). Proposed by Nybo *et al.* (2005) and supported by previous research in rats (Guezennec *et al.*, 1998), a minimum duration and exercise intensity is necessary to exceed the ammonia removal capacity of the brain. Accumulation of ammonia in cerebral spinal fluid could cause decreased cortical

glutamate concentration since ammonia is condensed with glutamate to produce glutamine during ammonia removal. Consequently, GABA concentration would decrease, resulting in decreased cortical inhibition. Whether this mechanism may explain the observed reduction of intracortical inhibition during prolonged exercise requires further investigation. The lack of difference between CSP shortening in CO and SD indicates that any mechanism contributing to shorter CSPs during exercise is unaffected by SD.

Cognitive performance, sleep deprivation and exercise

This study reproduced cognitive deficits widely reported after one night of SD, notably slowed response speed and increased number of omitted responses (*e.g.* (Tsai *et al.*, 2005)). No evidence of decreased response inhibition was observed in SD as demonstrated by the lack of primary interaction between congruency and condition, or second-order interaction with the addition of time points (D1, PRE, $CYCL_{20\text{-}40}$, POST TF). Using three short Stroop tasks (Color-Word, Emotional, and Specific), Sagaspe *et al.* (2006) similarly observed that 36 h of SD did not affect cognitive control. Cognitive control was also unaffected by exercise as there was no interaction between congruency and time points. In conjunction with the lack of significant interactions involving mean RT or decision error, these results suggest neither SD or exercise, nor their interaction, influenced cognitive control. The present study is consistent with Killgore (2010) and suggests that cognitive processes are differentially sensitive to SD as some cognitive functions were impaired (*e.g.* slowing of response speed) whereas others were unaffected (*e.g.* selective response inhibition).

Shorter RT during exercise was not associated with increased decision error, indicating that the response strategy (*i.e.* speed-accuracy trade-off) did not change and that exercise specifically caused increased performance. In accordance with our hypothesis, this positive effect of acute submaximal exercise also counteracted the negative effects of SD and restored information processing efficiency (*i.e.* faster RT, fewer omissions) to baseline levels. This benefit could have been due to greater exercise-induced nervous system activation (*e.g.* increased HR (Davranche *et al.*, 2005, 2006b), increased plasma catecholamines (Chmura *et al.*, 1994)), which could have temporarily negated the decreased alertness and attentional capacities caused by SD. This gain may have endured for a short duration; however it was no longer observed at POST TF, reinforcing the established transient post-exercise benefits of exercise on cognitive performance (Chang *et al.*, 2012). The exact mechanism(s) for transient improvements in cognitive performance during exercise remain to be elucidated.

Limitations

Without the availability of electroencephalography the effects of possible microsleeps are unknown despite constant subject supervision. Effects of subjects being exposed to low levels of light and being more active in SD may also have influenced results. The performance measure of TTF was chosen despite its limited application to real-world exercise performance, greater variability and important motivational component. The primary goal was to exhaust the subject and if a time trial was employed, the associated pacing strategies may have complicated interpretation of the results. Neuromuscular assessment was not conducted on the same apparatus as cycling, thus there was a delay from exercise termination to neuromuscular evaluation meaning that changes in neuromuscular measures immediately post-exercise would not have been identified. Measurement of electromyography was not conducted during the exercise bouts, thus preventing neuromuscular evaluation of the effects of SD during exercise. Further studies are required to investigate combined PNS and TMS measures during exercise with SD.

Conclusion

In summary, one night of complete SD resulted in decreased cycling time to task failure compared to a control condition. Self-reported measures, including RPE, were altered in SD, confirming the importance of emotional processing in SD-induced performance deficits. Cognitive processes appear to be differentially sensitive to SD as only some cognitive functions were impaired. Furthermore, the compensatory effect of acute submaximal exercise on cognitive deficits induced by sleep loss was demonstrated. Neuromuscular function 3-4 min after cycling cessation was similar between CO and SD, indicating that changes in the muscle and to the motor nervous system likely cannot explain any of the decrement in exercise performance with SD. Thus, the hypothesis that increased central fatigue after one night complete SD contributes to decreased exercise performance is unsupported.

STUDY 4

Central fatigue assessed by transcranial magnetic stimulation in ultra-trail running

Fatigue centrale évaluée par stimulation magnétique transcrânienne en ultra-trail

John Temesi[1], Thomas Rupp[2,3], Vincent Martin[4], Pierrick J. Arnal[1], Léonard Féasson[1,5], Samuel Verges[2,3], Guillaume Y. Millet[1,3]

[1] Université de Lyon, F-42023, Saint-Etienne, France

[2] University Grenoble Alpes, Laboratoire HP2, F-38000, Grenoble, France

[3] INSERM, U1042, F-38000, Grenoble, France

[4] Clermont Université, Université Blaise Pascal, EA 3533, Laboratoire des Adaptations Métaboliques à l'Exercice en Conditions Physiologiques et Pathologiques, BP 10448, F-63000 Clermont Ferrand, France

[5] Unité de Myologie, Centre Référent Maladies Neuromusculaires Rares Rhône-Alpes, Centre Hospitalier Universitaire de Saint-Etienne, F-42023, Saint-Etienne, France

ABSTRACT

The underlying mechanisms of the well-established central deficit in ultra-endurance running races are not understood. The use of transcranial magnetic stimulation (TMS) in parallel with peripheral nerve stimulation provides insight into the source of these central changes. The aims of this study were to determine the presence and magnitude of cortical and peripheral voluntary activation deficits after a mountain trail-running race and whether this can be explained by simultaneous changes in corticospinal excitability and intracortical inhibition. Neuromuscular function (TMS and femoral nerve electrical stimulation) of the knee extensors was evaluated before and after a 110-km ultra-trail in 26 experienced ultra-endurance trail runners during maximal and submaximal voluntary contractions and in relaxed muscle. Both peripheral (-26%) and cortical (-16%) voluntary activation decreased and were correlated ($P < 0.01$). Decreases in potentiated twitch and doublet amplitudes were correlated with decreased cortical voluntary activation ($P < 0.05$). There was increased motor-evoked potential (MEP) amplitude ($P < 0.05$) without change in cortical silent period (CSP) elicited by TMS at optimal stimulus intensity. Conversely, CSP at sub-optimal TMS intensity increased ($P < 0.05$) without concurrent change MEP amplitude. MEP and CSP responses suggest a shift in the sigmoidal MEP-stimulus-intensity relationship towards larger MEPs at great TMS intensity without change in inflection point of the curve and a left-shift in the CSP-stimulus-intensity relationship. These changes may contribute to the impaired motor command observed after the ultra-trail. The presence of peripheral changes, correlated with decreased cortical voluntary activation, suggests contribution of group III and IV afferents to central deficits during ultra-endurance running exercise.

Keywords: cortical voluntary activation, corticospinal excitability, intracortical inhibition, neuromuscular fatigue

RÉSUMÉ

Les mécanismes sous-jacents au déficit central bien décrit dans la course à pied d'ultra-endurance restent à éclaircir. L'utilisation de la stimulation magnétique transcrânienne (TMS) en parallèle de la stimulation nerveuse périphérique peut permettre de mieux comprendre l'origine de ces changements centraux. Les objectifs de cette étude étaient de déterminer la présence et l'importance des déficits d'activation volontaire corticale et

périphérique après un ultra-trail et si ces modifications peuvent être expliquées par des modifications simultanées d'excitabilité corticospinale et d'inhibition intracorticale. La fonction neuromusculaire (TMS et stimulation électrique du nerf fémoral) des extenseurs du genou a été évaluée avant et après un ultra-trail de 110 km chez 26 coureurs de trail d'ultra-endurance expérimentés pendant des contractions volontaires maximales et sous-maximales et sur muscle relâché. L'activation volontaire périphérique (-26%) et corticale (-16%) ont diminué et étaient corrélées (P < 0,01). La diminution des amplitudes de la secousse simple et des doublets potentiés étaient corrélés à la diminution de l'activation volontaire corticale (P < 0,05). L'amplitude des potentiels moteurs évoqués (MEP) (P < 0,05) a augmenté sans changement de la période de silence corticale (CSP) provoquée par la TMS à intensité optimale. Inversement, les CSP à intensité de TMS sous-optimale ont augmenté (P < 0,05) sans changement de l'amplitude des MEP. Les modifications de MEP et CSP observées suggèrent un changement dans la relation sigmoïdale entre l'amplitude des MEP et l'intensité de stimulation par TMS avec des MEP plus importants pour des intensités de TMS élevées sans changement du point d'inflexion de la courbe, ainsi qu'avec un décalage vers la gauche de la relation entre CSP et intensité de stimulation. Ces changements pourraient contribuer à la diminution de la commande motrice observée après un ultra-trail. La présence de changements périphériques, corrélés à la diminution de l'activation volontaire corticale, suggère la contribution des afférences de groupes III et IV dans les déficits centraux au cours d'une course à pied d'ultra-endurance.

Mots clés : *activation volontaire corticale, excitabilité corticospinale, inhibition intracorticale, fatigue neuromusculaire*

INTRODUCTION

Probably due to the explosion of ultra-endurance running participation, a large amount of research on the physiological consequences of ultra-marathons has been conducted recently (Millet *et al.*, 2002; Easthope *et al.*, 2010; Martin *et al.*, 2010; Millet *et al.*, 2011c). This type of event permits investigation and greater understanding of the limits of human performance (Millet & Millet, 2012). The origins of fatigue are dependent on numerous factors, including the type of exercise, making the ultra-trail running sub-group of endurance-running a unique field of study. With substantial elevation changes and long duration, ultra-trails combine a range of specific exercise intensities and employ various muscle groups, activation patterns and types of muscle contraction (*i.e.* combining concentric and severe eccentric loads).

Neuromuscular fatigue is an exercise-related decrease in the maximal voluntary torque of a muscle or muscle group, regardless of whether or not a task can be sustained. This may involve processes at all levels of the motor pathway from the brain to skeletal muscle. Large central fatigue (*i.e.* reduced maximal voluntary activation) has been observed in running bouts longer than 5 h (Millet *et al.*, 2002; Place *et al.*, 2004; Martin *et al.*, 2010; Millet *et al.*, 2011c). The presence of central fatigue does not however mean an absence of peripheral fatigue although compared to the central component peripheral fatigue appears to be only of moderate importance in extremely long-duration exercise. Only a few studies have investigated central fatigue in running exercise longer than 12 h in duration (Martin *et al.*, 2010; Millet *et al.*, 2011c; Saugy *et al.*, 2013), and only two have combined elevation change and extreme duration (Millet *et al.*, 2011c; Saugy *et al.*, 2013). All these studies used the classical peripheral electrical stimulation techniques of twitch interpolation and central activation ratio to assess voluntary activation (Merton, 1954). The major issue with these techniques is that they do not permit the differentiation between spinal and supraspinal components of central fatigue.

Using transcranial magnetic stimulation (TMS) in parallel with peripheral nerve stimulation during voluntary isometric contractions, Gandevia *et al.* (1996) observed that as exercise duration increases, the role of supraspinal factors in fatigue increases and that supraspinal deficits and failure are not necessarily paralleled by impairment of motor cortical excitability. Furthermore, the presence of central fatigue does not mean that both spinal and supraspinal fatigue are certainties.

Dynamic whole-body exercise has only recently been investigated with TMS, predominantly in cycling studies (*e.g.* (Goodall *et al.*, 2012; Sidhu *et al.*, 2012a; Temesi *et al.*,

2013)). Only one published study has employed TMS with running (Ross *et al.*, 2007), observing decreased cortical voluntary activation (VAc) of the dorsiflexors after a treadmill marathon. This study also observed decreased motor-evoked potential (MEP) amplitude of the *tibialis anterior* in relaxed muscle immediately post-marathon; however, MEP amplitude assessed in relaxed muscle limits interpretation because of both the greater MEP variability and lower corticospinal excitability in the relaxed muscle state (Gruet *et al.*, 2013a). All other whole-body investigations of VAc have been conducted with cycling and generally showed decreased VAc after exercise (Sidhu *et al.*, 2009b; Goodall *et al.*, 2012; Temesi *et al.*, 2013) although not 10 min after ~1.5 h of cycling (Klass *et al.*, 2012).

At similar exercise intensities, there is less central fatigue in cycling than running and this has been proposed to be related to increased influence of group III/IV afferents because of increased muscle damage in running (Millet & Lepers, 2004). Unlike the dorsiflexors which do not limit running performance (Fourchet *et al.*, 2012) and plantar flexors which display only moderate central deficits, the knee extensors demonstrate large central deficits after prolonged exercise (Martin *et al.*, 2010; Millet *et al.*, 2011c). Whether this manifests at the supraspinal level in trail running, particularly given the muscle damage associated with the eccentric nature of downhill running, remains to be determined. The use of TMS in parallel with peripheral nerve stimulation can provide greater insight into the source of these central changes.

Despite large central consequences, the effects of long running bouts on supraspinal activity and any subsequent effect on knee extensor function are unknown. Specifically, whether a supraspinal deficit occurs with an ultra-endurance trail running race and whether or not any such deficit is associated with changes in corticospinal excitability and/or intracortical inhibition remain to be determined. The aim of this study was thus to test the hypotheses that (i) an ultra-trail decreases VAc, and (ii) corticospinal fatigue occurs despite no change or increased MEP amplitude and unchanged cortical silent period (CSP).

METHODS

Subjects

Thirty-five healthy experienced ultra-endurance trail runners (15 females and 20 males) were recruited to participate in this study. Six subjects (3 females and 3 males) did not complete the ultra-trail and 3 others (1 female and 2 males) did not perform post-race testing due to time constraints. Thus, 26 subjects (11 females and 15 males) participated in all aspects of this

study (mean ± standard deviation: age, 43 ± 9 years; height, 172 ± 9 cm; body mass, 66.5 ± 10.9 kg; maximal oxygen consumption (VO_{2max}), 56.2 ± 6.3 $ml \cdot kg^{-1} \cdot min^{-1}$). Subjects were informed of the experimental protocol and all associated risks prior to giving written informed consent as part of a medical inclusion. All procedures conformed to the Declaration of Helsinki and were approved by the local ethics committee. All subjects were experienced ultra-endurance trail runners since participation in the partner ultra-trail (the North Face® Ultra-Trail du Mont-Blanc® 2012) required completion of a minimum of two demanding trail-running races with significant elevation change in the two years preceding the race.

Experimental design

Each subject completed one preliminary session and two experimental sessions. During the preliminary session, subjects completed a maximal incremental running test and were introduced to all experimental procedures and repeated trials until they were able to perform all tests consistently and as directed. The first experimental session (PRE) occurred on one of the three days before the North Face® Ultra-Trail du Mont-Blanc® 2012 and the second (POST) 1:01:30 ± 0:22:37 after completing the ultra-trail. Due to exceptional inclement weather conditions, the 2012 edition of the North Face® Ultra-Trail du Mont Blanc® involved running/walking 110 km with total positive elevation change of 5862 m (**Figure 26**). Under conditions of a mixture of rain, snow and clouds, the temperature reached a maximum of 12°C in Chamonix and decreased below 0°C at altitudes above 1800 m.

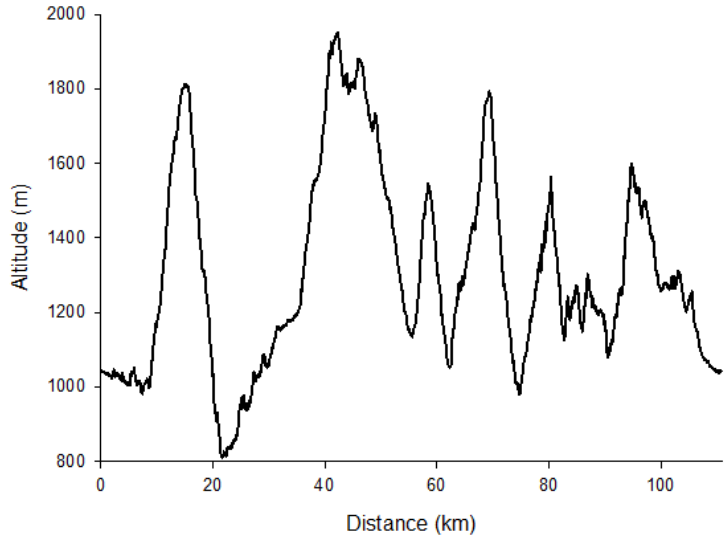

Figure 26. Course profile of Ultra-Trail du Mont-Blanc 2012.

Preliminary session

After a medical examination, subjects performed a maximal incremental running test to exhaustion on a treadmill (EF1800, HEF Tecmachine, Andrezieux-Boutheon, France). The subjects began the test at 10% grade and a speed of 4-6 $km \cdot h^{-1}$, with starting speed corresponding to running ability. The speed was then increased by 1 $km \cdot h^{-1}$ until volitional exhaustion. Subjects ran 2 min 30 s at each speed and then stopped for 30 s for a blood sample for lactate measurement. Respiratory measures were assessed breath-by-breath by an online system (Ergocard, Medisoft, Sorinnes, Belgium) and averaged every 30 s. VO_{2max} was considered as the oxygen consumption during the last 30 s prior to exhaustion.

The familiarization portion of the preliminary visit included maximal and submaximal contractions of the knee extensors with and without femoral nerve electrical stimulation (PNS) and TMS (see Neuromuscular testing protocol section). For TMS, this included training subjects to return to the pre-stimulus torque as soon as possible after the stimulus to permit accurate measurement of the CSP.

Neuromuscular testing protocol

Neuromuscular measures (**Figure 27**) were assessed PRE and POST with real-time visual feedback. Maximal torque was determined from 3 MVCs separated by 30 s with PNS (100-Hz paired pulses and single pulses) delivered at peak torque and immediately after in the relaxed state (100- and 10-Hz paired pulses and single pulses). Then three series of four contractions were performed with TMS delivered at the desired torque level (100, 75 and 50% MVC at optimal stimulus intensity (Todd *et al.*, 2003) and 50% MVC at sub-optimal stimulus intensity; see below for further details). Contractions were separated by 15 s and series by 30 s.

Force and electromyographic recordings

Knee extensor force was measured during voluntary and evoked contractions by a calibrated force transducer (Meiri F2732 200 daN, Celians, Montauban, France) with amplifier attached by a non-compliant strap to the right leg just proximal to the malleoli of the ankle joint. Subjects were seated upright in a custom-built chair with both right knee and hips at 90° of flexion. The load cell was fixed to the chair such that force was measured in direct line to the applied force. Torque was calculated as force measured by the force transducer multiplied by the length of the lever arm (*i.e.* distance from the tibial condyles to where the force transducer was attached to the leg).

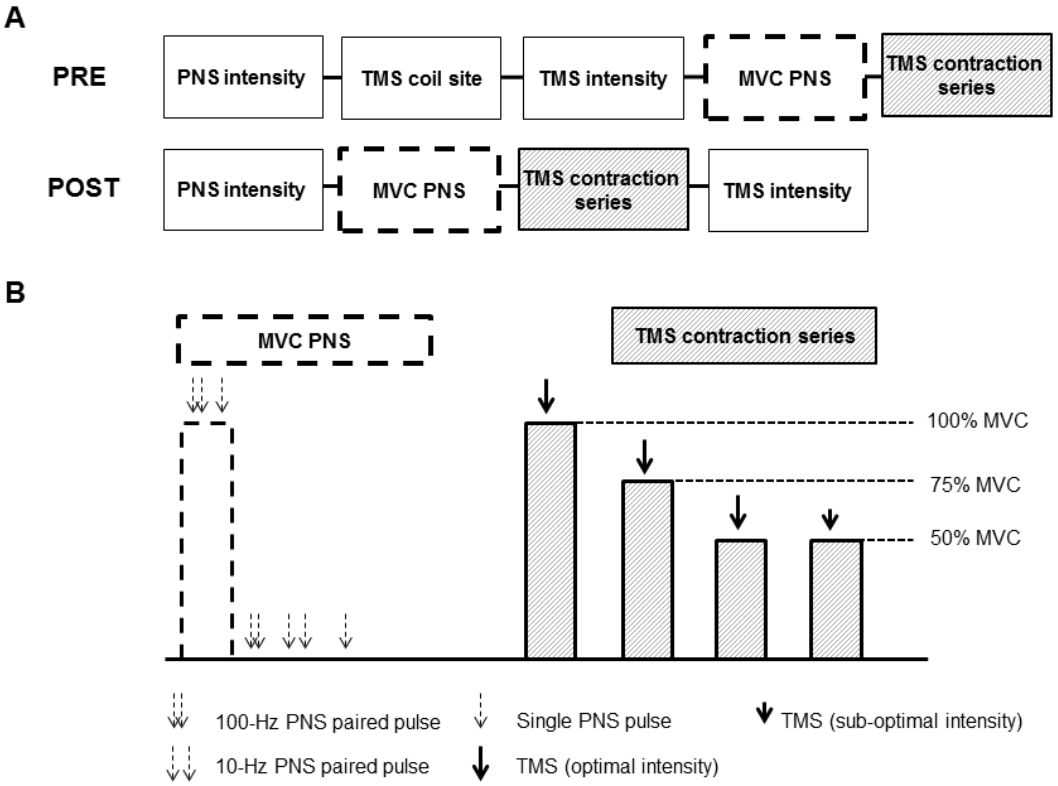

Figure 27. Panel **A**) Neuromuscular testing order PRE and POST ultra-trail for PNS and TMS. Panel **B**) Neuromuscular testing protocol for PNS MVCs and TMS contraction series.

Electromyographic activity (EMG) of the right knee extensors (*vastus lateralis*) was recorded with a pair of self-adhesive surface electrodes (Meditrace 100, Covidien, Mansfield, USA) in bipolar configuration with a 30-mm interelectrode distance and the reference on the patella. Low impedance (<5 kΩ) between electrodes was obtained by shaving, gently abrading the skin and then cleaning it with isopropyl alcohol. Signals were analogue-to-digitally converted at a sampling rate of 2000 Hz by PowerLab system (16/30-ML880/P, ADInstruments, Bella Vista, Australia) and octal bio-amplifier (ML138, ADInstruments; common mode rejection ratio = 85 dB, gain = 500) with bandpass filter (5-500 Hz) and analyzed offline using Labchart 7 software (ADInstruments).

Femoral nerve electrical stimulation

Single electrical stimuli of 1-ms duration were delivered via constant-current stimulator (DS7A, Digitimer, Welwyn Garden City, Hertfordshire, UK) to the right femoral nerve via a 30-mm diameter surface cathode manually pressed into the femoral triangle (Meditrace 100, Covidien, Mansfield, USA) and 50 x 90 mm rectangular anode (Durastick Plus, DJO Global,

Vista, USA) in the gluteal fold. Single stimuli were delivered incrementally until maximal M-wave (Mmax) and twitch amplitudes plateaued. Stimulus intensity of 130% of the intensity to produce Mmax and maximal twitch responses was employed to confirm supramaximality. Stimulus intensity was determined at the start of each session. Supramaximal PNS intensity increased from PRE (60 ± 18 mA) to POST (67 ± 21 mA; $P = 0.025$).

Transcranial magnetic stimulation

Single TMS pulses of 1-ms duration were manually delivered to elicit MEPs and superimposed twitches (SITs) during voluntary isometric knee extension. The contralateral motor cortex was stimulated by a magnetic stimulator (Magstim 200², The Magstim Company Ltd, Whitland, UK) with a 110-mm double-cone coil (maximum output of 1.4 T) to induce a postero-anterior current. The coil was manually controlled by an experienced investigator throughout the protocol. Subjects wore a cervical collar during all TMS measures to stabilize the head and neck. Subjects also wore a latex swim cap on which lines were drawn between the preauricular points and from nasion to inion to identify the vertex. Every centimeter was demarcated from the vertex to 2 cm posterior to the vertex along the nasal-inion line and also to 1 cm over the left motor cortex. At each point a stimulus was delivered at 50% maximal stimulator output during brief voluntary contractions of the knee extensors at 10% maximal voluntary contraction (MVC) torque to determine the optimal stimulus site. The coil was positioned at the site evoking the largest MEP amplitude and SIT throughout the protocol. Stimulus intensity was determined from a stimulus-response curve determined from MEP responses evoked during brief (~2-3 s) voluntary contractions at 20% MVC. TMS was delivered during 2 consecutive contractions at each of the randomly-ordered stimulus intensities of 40, 50, 60 and 70% maximal stimulator output. Stimuli were delivered at 15-s intervals. Optimal stimulus intensity was defined as the lowest stimulus intensity eliciting maximal MEP amplitudes (Groppa *et al.*, 2012). If a plateau was not confirmed from these intensities, higher intensities were investigated. A sub-optimal stimulus intensity equivalent to 60% of the optimal intensity (*i.e.* corresponding to the rising part of the stimulus-response curve) was also selected to identify any shift in the stimulus-response curve. Mean stimulus intensities PRE were 67 ± 9% and 40 ± 5% maximal stimulator output for optimal and sub-optimal stimulus intensities, respectively. Coil position in relation to the vertex was noted because identical coil position and TMS intensities were utilized PRE and POST. Immediately after POST evaluation, optimal stimulus intensity was re-determined in subjects still physically capable of sustaining the target torque level (20% MVC POST) ($n = 21$).

Optimal stimulus intensity in these subjects was similar PRE and POST (66 ± 9% versus 67 ± 6% maximal stimulator output, respectively; $P = 0.54$). During voluntary contractions, TMS was always delivered once the subject had contracted to the appropriate torque level and the torque had stabilized. Subjects were also instructed to re-contract to the pre-stimulus torque level immediately after TMS delivery.

Data analysis

EMG and femoral nerve electrical stimulation

M-wave peak-to-peak amplitude and duration were calculated from PNS in both relaxed (Mmax) and contracted (Msup at 100% MVC) muscles. Maximal torque was calculated as the mean peak torque from three MVCs. EMG root mean square (RMS) was calculated as the mean from three MVCs over a 200-ms period after the torque had reached a plateau and before PNS was delivered (RMS_{MVC}). Then RMS_{MVC} was normalized to both Mmax and Msup. The amplitudes of the potentiated peak twitch (TwPot) and doublet (100-Hz paired pulse, Db100; 10-Hz paired pulse, Db10) torques were also determined.

Peripheral voluntary activation (VAp) was assessed by twitch interpolation using the superimposed and potentiated doublet amplitudes elicited by 100-Hz paired pulses during and after MVCs and calculated from the equation: $[1 - (\text{PNS 100-Hz superimposed doublet amplitude}) \cdot Db100^{-1}] \times 100$. The presence of low- and high-frequency fatigue POST was evaluated from the change in the ratio of Db10 to Db100 (Verges *et al.*, 2009).

Transcranial magnetic stimulation

Peak-to-peak amplitude of MEPs were measured and normalized to Msup measured at the same time point. VAc during maximal effort was measured with TMS by modified twitch interpolation. For each series of contractions, estimated resting twitch (ERT) was determined by linear regression of the relation between SIT amplitude evoked when optimal intensity TMS was delivered at 100, 75 and 50% MVC and voluntary torque (Todd *et al.*, 2003). This relation was extrapolated and the y-intercept was interpreted as the ERT amplitude. In cases where the linear regression was not linear ($r < 0.9$), ERT was excluded and VAc was not calculated for the series (Hunter *et al.*, 2006). ERT was linear for all subjects for at least one series at both PRE and POST, thus permitting VAc to be determined in all subjects. VAc was assessed with the equation: $[1 - (SIT \cdot ERT^{-1})] \times 100$. The reliability of this method has recently been validated in the knee extensors (Goodall *et al.*, 2009; Sidhu *et al.*, 2009a). The

duration of the CSP was determined visually and defined as the duration from the stimulus to the return of continuous voluntary EMG (Sidhu *et al.*, 2009b).

Statistics

Statistical analyses were performed with Statistica (version 8, Tulsa, USA). The Shapiro-Wilk test was used to verify data normality. Paired T-tests were used to evaluate differences between PRE and POST. The relationships between percentage change (Δ) PRE-POST in selected central and peripheral parameters were determined by Pearson product correlation. Statistical significance was set at $P < 0.05$. All data are presented as mean ± standard deviation.

RESULTS

Performance

Subjects completed the 110-km ultra-trail in a mean time of 20:13:03 ± 3:22:34 (range: 13:49:31 - 25:49:23), equivalent to 192 ± 32% of the overall winning time (range: 131 - 245%).

Maximal voluntary torque and evoked responses

Two subjects with very large central deficits were outliers and excluded from MVC and VA analyses only. There was a significant 34% decrease in MVC post-race (**Figure 28**). Peripheral potentiated twitch and doublet (100 and 10 Hz) amplitudes decreased significantly by 11, 10 and 14%, respectively (**Figure 28**). There was also a tendency for Db10/Db100 to decrease from PRE to POST although it did not reach the level of significance (**Figure 28**, $P = 0.096$).

M-waves and RMS

M-wave amplitudes were unchanged although there was a tendency for both Mmax and Msup to be smaller POST (**Table 10**). Peak-to-peak M-wave duration was also unchanged although there was a trend for Msup to be longer POST (**Table 10**). RMS_{MVC}, both raw and normalized, significantly decreased from PRE to POST (**Table 10**).

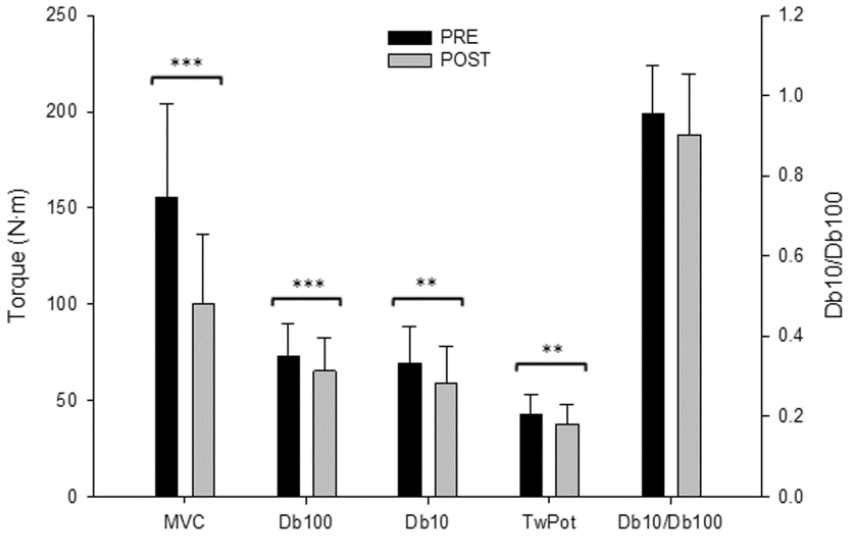

Figure 28. MVC and electrically-evoked mechanical responses PRE and POST ultra-trail. Values are presented as mean ± standard deviation. Significant difference PRE-POST, ** $P < 0.01$ and *** $P < 0.001$.

Table 10. *Vastus lateralis* M-wave amplitude and duration and RMS

	PRE	POST	*P*-value
Mmax amplitude (mV)	13.2 ± 3.8	12.4 ± 4.0	0.09
Msup amplitude (mV)	13.0 ± 3.6	12.0 ± 4.3	0.10
Mmax peak-to-peak duration (ms)	9.6 ± 2.2	9.5 ± 2.4	0.45
Msup peak-to-peak duration (ms)	6.8 ± 1.5	7.2 ± 1.4	0.06
RMS_{MVC} (mV)	0.63 ± 0.31	0.36 ± 0.15	< 0.001
$RMS_{MVC} \cdot Mmax^{-1}$	0.047 ± 0.014	0.030 ± 0.009	< 0.001
$RMS_{MVC} \cdot Msup^{-1}$	0.048 ± 0.016	0.034 ± 0.021	0.009

Values are presented as mean ± standard deviation.

Voluntary activation

There was a mean decrease of 16% for VAc (93 ± 7 to 80 ± 11%, $P < 0.001$) and 26% for VAp (91 ± 8 to 72 ± 14%, $P < 0.001$). There was a correlation between ΔVAp and ΔVAc (**Figure 29**). ΔVAc and ΔVAp were correlated with ΔMVC ($r = 0.61$, $P = 0.002$ and $r = 0.79$, $P < 0.001$, respectively). ΔVAc was also correlated with ΔTwPot ($r = 0.53$, $P = 0.008$) and ΔDb10 ($r = 0.45$, $P = 0.028$) and there was a trend for ΔVAc to be correlated with ΔDb100 ($r = 0.37$, $P = 0.074$).

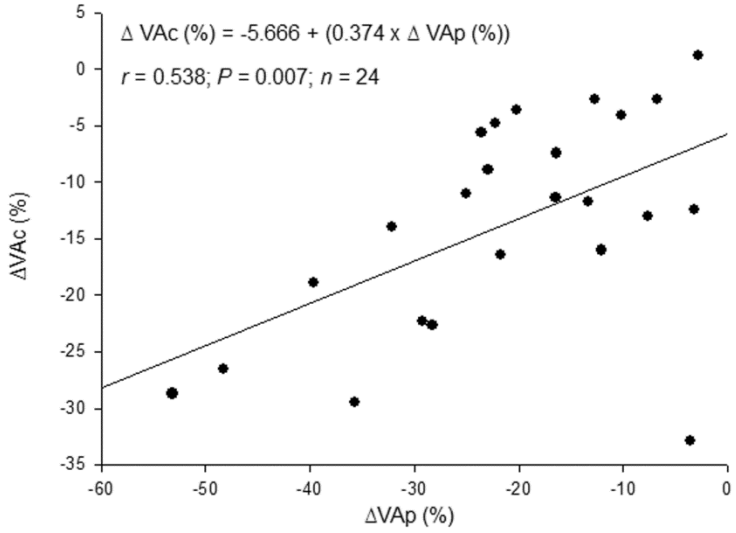

Figure 29. Correlation between the change in ΔVAc and ΔVAp.

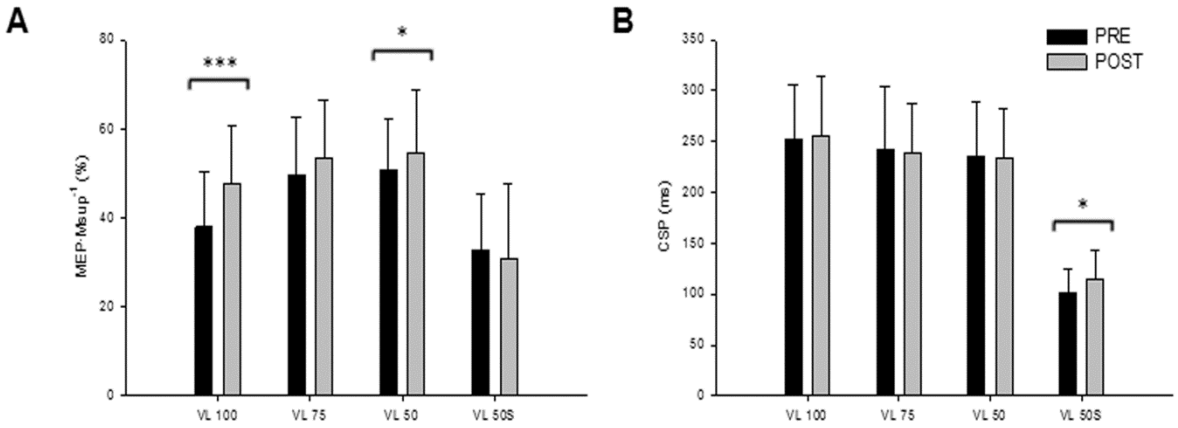

Figure 30. *Vastus lateralis* (Panel **A**) MEP amplitude normalized to Msup and (Panel **B**) CSP elicited by optimal-intensity TMS during contractions at 50, 75 and 100% MVC and by sub-optimal TMS intensity at 50% MVC (50S) PRE and POST ultra-trail. Values are presented as mean ± standard deviation. Significant difference PRE-POST, * $P < 0.05$ and *** $P < 0.001$.

Motor-evoked potentials

At 50 and 100% MVC, MEP·Msup^{-1} increased significantly from PRE to POST (**Figure 30A**) and there was a tendency for MEP·Msup^{-1} at 75% MVC to increase ($P = 0.099$). Conversely, the amplitude of MEPs elicited at sub-optimal stimulus intensity was unchanged ($P = 0.59$).

Cortical silent period

There were no changes in CSP during contractions at 50, 75 or 100% MVC elicited by optimal stimulus intensity (**Figure 30B**, $P > 0.05$). Conversely, CSPs at sub-optimal stimulus intensity increased from PRE to POST (**Figure 30B**). At sub-optimal TMS intensity, no CSP was elicited in at least one contraction in 20% of subjects PRE and in 5% of subjects POST.

DISCUSSION

Central fatigue has been reported to be the main cause of knee extensor strength loss after prolonged running. The primary aim of the present study was to determine whether at least part of this central fatigue was supraspinal. The main results are that after a 110-km ultra-trail (i) there were significant and correlated decreases in cortical and peripheral VA, (ii) there were correlations between peripheral changes and decreases in VA, suggesting that the large central deficits consistently observed after extreme duration running exercise have both central and peripheral origins and (iii) there was increased corticospinal excitability (as indicated by greater MEP amplitude) and no change in intracortical inhibition (as indicated by unchanged CSP) at optimal TMS intensity. There was also increased CSP duration and unchanged MEP amplitude at sub-optimal TMS intensity. These suggest a shift in the MEP-stimulus-intensity relationship towards larger MEP amplitudes only at higher TMS intensities and a left-shift in the CSP-stimulus-intensity relationship.

Maximal torque and PNS measures: comparison with the literature

Only a couple studies (Martin *et al.*, 2010; Millet *et al.*, 2011c; Saugy *et al.*, 2013) have examined long-distance running comparable to that of the ultra-trail in the present study. Despite being shorter than these studies, mean MVC decrease was similar (Martin *et al.*, 2010; Millet *et al.*, 2011c) or greater (Saugy *et al.*, 2013), a finding compatible with the existence of a plateau in the strength loss-exercise duration relationship (Millet, 2011). In this study, ΔVAp was comparable to that reported after a 24-h treadmill run (Martin *et al.*, 2010), a 330-km ultra-trail (mean time ~122.5 h) with 24 000 m of elevation change (Saugy *et al.*, 2013) and a 166-km ultra-trail (mean time ~37.5 h) with 9500 m of elevation change (Millet *et al.*, 2011c). The latter study reported decreased *vastus lateralis* M-wave amplitude and increased *vastus lateralis* M-wave duration. A similar tendency for Mmax and Msup amplitude and Msup duration was observed in the present study. Similar to other long-distance running studies (Millet *et al.*, 2003a; Place *et al.*, 2004; Martin *et al.*, 2010; Saugy *et*

al., 2013), low-frequency fatigue was not observed in this study although there was a trend towards a reduction in the ratio Db10 to Db100 in accordance with Millet *et al.* (2011c). This suggests that at low intensities, extremely long-duration eccentric exercise is required to trigger low-frequency fatigue that is generally observed in much shorter distance and higher intensity (combination of speed and negative slope) downhill running. Finally, as with longer treadmill and ultra-trail runs (Martin *et al.*, 2010; Millet *et al.*, 2011c; Saugy *et al.*, 2013), potentiated twitch amplitude decreased, although to a lesser extent. With shorter trail (mean time < 9 h) and treadmill runs (mean time < 3.5 h), a change in twitch amplitude PRE to POST was not observed (Millet *et al.*, 2002; Millet *et al.*, 2003a; Ross *et al.*, 2007; Easthope *et al.*, 2010; Ross *et al.*, 2010a) suggesting an effect of distance and/or duration on twitch amplitude. The present results confirm previously published consequences of extreme running exercise on both global and peripheral fatigue.

Centrally- and peripherally-assessed voluntary activation

This study was the first to measure VAc of the knee extensors with extreme fatigue induced by long-distance running. Previously, Ross *et al.* (2007) observed decreased dorsiflexor VAc after a 42.2-km treadmill marathon but dorsiflexors are not considered limiting to trail running performance (Fourchet *et al.*, 2012). All other investigations of knee extensor VAc have been conducted with cycling and all showed decreased VAc after exercise (Sidhu *et al.*, 2009b; Goodall *et al.*, 2012; Temesi *et al.*, 2013) with the exception of Klass *et al.* (2012) where no decrease in VAc was observed 10 min after ~1.5 h of cycling.

In the present study, despite greater ΔVAp than ΔVAc (-26% versus -16%), these changes were well-correlated indicating that supraspinal fatigue plays an important role in the decrease of VAp. The correlation between ΔVAp in knee extensors and plantar flexors previously observed in an ultra-trail (Millet *et al.*, 2011c) suggests there is a common regulatory component independent of peripheral factors. Conversely, the significant correlations between central (ΔVA) and peripheral factors (ΔTwPot and ΔDb) in the present study suggest that afferent fibers are involved in central fatigue observed after an ultra-trail. This idea has previously been proposed for shorter endurance cycling exercise (Amann, 2011). Previous research suggests that neither acidosis nor potassium are major factors in ultra-endurance activities (Millet *et al.*, 2011c), indicating that group III and IV afferents likely respond to mechanical stimuli (*i.e.* stress and pressure) (Legramante *et al.*, 2000; Ge & Khalsa, 2003) and inflammatory processes (Hoheisel *et al.*, 2005; Schomburg *et al.*, 2012) in this type of event. After extreme endurance activities, inflammatory markers remain elevated

up to five days after the cessation of the exercise bout (Neubauer *et al.*, 2008; Millet *et al.*, 2011c), well beyond the 1 h delay to POST testing in the present study. The specific role of peripheral muscle afferents and direct spinal and supraspinal mechanisms remains to be determined.

Motor-evoked potentials and cortical silent periods

The TMS contraction series required maximal (100% MVC) or submaximal (50 and 75% MVC) isometric contractions. Increased MEP amplitude and unchanged CSP at optimal TMS intensity after the ultra-trail could indicate a transient increase in cortical excitability without change in inhibition that translates into a more effective corticospinal response to a given stimulus. The TMS intensities employed in isometric voluntary contraction evaluation, however, contrast dramatically with the realities of an ultra-trail and optimal TMS intensity likely exceeds normal motor command both during an ultra-trail and MVC.

Previous studies from other laboratories have not shown any change in MEP amplitude/area or CSP duration after cycling (intensity: 55-80% maximal power output; mean duration: 4-94 min) (Sidhu *et al.*, 2009b; Goodall *et al.*, 2012; Klass *et al.*, 2012) when investigated in a similar manner. These results contrast those observed in the present study. Two factors, exercise duration and TMS intensity, may contribute to these differences. Another study from our group observed MEP amplitude in the *vastus lateralis* and *vastus medialis* increase during 40 min of cycling at 65% maximal aerobic power output followed by an incremental cycling test to task failure (Temesi *et al.*, 2013). MEP amplitude also increased at optimal TMS intensity in the present study, after a more extreme activity in terms of duration. Together, these studies suggest that duration of effort and the associated consequences (*e.g.* hydration, glycaemia, pain and also sleep deprivation for extreme-duration exercise) play an important role in transient changes toward higher corticospinal excitability when tested at optimal stimulus intensity (67 ± 9% maximal stimulator output). The concurrent use of optimal and sub-optimal TMS intensities to evaluate MEP and CSP changes is novel, and our results suggest that selection of an appropriate TMS intensity is essential. Both Klass *et al.* (2012) and Sidhu *et al.* (2009b) performed TMS at intensities comparable to sub-optimal TMS intensities in the present study (*i.e.* 30-60% maximal stimulator output versus 40 ± 5% maximal stimulator output in the present study). At sub-optimal TMS intensity, the unchanged MEP amplitudes in the present study mirrored the aforementioned findings of these studies (Sidhu *et al.*, 2009b; Klass *et al.*, 2012). Furthermore, Sidhu *et al.* (2012a) found no change in MEP amplitude normalized to Msup at similar TMS intensities

(*i.e.* 41.4 ± 0.9% maximal stimulator output). This study is particularly activity-specific since this is the only one examining MEP changes during cycling. Unlike the observed increase in CSP at sub-optimal TMS intensity in the present study, these studies (Sidhu *et al.*, 2009b; Klass *et al.*, 2012) did not observe a change in CSP despite similar mean CSP durations and TMS intensities. This supports the proposal that corticospinal changes are related to exercise duration. The finding that increased corticospinal inhibition occurs during exercise at sub-optimal TMS intensity has previously been observed during cycling using a different method of evaluation (Sidhu *et al.*, 2013b).

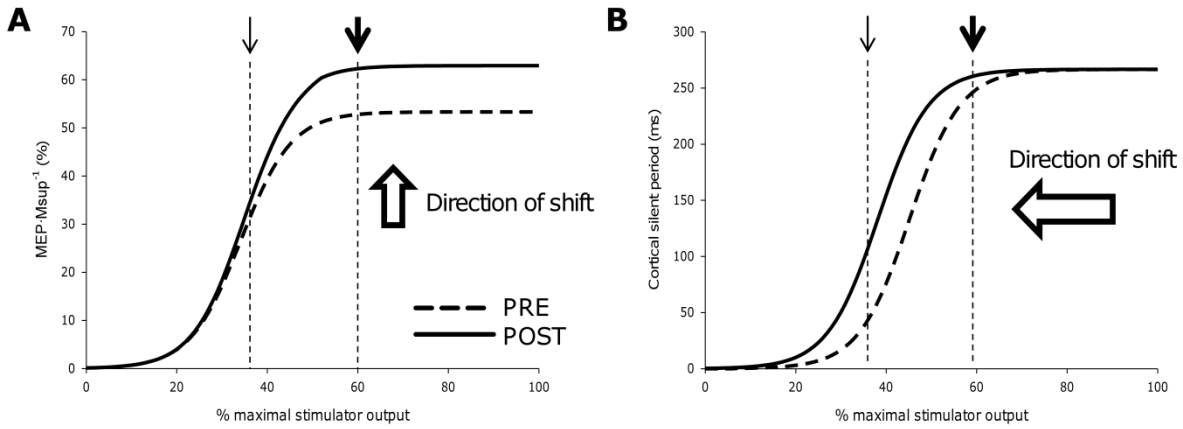

Figure 31. Proposed theoretical changes to the sigmoidal MEP and CSP stimulus-response curves from PRE to POST ultra-trail. Optimal and sub-optimal TMS intensities of 60 (thick arrow and dotted line) and 36% (thin arrow and dotted line), respectively, are used as examples. Optimal and sub-optimal stimulus intensities are based upon PRE evaluation for both PRE and POST. (Panel **A**) The large vertical arrow indicates the proposed shift at moderate to high TMS intensities in the MEP-stimulus-intensity relationship. There is also no change in inflection point of the relationship. (Panel **B**) The large horizontal arrow illustrates the proposed left-shift in CSP-stimulus intensity relationship.

Isometric voluntary contractions at 50% MVC with sub-optimal intensity TMS would probably be the contractions most physiologically representative of regulatory muscle control during an ultra-trail. Unlike the unchanged CSP and increased MEP amplitude at optimal TMS intensity, CSPs induced by TMS at sub-optimal intensity increased in duration while MEP amplitude remained unchanged. These results can be placed within the framework of the previously demonstrated sigmoidal MEP- (Devanne *et al.*, 1997; Duclay *et al.*, 2011) and CSP- (Kimiskidis *et al.*, 2005; Duclay *et al.*, 2011) stimulus-intensity relationships. In the present study, optimal TMS intensity was determined as the lowest stimulus intensity eliciting a maximal MEP response (*i.e.* the intensity at the start of the plateau in a MEP-stimulus-

intensity curve, **Figure 31A**). The observed changes in MEP amplitude and CSP duration suggest that both MEP and CSP stimulus-response curves underwent transformations and/or shifts but that these changes were different (**Figure 31**). It is proposed that after ultra-endurance running exercise, there is a shift of the MEP stimulus-response curve towards greater MEP amplitudes at higher TMS intensities without change in the lower inflection point (**Figure 31A**). Concurrently, it is proposed that there is a left-shift of the CSP stimulus-response curve (**Figure 31B**). This suggests that an ultra-trail may cause a decrease in the threshold to induce CSPs at a TMS intensity (sub-optimal) that may be physiologically representative of cortical drive during an endurance exercise. There are many demands on the brain during an ultra-trail (*e.g.* regulation of vital physiological systems, prevention of injury and long-term physical harm, comparison of perceived exertion to existing pacing templates). The combination of increased intracortical inhibition without a compensatory increase in corticospinal excitability and decreased voluntary activation may be a regulatory safety mechanism to prevent physical harm.

Limitations

Subjects were tested as soon as possible after they completed the ultra-trail competition. Despite efforts to conduct POST measures in a timely manner, there was a large delay and some variability in the time between race completion and the start of testing because of the distance from the finish to the testing site and the necessity of ensuring the safety of the subjects. The same coil position and TMS stimulus intensity were used at PRE and POST. This was done for a number of reasons: (i) given the physical state of the subjects at POST, including several that were unable to complete the three series of contractions with TMS, reassessment of TMS intensity prior to POST assessment would probably have reduced the number of subjects and impacted data analysis and (ii) to be able to compare CSP and MEP since changes to stimulus intensity influence both these parameters. Optimal stimulus intensity was similar PRE and POST. Due to the delay in conducting POST, MEP and CSP changes may be underestimated or masked. Previous studies have shown MEPs and CSPs to recover rapidly after isometric single-joint exercise (*e.g.* (Taylor *et al.*, 1996; Taylor *et al.*, 2000)). Due to the nature of the ultra-trail, it was impossible to test subjects at the same time of day. Nevertheless, this was unlikely to influence results as corticospinal and intracortical excitability have been shown to be unaffected by time of day (Doeltgen & Ridding, 2010). Finally, antagonist (*e.g. biceps femoris*) EMG was not measured due to time constraints.

Changes in the relative contribution of agonists and antagonists may affect measures such as VA.

Conclusion

This study was the first to combine TMS and extreme endurance to investigate the physiological consequences of an extreme duration exercise on central changes at the supraspinal level. The hypotheses that an ultra-trail decreases VAc and that corticospinal fatigue occurs with a concomitant increase in MEP amplitude and unchanged CSP duration were confirmed. However, CSP induced by sub-optimal TMS intensity increased without concurrent change in MEP amplitude at this intensity. This suggests a left-shift in the CSP-stimulus-intensity relationship and a shift in the MEP-stimulus-intensity relationship towards larger MEPs at higher TMS intensities without change in the inflection point of the curve. These changes may contribute to performance limitations during the ultra-trail. Peripheral changes were also observed and correlated with decreases in cortical and peripheral voluntary activation, supporting the proposed contribution of group III and IV afferents to central deficits during endurance exercise.

GENERAL DISCUSSION AND PERSPECTIVES

DISCUSSION

This thesis is comprised of two main parts, one methodological (Studies 1 and 2) and one applied to extreme exercise (Studies 3 and 4). The first two studies in this thesis indicate that the method in which TMS is employed is extremely important and that methodological differences may be a source of discrepancies between studies. Together Studies 1 and 2 contribute to the development of evaluation methods that can offer reliable results and permit the investigation of the desired TMS parameters.

The primary result from Study 1 is that a stable contraction force level is essential before the delivery of a TMS pulse. As such, all subsequent studies forming part of this thesis ensured that TMS was delivered after the force had stabilized at the target force level. This study further suggests that because differential responses in the approach to a target force only occurred during weak contractions, transient changes in corticospinal excitability may have influenced the elicited responses. As contraction intensity increases from 0% MVC (*i.e*. rest), corticospinal excitability increases rapidly (Ugawa *et al.*, 1995; Taylor *et al.*, 1997). Contraction intensity corresponding to maximal corticospinal excitability varies by muscle according to their unique spatial recruitment patterns, plateauing around 5% MVC in *adductor pollicis* and 50% MVC in *biceps brachii* and *brachioradialis* (Taylor *et al.*, 1997). This is characterized by *adductor pollicis* recruiting almost all motoneurons at a contraction intensity <30% MVC while in *biceps brachii*, approximately 20% of motoneurons are recruited at intensities >50% MVC (Kukulka & Clamann, 1981). Given that both Studies 3 and 4 employed a stimulus-response curve at 20% MVC to determine TMS intensity, increasing or decreasing to the target force may have caused either over- or under-estimation of the intensity to elicit maximal MEP amplitude, and would thus have influenced the selected TMS intensity. The results of Study 1 are also imperative for all other published studies that have delivered TMS during voluntary contractions, particularly at low contraction intensities.

The main result from Study 2 is that the use of commonly employed methods of determining TMS intensity (*e.g*. stimulus-response curves or a percentage of AMT or RMT) results in the selection of different stimulus intensities. This presents difficulties when comparing the results of studies (see **Table 1** for a summary of methods in major lower-limb TMS studies) and determining possible reasons for incoherent findings. It is recognized that

standardizing TMS methodology is challenging because different research and clinical fields are interested in different parameters. This thesis is focused on the use of TMS to evaluate the effects of central fatigue, particularly its supraspinal component, and central perturbations with endurance and ultra-endurance exercise. By its very definition, fatigue is related to what happens during physical activity; thus, connections between the brain and muscle at rest are not of primary importance. The connections that exist, their ability to be recruited during muscular contraction and any changes that may influence exercise performance, on the other hand, are of utmost importance. Similarly, the muscle chosen for investigation is vital when determining TMS methodology. In this thesis, all studies have investigated the *quadriceps femoris* due to its functional importance in locomotor activities and daily life. Many initial TMS studies, however, employed upper-limb muscles because of the ease with which these muscles could be stimulated and the clarity of the responses.

Many other fundamental methodological aspects of TMS in the evaluation of fatigue remain to be elucidated including stimulator/coil differences and the best method of determining of optimal coil position. First, most magnetic stimulators used in research are Magstim stimulators, theoretically making comparisons between studies easier. However, there are Magstim models that deliver monophasic pulses while others deliver biphasic pulses. As laboratories strive to economize on tight budgets, laboratories may opt to purchase a one-size-fits-all stimulator and it is unknown how this might affect research results (see Literature Review for details on stimulator differences). Another important methodological issue is the determination of optimal coil position. This was not evaluated for this thesis despite being extensively considered. It was decided that a grid pattern would be employed and the response from a single stimulus at each point during a voluntary contraction at 10% MVC would be sufficient to determine the optimal coil position. It is unknown whether identical or similar coil positions elicit maximal responses in both the relaxed and contracted muscular states, and subsequently how this might influence the selection of optimal coil position. Preliminary investigations in our laboratory suggest that for some subjects there can be a large difference in optimal coil position between relaxed and contracting muscular states. Furthermore, whether it is appropriate to use one response at each site to select the position with the greatest response, especially in the relaxed muscle given the increased response variability when the target muscle is relaxed (Kiers *et al.*, 1993), is unknown. It is also unknown if an optimal coil position exists. Whether homogeneous changes to motor cortical excitability occur as contraction intensity increases is an important factor to consider. While it would be straightforward if the presence of a MEP is sufficient to select a coil position, it neglects to

account for possible effects this might have on initial and subsequent measures in the evaluation of fatigue. For example, the selected coil position likely influences the stimulus intensity (*i.e.* increased TMS intensity the further the coil is from the relevant motor cortical area, or optimal position) and subsequently the elicited measures (*i.e.* CSP is largely stimulus-intensity dependent independent of other factors (Saisanen *et al.*, 2008)) or the selection of sub-optimal TMS intensities if these are to be employed.

Choosing an appropriate TMS intensity is essential and the results of the two applied studies (Studies 3 and 4) illustrate this point. In both studies, two TMS intensities were employed to investigate changes in MEP amplitude and in Study 4 two intensities for CSP duration. The rationale for employing a second, lower, stimulus intensity is that real changes in corticospinal excitability or inhibition may potentially be overlooked at optimal stimulus intensity. The sigmoidal stimulus-response relationship for both CSPs and MEPs is well-established (Devanne *et al.*, 1997; Kimiskidis *et al.*, 2005; Duclay *et al.*, 2011). When only an optimal intensity corresponding to a maximal response on a stimulus-response curve is selected, identification of real changes to the curve may be impeded due to the absence of an adequate number of data points or a single inappropriate data point. In Study 4 there were contrasting results for MEP amplitude and CSP duration at the two selected stimulus intensities. At optimal TMS intensity, MEP amplitude increased while CSP duration was unchanged and at sub-optimal TMS intensity MEP amplitude remained unchanged while CSP duration increased. In conjunction with the lack of pre-post ultra-trail change in optimal TMS intensity, this suggests that there was a left-shift of the CSP stimulus-response curve and a shift to greater MEP amplitudes at higher stimulus intensities only. Conversely, MEP amplitudes in Study 3 at both selected TMS intensities demonstrated the same changes (*i.e.* during voluntary contractions at 50% MVC, *vastus lateralis* MEP amplitude increased and both *rectus femoris* and *vastus medialis* MEP amplitudes remained unchanged). This may indicate a shift to greater MEP amplitudes at all stimulus intensities in *vastus lateralis* without any change in the *rectus femoris* or *vastus medialis* stimulus-response curves at 50% MVC. Interestingly, the increased MEP amplitude in *vastus medialis* post-exercise in Study 3 may be driven by greater contraction intensity and not TMS intensity since it was only observed at optimal TMS intensity in MEP·Mmax^{-1} at 100% and 75% MVC and MEP·Msup^{-1} at 100% MVC. This is the only study that we are aware of that has shown any type of intervention (*e.g.* exercise (Goodall *et al.*, 2012), hypoxia (Goodall *et al.*, 2012; Rupp *et al.*, 2012), passive hyperthermia (Ross *et al.*, 2012)) to result in differential changes in corticospinal excitability by contraction intensity; however, very few studies have reported changes in MEP amplitude

or area at more than one contraction intensity. Whether changes in corticospinal excitability are linked to contraction intensity remains to be determined. Although there are no scientific studies supporting this suggestion, this may potentially allow individuals to transiently perform at a very high intensity when sufficiently motivated despite the deleterious effects of fatigue. Such situations may include athlete being able to sprint the last part of a competition such as an ultra-trail or military personnel rescuing a colleague from a dangerous situation in a war zone despite having been in intense combat for many hours.

The results of Study 3 also raise the question of differences in the development of fatigue between the three *quadriceps femoris* muscles. Although Study 2 did not observe a difference in optimal stimulus intensity determined from *vastus lateralis, rectus femoris* or *vastus medialis*, changes in corticospinal excitability in Study 3, as assessed by MEP amplitude, differed between the three investigated quadriceps muscles. It appears that there was a spectrum from no change in *rectus femoris* to consistently increased corticospinal excitability in *vastus lateralis* with *vastus medialis* in between these two extremes. This may partially reflect that the *rectus femoris* is biarticular and both the *vastus lateralis* and *vastus medialis* monoarticular or it may represent differences in corticospinal projections to the individual quadriceps muscles. Although the regional differences in fatigability previously observed in the *rectus femoris* (Watanabe *et al.*, 2013) are peripheral, these results indicate that the proposed hard-wired difference remains a possibility. It also demonstrates there is much to be learnt about how fatigue manifests in the quadriceps.

In fatigue evaluation, VA is recognized as the gold standard to identify a central deficit. Thus, to determine the presence and development of supraspinal deficits, the evaluation of VAc is required. Determination of VAp by electrical stimulation employs a stimulus intensity guaranteed to elicit the largest possible evoked response (*e.g.* SIT and potentiated twitch), thus supramaximal stimuli are delivered to account for any changes in the intensity to elicit a maximal response. A TMS intensity that elicits maximal MEP amplitude without a large increased in TMS-induced antagonist response is therefore believed to be essential. The potential influence of a moderate or large TMS-induced antagonist response on VAc evaluation has yet to be systematically investigated. It remains to be determined whether potential antagonist coactivation would be a greater problem than delivering TMS at an intensity that only elicits near-maximal responses. Questions pertaining to the selection of stimulus intensity in other TMS-induced parameters also exist. Given the differential MEP and CSP responses by TMS intensity in Study 4, it remains to be determined whether the lowest stimulus intensity to elicit MEPs of maximal amplitude is ideal for investigating other

central parameters, particularly in the context of fatigue. It is also unknown if VAc must be determined at an intensity to elicit maximal responses. The effect of a sub-optimal TMS intensity on SIT at three different contraction intensities for the determination of estimated resting twitch and the subsequent VAc calculation are unknown.

A previous study (McNeil *et al.*, 2011a) observed differential responses to both strong and weak TMS and cervicomedullary junction stimuli during a 10-min weak iso-EMG voluntary contraction of the elbow flexors. While weak single-pulse stimuli elicited similar MEP and CMEP changes (*i.e*. decreased amplitude), there were differential responses to a strong stimulus intensity (*i.e*. decreased CMEP amplitude and unchanged MEP amplitude). The authors suggest that both weak TMS and cervicomedullary junction stimuli and strong cervicomedullary junction stimuli were unable to overcome a reduction in spinal excitability. The unchanged MEP amplitude to strong TMS may indicate a capacity for cortical facilitation or that the TMS intensity was initially supramaximal and remained supramaximal, although to a lesser extent, throughout the protocol. The latter possibility may have occurred in at least some of the subjects since the strong TMS pulses were delivered at 155.8 ± 43.0% RMT. Study 2 demonstrated that TMS intensities to elicit maximal MEP responses in the quadriceps at contraction intensities of 10, 20 and 50% MVC are similar to or lower than at 120 or 130% RMT. McNeil *et al.* (2011a) also reported that conditioned MEP and CMEP areas elicited by strong stimuli decreased with time although to a lesser extent than the area of MEPs and CMEPs elicited by weak stimuli. They proposed that this difference related to the composition of the motoneuronal pool activated during the submaximal contraction. Predominantly smaller motoneurons are active during the protocol and these motoneurons respond most readily to weak TMS and cervicomedullary junction stimuli. It also becomes increasingly difficult to excite these smaller motoneurons as fatigue develops. This resulted in the large decrease in MEP and CMEP areas in response to weak stimuli. Meanwhile, the larger motoneurons that only respond to strong stimuli appear to be essentially unaffected as demonstrated by the smaller decrease in MEPs and CMEPs elicited by strong stimuli, although this may also represent intrinsic motoneuronal changes. These results underscore the importance of stimulus intensity selection by presenting results that on first glance may appear inconsistent. Moreover, they emphasize the need to further investigate the effect of fatigue on TMS delivered at different intensities.

The few studies that have investigated changes to TMS parameters with exercise at different TMS intensities have sometimes (McNeil *et al*., 2001; Study 4) but not always (McNeil *et al*., 2011; Study 3) observed TMS intensity-dependent differences in responses.

The dearth of research in this area does not permit conclusions to be drawn with any degree of certainty. For example, McNeil *et al.* (2011a) proposed that the differential changes to single-pulse stimuli may be related to the prevalence of small motoneuron activity during their protocol and the predominance for these same motoneurons to be activated by weak TMS. The former situation is unlikely in Study 4 since an ultra-trail might be expected to activate at least a significant portion, if not all, of the motoneuron pool. Over the course of a sustained (Garland *et al.*, 1994) or series of intermittent (Carpentier *et al.*, 2001) isometric fatiguing contractions, motoneuronal derecruitment has been observed, particularly among motoneurons with a high activation threshold (Carpentier *et al.*, 2001). Furthermore, the activation threshold in high-threshold motoneurons was observed to decrease over the course of a series of fatiguing isometric contractions at 50% MVC (Carpentier *et al.*, 2001). Despite the relatively low exercise intensity, the extreme distance (110 km) and duration (13:49:31 - 25:49:23) and large decrease in VAc suggest that most or all motoneurons played an important role in race completion. Meanwhile, the contrasting results in Studies 3 and 4 may be associated with differences in distance, duration and/or exercise type (*i.e.* running versus cycling). While stimulus-response curves have been conducted in both the relaxed and contracting muscle, the effects of any possible changes to these curves have not been investigated with fatigue. As the results of Study 4 and McNeil *et al.* (2011a) suggest, especially when contrasted with the results of Study 3, changes to these curves may help explain some performance decrements. In lieu of the time-consuming nature of evaluating stimulus response curves and the rapid recovery of TMS parameters in many studies, utilization of multiple TMS intensities may be crucial to better understanding the supraspinal drive to the muscles and how this may impact fatigue.

The finding of decreased VA (VAp and VAc in Study 4 and VAp and a trend for VAc in Study 3) with endurance and ultra-endurance exercise is consistent with most related studies. The studies in the present thesis are the first to report increased MEP amplitude in any muscle after a dynamic whole-body exercise bout, with the exception of Fernandez-del-Olmo *et al.* (2013), who compared pre-post Wingate MEP changes at the same absolute, not relative, force levels. The previously discussed results of Study 3 further suggest that changes in cortical excitability are muscle dependent. The CSP results from both Studies 3 and 4 are also novel. In previous dynamic whole-body exercise, CSP was unchanged in the *vastus lateralis* (Goodall *et al.*, 2012; Fernandez-del-Olmo *et al.*, 2013; Girard *et al.*, 2013), *rectus femoris* (Sidhu *et al.*, 2009b; Klass *et al.*, 2012) and *vastus medialis* (Klass *et al.*, 2012) after cycling and also in the *tibialis anterior* after a treadmill marathon run (Ross *et al.*, 2007). In

Study 4, CSP elicited by sub-optimal TMS intensity increased in duration during the ultra-trail yet remained unchanged at optimal TMS intensity. While fatiguing isometric contraction protocols in both upper and lower limbs report increasing CSP duration, this has not been previously observed in locomotor exercise. The fact that shorter duration cycling studies performed at moderate to high intensities with TMS intensities comparable to the sub-optimal TMS intensity employed in this study reported unchanged CSP (Sidhu *et al.*, 2009b; Klass *et al.*, 2012) supports the idea that exercise duration and/or intensity are factors influencing CSP. Study 3, meanwhile, is the only published study reporting decreased CSP duration after an acute exercise bout. While total exercise duration in Study 3 was comparable to others (Sidhu *et al.*, 2009b; Klass *et al.*, 2012), the combination of exercise duration and exercise intensity was different. Furthermore, the TMS intensity in Study 3 was greater than that employed by either Klass *et al.* (2012) or Sidhu *et al.* (2009b). The combination of these factors may have contributed to this intriguing finding.

Another important factor to consider when comparing and interpreting the reported CSP and MEP changes, or lack thereof, is the delay between exercise cessation (whether due to task failure or protocol design) and post-intervention evaluation. Cortical silent period recovery is extremely rapid with significant recovery occurring within as few as 5 s and complete recovery within as little as 15 s during intermittent isometric maximal contractions over various durations and duty cycles (total protocol time from 3.5-7.5 min) (Taylor *et al.*, 2000). Similarly, MEP recovery has been shown to recover within the first ~30 s after exercise (Taylor *et al.*, 1999; Taylor *et al.*, 2000; Sogaard *et al.*, 2006). There inevitably must be a delay between exercise cessation and isometric evaluation in whole-body dynamic exercise protocols due to the necessity of installing the subject on an ergometer. In Study 4, there was a delay to post-exercise measurements of 1:01:30 ± 0:22:37. Thus, there is great confidence that the reported increase in CSP at sub-optimal TMS intensity and the reported increase in MEP amplitude at optimal TMS intensity are real changes. The lack of change to MEP amplitude at sub-optimal TMS intensity and CSP at optimal TMS intensity may be truly representative of the effects of an ultra-trail, or these findings may be the result of transient effects that recover more rapidly than the observed changes. Regardless, Study 4 suggests that the duration, and possibly the intensity, of the exercise bout have a role to play in the post-exercise duration of TMS-induced effects.

It is important to link these findings to real-world activities. For example, does the lack of MEP amplitude change at optimal TMS intensity have real implications to performance? The question of what experimental conditions are most representative of those

during exercise, especially endurance and ultra-endurance exercise is important. Both steady-state and time-trial protocols have a role in expanding the understanding of central perturbations and fatigue. Study 3, while not entirely steady-state, followed a strict protocol. This avoided pacing strategies as a confounding factor. A real-world time-trial such as the North Face® Ultra-Trail du Mont Blanc® in Study 4 may be more realistic because it includes the management of pacing and feeding strategies, the possibility of changing meteorological conditions, the presence and actions of other competitors, the control of cortical drive to exercising muscles and the regulation of other tasks, all of which influence perception of task effort and eventually performance (Millet, 2011). Thus, the use of an optimal TMS intensity as denoted by Groppa *et al.* (2012) (*i.e*. the transition from the rising slope to the flat portion of the sigmoid stimulus-response curve) may not be the most physiologically relevant to the real-world. Instead the changes observed at a sub-optimal TMS intensity in Study 4 (*i.e*. increased CSP and unchanged MEP) may be may be more indicative of changes contributing to the observed supraspinal fatigue since supraspinal drive to the muscles during a race may be limited by the complex regulatory demands of the body.

Initially, it appears reasonable to observe decreased VAc in the presence of unchanged corticospinal excitability and increased intracortical inhibition, as occurred in Study 4 in response to sub-optimal TMS intensity. A logical continuation is that these may contribute to decreased endurance performance. Meanwhile, the increased cortical excitability and unchanged intracortical inhibition at optimal TMS intensity would appear to suggest the possibility of a transient capability to improve or maintain performance since a smaller input to the motor cortex may be needed to produce the same central motor command. This scenario, however, is in opposition to the findings of Gandevia *et al.* (1996), who previously demonstrated that there are independent mechanisms contributing to the supraspinal deficit and decreased MVC and changes to CSPs and MEPs. During a 2-min MVC of the elbow flexors, SIT, MEP area and CSP duration increased while MVC decreased. Thirty seconds after exercise cessation, recovery had started as demonstrated by decreased SIT and increased MVC. The same protocol was repeated with a cuff inflated to maintain ischemia during the first minute post-exercise. During the ischemic period, neither MVC nor SIT recovered; however, CSP and MEP both returned to baseline levels within 30 s. The results of this study indicate that under ischemic conditions, the motor cortex failed to drive corticospinal motoneurons that were of normal excitability and that muscle fatigue was directly responsible for this continued central failure, likely due to input from group III/IV afferents. Thus, the results of Study 4 indicate that the motor cortex failed to drive corticospinal motoneurons that

were of increased excitability when they were stimulated maximally (*i.e.* at optimal TMS intensity). Muscle fatigue, via group III/IV afferents, may be responsible for this failure. In an ultra-trail, participants are unlikely to attempt maximal physical performance at any one moment in order to achieve the best global performance (*i.e*. best finishing time). Given the numerous demands on the body and the low exercise intensity, there does not appear to be a need or desire to maximally drive the motoneurons to the legs. Thus, a sub-optimal TMS intensity may be more representative of the drive to the muscles during an ultra-trail. In this scenario, corticospinal excitability is unchanged while increased intracortical inhibition may indicate a greater difficulty to initiate drive to the muscles. In combination with group III/IV afferent input contributing to central fatigue, it would be expected to observe decreased exercise performance.

In Study 3, increased cortical excitability, as denoted by increased MEP amplitude in *vastus lateralis* and *vastus medialis*, and decreased intracortical inhibition, as denoted by decreased CSP duration in all quadriceps muscles, were both observed. Reaction time was quicker during exercise; however this effect was transient and 15 min after exercise cessation this effect was no longer observed. These results reiterate that in short endurance exercise, exercise can act to facilitate cortical processes (*i.e*. cognitive processes and neuromuscular processes). These results may signify a link between cortical facilitation and cognitive benefits during exercise; however, further investigations need to be conducted to determine if this is the case.

Finally, it can be concluded that that in endurance and ultra-endurance exercise, there is evidence of supraspinal fatigue and changes in both corticospinal excitability and inhibition. The real-world relevance of these findings and the role of TMS intensity in the evaluation of fatigue remain to be elucidated.

PERSPECTIVES

There are many exciting research areas involving TMS, supraspinal fatigue and neuromuscular changes and/or adaptations that remain to be explored and elucidated. While there is tremendous interest in clinical populations and funding opportunities to drive such research, the healthy active human remains an interesting model. This population must have a vital role in the development of sound methodological approaches for evaluating fatigue and corticospinal changes with exercise interventions. Furthermore, it must represent a baseline

for comparison in order that potential deficiencies in clinical populations or a sedentary population can be identified. Booth and Laye (2009) encapsulate this up by stating that "[m]edicine must know what biologically normal physiology is in order to know how to prescribe the most optimal treatments to maintain optimal health of all organ systems." Therefore, a healthy and active brain and body must be the basis for understanding central and supraspinal fatigue and corticospinal changes with physical activity. Within the context of this target population, the following perspectives are of personal interest.

One of the most important factors affecting the interpretation of all results in the investigation of fatigue is the delay between the post-intervention measures and the end of the exercise intervention (*i.e*. task failure, the end of a race or a predetermined protocol). Results of upper-body isometric-contraction protocols show that the predominant measures investigated with TMS (CSP, MEP and SIT, the latter frequently used to determine VAc) recover extremely quickly (within ~30 s) after exercise cessation (Taylor *et al.*, 2000; Sogaard *et al.*, 2006; Szubski *et al.*, 2007). Further research must be conducted into whether recovery kinetics of all TMS parameters is similar and how recovery kinetics might be affected by the duration, intensity and type of activity. Due to the rapid recovery of TMS parameters to, but not below, baseline in isometric contraction protocols, and the lack of exercise-induced effects on MEP and CSP (Sidhu *et al.*, 2009b; Goodall *et al.*, 2012; Klass *et al.*, 2012), no change is interpreted as exactly that when it may reflect an inability to observe a real change. Results from both Studies 3 and 4 suggest that whole-body dynamic exercise causes changes to both corticospinal excitability and inhibition. Furthermore, Study 4 suggests that an ultra-trail has longer-term post-exercise effects than single-joint isometric protocols, regardless of their intensity or duration, as the mean time to the start of post-ultra-trail measures was more than 1 h after the end of the exercise bout. Whether this may be linked to central and/or peripheral changes that potentially arise from factors such as the persistent effects of inflammatory processes after ultra-endurance exercise (see Study 4 discussion) remains to be investigated.

Recently, TMS evaluation during cycling bouts has been employed to evaluate the effects of fatigue (Sidhu *et al.*, 2012a; Sidhu *et al.*, 2013b). This enables the rapid recovery of TMS parameters after exercise cessation to be overcome. It also permits better understanding of fatigue during exercise. In the future, this must be expanded to include activities of daily living and functional importance. The necessity and relevance of progressing beyond single-joint protocols and into the area of locomotion has been discussed by Sidhu *et al.* (2013a) in a recent review. Without question, the major current limitations to locomotor TMS

investigations are methodological. As such few studies to date have investigated TMS-induced parameters during walking (*e.g.* (Schubert *et al.*, 1999; Petersen *et al.*, 2001)) and none of these have examined the effects of fatigue. Furthermore, no study has utilized TMS during running. There are obvious difficulties in employing TMS during locomotor activities, especially walking and running; however, the fundamental nature of these activities to human life implores us to overcome these difficulties to better understand them. Given the very few studies employing TMS during locomotion, EMG suppression has been frequently utilized (Petersen *et al.*, 2001; Sidhu *et al.*, 2013b). Since EMG suppression must be evaluated during exercise, its potential to clarify the role and influence of inhibitory mechanisms during fatigue must be explored. However, a major limit to this method is that it must be subthreshold, and should facilitation develop over the course of the exercise bout, valid comparisons cannot be made. In Sidhu *et al.* (2013b), approximately half of the 16 subjects were excluded from analysis of the effects of cycling and subsequent recovery. The number of excluded subjects ranged from a low of 6 (38%) subjects in the *vastus lateralis* before exercise to a high of 13 (81%) subjects in the *tibialis anterior* during recovery. The high rate of subject exclusion demands attention and poses the question of whether a method that eliminates half of all subjects is viable, or whether it may in fact hide more than it reveals. In our laboratory, we are beginning to discuss the methodological questions that must be overcome to build upon the scant literature of fatigue development as assessed by TMS during locomotor activities, and also how to investigate the functionally important activities that are walking and running.

Just as Study 4 examined the extreme conditions of an ultra-trail, other studies can use extreme conditions as a model to examine neuromuscular and corticospinal changes. The use of one night of SD in Study 3, while unusual is not that uncommon. There are many individuals that chronically function with inadequate sleep or groups such as military personnel that push the limits of sleeplessness to the extreme, and often in high-risk situations. It is unknown whether the extension of SD to two, three or more nights would permit identification of central and neuromuscular deficits. Given the previously outlined importance of the delay between exercise cessation and post-exercise evaluation, if there were small differences in central fatigue or corticospinal changes between SD and control conditions in Study 3, they may have masked. By extending the period of SD, there would probably be a greater chance of identifying differences if there are in fact any. Furthermore, while Study 3 was methodologically sound and well-controlled, one study does not provide definitive answers, even in areas with a paucity of research.

It is also unknown whether the initiation and evolution of either central or supraspinal fatigue and associated parameters are different between men and women. Most studies have employed exclusively male subject groups although the few studies comparing maximal force and fatigue development in males and females have observed several differences. Women have been reported to perform submaximal intermittent or sustained isometric contraction protocols at the same relative intensity for longer duration than men in upper- (Hunter & Enoka, 2001; Hunter *et al.*, 2006; Yoon *et al.*, 2009) and lower- (Clark *et al.*, 2005; Bachasson *et al.*, 2013b) limb muscles. It has also been observed that maximal force decreases less in women during intermittent (Russ & Kent-Braun, 2003; Hunter *et al.*, 2006) and sustained (Martin & Rattey, 2007) MVC protocols and after ≥2 h cycling (Glace *et al.*, 2013) and running (Glace *et al.*, 1998). In Glace *et al.* (1998), the decreased maximal knee extension and flexion strength evaluated at $60°\cdot s^{-1}$ after 2 h running at ventilatory threshold in men was remarkably not present in women. Several studies have suggested that the proportion of fatigue attributable to peripheral and central mechanisms varies with men and women (Russ & Kent-Braun, 2003; Martin & Rattey, 2007; Keller *et al.*, 2011; Glace *et al.*, 2013) with contradictory results. The diversity of protocols employed suggests that factors such as the type of protocol (*e.g.* intermittent versus continuous), exercise (*e.g.* isometric contractions versus dynamic whole-body exercise) and muscles investigated (*e.g.* elbow flexors versus knee extensors) may contribute to the variable results. Glace *et al.* (2013) reported that following a 2-h cycling bout at ventilatory threshold immediately preceding a 3-km time-trial, MVC loss is attributable solely to central mechanisms in women while both central and peripheral mechanisms contribute in men. Whether this difference applies to running and longer running and cycling bouts remains to be determined. The two studies that investigated supraspinal sex differences failed to observe any (Hunter *et al.*, 2006; Keller *et al.*, 2011). Since both studies employed isometric contraction protocols, future investigations must examine whether this is also the case with whole-body exercise. Although initial analysis of the data from Study 4 suggested that there were no differences between men and women for any analysed parameter, further analyses are planned to account for performance differences.

An important and developing area that fell outside the scope of this thesis is the use of TMS to evaluate training adaptations. Previous resistance training studies have shown neural adaptations with TMS in both the upper (Carroll *et al.*, 2002; Jensen *et al.*, 2005) and lower limbs (Beck *et al.*, 2007; Griffin & Cafarelli, 2007). These adaptations have been primarily changes in MEP amplitude at a given contraction intensity. More recent studies have suggested that the interaction between training-induced changes in MEP amplitude and CSP

duration can also be modulated (Kidgell & Pearce, 2010; Kidgell *et al.*, 2010) depending on the type of training performed (*i.e*. maximal isometric contractions versus high-intensity speed-controlled contractions). The influence of strength training on neural adaptation is further supported by a recent study that demonstrated that 3 weeks of arm immobilization (15 $h \cdot day^{-1}$) resulted in decreased MEP amplitude at various stimulus intensities during voluntary contraction of the *biceps brachii* (Pearce *et al.*, 2012). Conversely, MEP amplitude was unchanged in both a control group and a group that underwent arm immobilization in conjunction with thrice weekly heavy-load strength training, indicating that strength training may counteract inactivity and prevent negative neural changes.

Little is known about whether aerobic training causes neural adaptations in healthy subjects. In one of the few studies to examine the effects of aerobic training on neuromuscular parameters, Cafarelli *et al.* (1995) observed $EMG \cdot force^{-1}$ to increase during 20 min of single-leg cycling at 70% VO_{2max} before a training program. After an 8-week single-leg cycling training program, $EMG \cdot force^{-1}$ remained stable throughout the 20-min cycling bout at the same power output (70% pre-training VO_{2max}) despite unchanged pre- to post-training program EMG during brief cycling bouts at various submaximal intensities. The authors concluded that changes in muscle activation occur due to an increased capacity of the muscle to perform prolonged exercise. More recently, Vila-Cha *et al.* (2010) observed that 6 weeks of either endurance or strength training caused increased motor unit conduction velocity during knee extensor contractions at 30% MVC in both conditions. Meanwhile, motor unit discharge rate decreased after endurance training and increased after strength training.

To date there have been no published studies investigating the effects of aerobic/endurance exercise training on TMS-evoked parameters in this population. Several studies have investigated whether neural adaptations occur in populations affected by Parkinson disease and after the occurrence of a stroke (Forrester *et al.*, 2006; Fisher *et al.*, 2008; Yang *et al.*, 2010). These studies have all observed neural changes as demonstrated by changes in TMS parameters. In patients with Parkinson disease, Fisher *et al.* (2008) observed increased maximal CSP duration in the *first dorsal interosseous* without change in the TMS intensity to elicit a CSP of half maximal duration or the slope of a CSP stimulus-response curve after 8 weeks of high- but not low-intensity body-weight-supported treadmill training. This finding is significant because shorter CSPs are consistently associated with increased Parkinson symptom severity (Lefaucheur, 2005) and because this neural adaptation was found in a muscle that was not trained. In stroke patients after 4-week body weight-supported treadmill training program, Yang *et al.* (2010) reported decreased RMT in the *abductor*

hallucis in patients <6 months but not in patients >12 months post-stroke. More interesting is that they also found that both population groups increased the map area (*i.e*. the area where 1 of 4 stimuli at 110% RMT elicited a MEP), indicating cortical plasticity and that this increase was much greater than with a general exercise program. Meanwhile, Forrester *et al.* (2006) only found exercise training-induced changes in TMS parameters in the paretic side in a group of 3 stroke patients. In this case, *vastus medialis* MEP amplitude increased after a single 20-min treadmill walking bout whereas it was unchanged in the non-training group. The type of exercise training programs evaluated in these clinical populations were adapted to the target populations and are very different from the aerobic/endurance training programs in healthy and athletic populations. Further research is required to determine whether an aerobic/endurance exercise training program induces neural adaptions similar to those seen with resistance training programs and also the possible neural adaptations of more commonly-employed mixed training programs (*i.e*. combination of resistance and aerobic training). Any neural adaptations may be dependent upon the type of training program employed, similar to effects of different resistance-only training interventions (Kidgell & Pearce, 2011).

All subjects in Study 4 were trained ultra-endurance runners that had completed a number of qualifying races of prescribed distance and elevation change in order to be eligible to compete at the North Face® Ultra-Trail du Mont-Blanc® 2012. Time to complete the ultra-trail was not correlated with the changes in VAc, CSP or MEP (data not presented) suggesting that differences in performance did not influence changes in TMS-induced parameters. This may also be related to the high level of fitness required of all subjects. Similarly, all subjects in Study 3 were trained, as were subjects in many of the studies evaluating supraspinal fatigue with whole-body exercise (Goodall *et al.*, 2012; Klass *et al.*, 2012; Girard *et al.*, 2013). Only a couple studies employed moderately active subjects (Sidhu *et al.*, 2009b; Fernandez-del-Olmo *et al.*, 2013). This raises the question of whether these findings are applicable to the general population although Study 4 would have been impossible to conduct in an untrained population. More importantly, can aerobic/endurance training facilitate neural adaptations and would any such neural adaptations have a role to play in developing healthy lifestyles and/or reducing risk factors for disease? Or, returning to Booth and Laye (2009) and in light of the results of Pearce *et al.* (2012), should we look at endurance- and/or resistance-trained individuals as a normal baseline? From this point, one can ask if a lack of aerobic physical activity causes corticospinal de-adaptation.

DISCUSSION GENERALE ET PERSPECTIVES

DISCUSSION

Cette thèse est composée de deux parties principales, une partie méthodologique (Etudes 1 et 2) et une autre partie appliquée à l'exercice extrême (Etudes 3 et 4). Les deux premières études contribuent au développement méthodologique de la TMS pour évaluer la fatigue. Le résultat principal de l'Etude 1 est qu'une contraction maintenue à un niveau de force stable est indispensable avant l'application d'une impulsion TMS. Ceci est impératif pour toutes les autres études qui appliquent la TMS pendant des contractions volontaires, en particulier à faibles intensités de contraction. Le résultat principal de l'Etude 2 est que les méthodes fréquemment utilisées pour déterminer l'intensité de TMS conduisent à la sélection d'intensités de stimulation différentes. Ce résultat indique la nécessité de choisir une méthode de détermination de l'intensité de TMS liée aux objectifs spécifiques de l'étude, ce qui a été réalisé dans le cadre des Etudes 3 et 4. De plus, l'emploi d'une deuxième intensité de stimulation plus faible dans ces études s'est justifié par le fait que des changements de l'excitabilité ou de l'inhibition corticospinales réels peuvent ne pas être pris en compte si seulement une intensité correspondant à une réponse maximale sur la courbe stimulus-réponse est sélectionnée. L'identification des vraies modifications de la courbe stimulus-réponse peut être entravée du fait de l'absence d'un nombre suffisant de points sur la courbe ou de la sélection d'un seul point de données inapproprié. Les résultats opposés quant aux modifications de MEPs et CSPs dans l'Etude 4 avec les deux intensités de TMS sélectionnées et l'absence de différence avec les MEPS aux deux intensités de TMS sélectionnées dans l'Etude 3 soulignent cette importance. L'Étude 3 pose aussi la question des différences de développement de la fatigue entre les trois muscles du quadriceps mesurés. Alors que l'Etude 2 n'a pas observé de différence à intensité de stimulation optimale entre ces muscles, les changements d'excitabilité corticospinale observés dans l'Étude 3 étaient différents entre les muscles.

Les différences de résultats entre les Etudes 3 et 4 concernant l'impact de l'intensité de TMS sur les paramètres mesurés peuvent être liées à des différences de distance, durée et/ou au type d'exercice réalisé. Elles suggèrent aussi que des changements peuvent se produire au niveau des courbes stimulus-réponse. Du fait du long temps d'évaluation nécessaires pour la réalisation de courbes stimulus-réponse et du fait de la récupération

rapide des paramètres mesurés par TMS, l'utilisation de plusieurs intensités de TMS peut être intéressant pour mieux comprendre la commande supraspinale en direction des muscles et ses modifications avec la fatigue. Dans l'évaluation de la fatigue, VA est utilisé pour identifier un déficit central. Aussi, une intensité de TMS qui induit un MEP de l'amplitude maximale est considérée comme essentielle pour l'évaluation de VAc. Du fait de la différence de réponses des MEPs et CSPs selon l'intensité de TMS dans l'Etude 4, il reste à déterminer si l'intensité de stimulation la plus faible qui induit des MEPs maximales est appropriée pour étudier d'autres paramètres centraux telles le VAc. Les différences dépendantes de l'intensité de TMS observées dans cette thèse n'ont pas toujours été observées dans d'autres études et appellent à des recherches supplémentaires sur cette question.

L'observation d'une diminution de VAc avec l'exercice d'endurance est conforme à de précédentes études. Les Études 3 et 4 sont cependant les premières à rapporter une augmentation de l'amplitude des MEPs après un exercice d'endurance et les changements de CSP observées dans ces deux études sont nouveaux. Toutes les études faites en course à pied ou vélo ont trouvé une CSP inchangée à intensité de TMS optimale. Dans l'Etude 4, la CSP induite par intensité de TMS sub-optimale a augmenté et est restée inchangée à intensité de TMS optimale. Bien que des protocoles isométriques fatigants rapportent une augmentation de la durée de CSP, cela n'a pas été observé précédemment avec un exercice locomoteur. L'Etude 3 est la seule étude publiée qui a observé une réduction de la durée de la CSP après un exercice aigu. La combinaison de la durée, de l'intensité et/ou le mode d'exercice ainsi que l'intensité de la TMS sont des facteurs qui peuvent expliquer les réponses contradictoires de CSP. Dans l'Etude 4, il y avait un délai relativement important avant les mesures après exercice. Les changements observés dans cette étude suggèrent que la durée de l'exercice pourrait influencer le temps de persistance des effets induits par la TMS après la fin de l'exercice. Enfin, on peut conclure qu'avec l'exercice d'endurance et d'ultra-endurance, il existe des preuves de présence de fatigue supraspinale et des changements de l'excitabilité et de l'inhibition corticospinale. La pertinence de ces observations et le rôle de l'intensité de la TMS dans l'évaluation de la fatigue restent à élucider.

PERSPECTIVES

Il y a de nombreux domaines de recherche intéressants en lien avec la TMS, la fatigue supraspinale et des changements et/ou adaptations neuromusculaires qui restent à explorer et

élucider. Bien qu'il y ait beaucoup d'intérêt envers les situations cliniques, l'homme sain et actif reste un modèle très intéressant. Cette population doit jouer un rôle important dans le développement d'approches méthodologiques solides pour évaluer la fatigue et les changements corticospinaux associés à l'exercice. Cette population doit également représenter un groupe de comparaison afin que des altérations potentielles dans des populations de patients ou de sujets sédentaires puissent être identifiées. Dans le cadre de cette population de sujet sain, les perspectives suivantes sont d'un intérêt personnel.

Un facteur important qui influence l'interprétation des résultats de l'évaluation de la fatigue est le délai entre les mesures après l'intervention et la fin de de l'exercice. Les résultats des protocoles isométriques montrent que les modifications observées par TMS récupèrent rapidement après l'arrêt de l'exercice. Des recherches supplémentaires sont nécessaires pour déterminer si la cinétique de récupération de tous les paramètres évalués par TMS sont similaires ainsi que pour évaluer les effets de la durée, de l'intensité et du type d'activité sur la cinétique de récupération. Récemment, l'évaluation par TMS au cours du pédalage sur vélo a été utilisée pour explorer les effets de la fatigue. Ceci permet d'éviter le problème de la récupération rapide des paramètres évalués par TMS et autorise une meilleure compréhension de la fatigue au cours même de l'exercice. Ces études doivent être développées pour inclure la marche et la course à pied en raison de leur importance fonctionnelle dans la vie quotidienne. Les limites principales actuelles à l'utilisation de la TMS pendant la locomotion sont d'ordre méthodologique ; par conséquent, la recherche doit à l'avenir développer des solutions pour que la TMS soit un outil viable pour explorer ces conditions.

La plupart des études ont évalué des sujets exclusivement masculins. Peu d'éléments sont disponibles concernant le développement et l'évolution de la fatigue centrale et supraspinale chez les femmes et l'existence de différences entre les sexes. Les études qui comparent la force maximale et le développement de la fatigue ont observé des différences de sexe, mais la diversité des protocoles utilisés ne permet pas de conclusions définitives. Les seules études qui ont étudié les différences supraspinales en fonction du sexe n'en n'ont pas mis en évidence de différences au cours de protocoles isométriques. Les investigations futures doivent examiner si ceci est également vrai lors de locomotions. Bien que l'analyse initiale des données de l'Etude 4 suggère qu'il n'y a pas de différences entre les hommes et les femmes pour tous les paramètres analysés, des analyses supplémentaires sont prévues pour tenir compte des différences de performance.

Un domaine important qui est en train de se développer est l'utilisation de la TMS pour évaluer les adaptations liées à l'entrainement. Des études ont montré des adaptations neuromusculaires identifiées par TMS suite à un entraînement en force. Des études plus récentes ont suggéré que l'interaction entre les changements de MEP et CSP induits par l'entrainement sont dépendent du type d'entrainement effectué et que l'entraînement en force peut contrer les effets de l'inactivité et les altérations neurales associées. Peu d'éléments sont disponibles quant aux adaptations neurales provoquées par l'entraînement en endurance chez le sujet sain. Aucune étude publiée ne s'est intéressé aux effets de l'entrainement physique de type aérobie sur les paramètres TMS dans cette population. Des recherches sont nécessaires pour déterminer si l'entrainement physique de type aérobie induit des adaptations neurales similaires à celles d'associées à l'entraînement en force. Il reste aussi à déterminer la possibilité d'adaptations neurales spécifiques à des programmes d'entrainement de type mixtes tels que souvent employées. Tous les sujets des Etudes 3 et 4 étaient bien entrainés de même que les sujets de la plupart des études évaluant la fatigue supraspinale. Cela pose la question de l'applicabilité de ces résultats à la population générale. Plus important encore, l'entrainement de type aérobie/endurance pourrait faciliter les adaptations neurales et en conséquence jouer un rôle dans le développement d'une meilleure qualité de vie et/ou réduire les facteurs de risque de certaines maladies ? Faut-il considérer des individus entrainés en endurance et/ou en force comme norme de comparaison? Ainsi, on peut se demander si un manque d'activité physique de type aérobie provoque une désadaptation corticospinale.

REFERENCES

Amann M. (2011). Central and peripheral fatigue: interaction during cycling exercise in humans. *Med Sci Sports Exerc* **43,** 2039-2045.

Amann M & Dempsey JA. (2008). Locomotor muscle fatigue modifies central motor drive in healthy humans and imposes a limitation to exercise performance. *J Physiol* **586,** 161-173.

Amann M, Romer LM, Pegelow DF, Jacques AJ, Hess CJ & Dempsey JA. (2006). Effects of arterial oxygen content on peripheral locomotor muscle fatigue. *J Appl Physiol* **101,** 119-127.

Arai N, Okabe S, Furubayashi T, Terao Y, Yuasa K & Ugawa Y. (2005). Comparison between short train, monophasic and biphasic repetitive transcranial magnetic stimulation (rTMS) of the human motor cortex. *Clin Neurophysiol* **116,** 605-613.

Awiszus F & Feistner H. (1994). Quantification of D- and I-wave effects evoked by transcranial magnetic brain stimulation on the tibialis anterior motoneuron pool in man. *Exp Brain Res* **101,** 153-158.

Awiszus F, Wahl B & Meinecke I. (1997). Influence of stimulus cross talk on results of the twitch-interpolation technique at the biceps brachii muscle. *Muscle Nerve* **20,** 1187-1190.

Azboy O & Kaygisiz Z. (2009). Effects of sleep deprivation on cardiorespiratory functions of the runners and volleyball players during rest and exercise. *Acta Physiol Hung* **96,** 29-36.

Bachasson D, Guinot M, Wuyam B, Favre-Juvin A, Millet GY, Levy P & Verges S. (2013a). Neuromuscular fatigue and exercise capacity in fibromyalgia syndrome. *Arthritis Care Res (Hoboken)* **65,** 432-440.

Bachasson D, Millet GY, Decorte N, Wuyam B, Levy P & Verges S. (2013b). Quadriceps function assessment using an incremental test and magnetic neurostimulation: a reliability study. *J Electromyogr Kinesiol* **23,** 649-658.

Bachasson D, Temesi J, Bankole C, Lagrange E, Boutte C, Millet GY, Verges S, Levy P, Feasson L & Wuyam B. (2013c). Assessment of quadriceps strength, endurance and fatigue in FSHD and CMT: benefits and limits of femoral nerve magnetic stimulation. *Clin Neurophysiol*. DOI: 10.1016/j.clinph.2013.08.001.

Badawy RA, Curatolo JM, Newton M, Berkovic SF & Macdonell RA. (2006). Sleep deprivation increases cortical excitability in epilepsy: syndrome-specific effects. *Neurology* **67,** 1018-1022.

Bailey SP, Hall EE, Folger SE & Miller PC. (2008). Changes in EEG during graded exercise on a recumbent cycle ergometer. *J Sports Sci Med* **7,** 505-511.

Bainbridge FA. (1919). *The physiology of muscular exercise.* Longman's Green, London, UK.

Balconi M & Ferrari C. (2012). rTMS stimulation on left DLPFC affects emotional cue retrieval as a function of anxiety level and gender. *Depress Anxiety* **29,** 976-982.

Balkin TJ, Rupp T, Picchioni D & Wesensten NJ. (2008). Sleep loss and sleepiness: current issues. *Chest* **134,** 653-660.

Balslev D, Braet W, McAllister C & Miall RC. (2007). Inter-individual variability in optimal current direction for transcranial magnetic stimulation of the motor cortex. *J Neurosci Methods* **162,** 309-313.

Barker AT. (1999). The history and basic principles of magnetic nerve stimulation. *Electroencephalogr Clin Neurophysiol* **51 Suppl,** 3-21.

Barker AT, Jalinous R & Freeston IL. (1985). Non-invasive magnetic stimulation of human motor cortex. *Lancet* **1,** 1106-1107.

Baudry S, Klass M, Pasquet B & Duchateau J. (2007). Age-related fatigability of the ankle dorsiflexor muscles during concentric and eccentric contractions. *Eur J Appl Physiol* **100,** 515-525.

Beck S, Taube W, Gruber M, Amtage F, Gollhofer A & Schubert M. (2007). Task-specific changes in motor evoked potentials of lower limb muscles after different training interventions. *Brain Res* **1179,** 51-60.

Beelen A & Sargeant AJ. (1991). Effect of fatigue on maximal power output at different contraction velocities in humans. *J Appl Physiol* **71,** 2332-2337.

Belanger AY & McComas AJ. (1981). Extent of motor unit activation during effort. *J Appl Physiol* **51,** 1131-1135.

Berardelli A, Inghilleri M, Rothwell JC, Cruccu G & Manfredi M. (1991). Multiple firing of motoneurones is produced by cortical stimulation but not by direct activation of descending motor tracts. *Electroencephalogr Clin Neurophysiol* **81,** 240-242.

Berchicci M, Menotti F, Macaluso A & Di Russo F. (2013). The neurophysiology of central and peripheral fatigue during sub-maximal lower limb isometric contractions. *Front Hum Neurosci* **7,** 135.

Berger LL, Regueme SC & Forestier N. (2010). Unilateral lower limb muscle fatigue induces bilateral effects on undisturbed stance and muscle EMG activities. *J Electromyogr Kinesiol* **20,** 947-952.

Berlim MT, Van den Eynde F & Daskalakis ZJ. (2013). High-frequency repetitive transcranial magnetic stimulation accelerates and enhances the clinical response to antidepressants in major depression: a meta-analysis of randomized, double-blind, and sham-controlled trials. *J Clin Psychiatry* **74,** e122-129.

Bigland-Ritchie B. (1984). Muscle fatigue and the influence of changing neural drive. *Clin Chest Med* **5,** 21-34.

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

Bigland-Ritchie B, Jones DA, Hosking GP & Edwards RH. (1978). Central and peripheral fatigue in sustained maximum voluntary contractions of human quadriceps muscle. *Clin Sci Mol Med* **54,** 609-614.

Bigland-Ritchie B & Woods JJ. (1984). Changes in muscle contractile properties and neural control during human muscular fatigue. *Muscle Nerve* **7,** 691-699.

Bigland B & Lippold OC. (1954). Motor unit activity in the voluntary contraction of human muscle. *J Physiol* **125,** 322-335.

Binks PG, Waters WF & Hurry M. (1999). Short-term total sleep deprivations does not selectively impair higher cortical functioning. *Sleep* **22,** 328-334.

Boerio D, Jubeau M, Zory R & Maffiuletti NA. (2005). Central and peripheral fatigue after electrostimulation-induced resistance exercise. *Med Sci Sports Exerc* **37,** 973-978.

Bond V, Balkissoon B, Franks BD, Brwnlow R, Caprarola M, Bartley D & Banks M. (1986). Effects of sleep deprivation on performance during submaximal and maximal exercise. *J Sports Med Phys Fitness* **26,** 169-174.

References

Booth FW & Laye MJ. (2009). Lack of adequate appreciation of physical exercise's complexities can pre-empt appropriate design and interpretation in scientific discovery. *J Physiol* **587,** 5527-5539.

Borg G. (1970). Perceived exertion as an indicator of somatic stress. *Scand J Rehabil Med* **2,** 92-98.

Borrani F, Candau R, Perrey S, Millet GY, Millet GP & Rouillon JD. (2003). Does the mechanical work in running change during the VO2 slow component? *Med Sci Sports Exerc* **35,** 50-57.

Brisswalter J, Arcelin R, Audiffren M & Delignieres D. (1997). Influence of physical exercise on simple reaction time: effect of physical fitness. *Percept Mot Skills* **85,** 1019-1027.

Brodan V, Vojtechovsky M, Kuhn E & Cepelak J. (1969). Changes of mental and physical performance in sleep deprivated healthy volunteers. *Act Nerv Super (Praha)* **11,** 175-181.

Brownsberger J, Edwards A, Crowther R & Cottrell D. (2013). Impact of mental fatigue on self-paced exercise. *Int J Sports Med*. DOI: 10.1055/s-0033-1343402.

Brummer V, Schneider S, Abel T, Vogt T & Struder HK. (2011). Brain cortical activity is influenced by exercise mode and intensity. *Med Sci Sports Exerc* **43,** 1863-1872.

Bulbulian R, Heaney JH, Leake CN, Sucec AA & Sjoholm NT. (1996). The effect of sleep deprivation and exercise load on isokinetic leg strength and endurance. *Eur J Appl Physiol Occup Physiol* **73,** 273-277.

Butler JE, Larsen TS, Gandevia SC & Petersen NT. (2007). The nature of corticospinal paths driving human motoneurones during voluntary contractions. *J Physiol* **584,** 651-659.

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

Butler JE, Taylor JL & Gandevia SC. (2003). Responses of human motoneurons to corticospinal stimulation during maximal voluntary contractions and ischemia. *J Neurosci* **23,** 10224-10230.

Cafarelli E, Liebesman J & Kroon J. (1995). Effect of endurance training on muscle activation and force sensation. *Can J Physiol Pharmacol* **73,** 1765-1773.

References

Cahill F, Kalmar JM, Pretorius T, Gardiner PF & Giesbrecht GG. (2011). Whole-body hypothermia has central and peripheral influences on elbow flexor performance. *Exp Physiol* **96,** 528-538.

Candau R, Belli A, Millet GY, Georges D, Barbier B & Rouillon JD. (1998). Energy cost and running mechanics during a treadmill run to voluntary exhaustion in humans. *Eur J Appl Physiol Occup Physiol* **77,** 479-485.

Carpentier A, Duchateau J & Hainaut K. (2001). Motor unit behaviour and contractile changes during fatigue in the human first dorsal interosseus. *J Physiol* **534,** 903-912.

Carroll TJ, Riek S & Carson RG. (2002). The sites of neural adaptation induced by resistance training in humans. *J Physiol* **544,** 641-652.

Cerri G, Cocchi CA, Montagna M, Zuin M, Podda M, Cavallari P & Selmi C. (2010). Patients with primary biliary cirrhosis do not show post-exercise depression of cortical excitability. *Clin Neurophysiol* **121,** 1321-1328.

Chang YK, Labban JD, Gapin JI & Etnier JL. (2012). The effects of acute exercise on cognitive performance: a meta-analysis. *Brain Res* **1453,** 87-101.

Chen HI. (1991). Effects of 30-h sleep loss on cardiorespiratory functions at rest and in exercise. *Med Sci Sports Exerc* **23,** 193-198.

Chin O, Cash RF & Thickbroom GW. (2012). Electromyographic bursting following the cortical silent period induced by transcranial magnetic stimulation. *Brain Res* **1446,** 40-45.

Chmura J, Krysztofiak H, Ziemba AW, Nazar K & Kaciuba-Uscilko H. (1998). Psychomotor performance during prolonged exercise above and below the blood lactate threshold. *Eur J Appl Physiol Occup Physiol* **77,** 77-80.

Chmura J, Nazar K & Kaciuba-Uscilko H. (1994). Choice reaction time during graded exercise in relation to blood lactate and plasma catecholamine thresholds. *Int J Sports Med* **15,** 172-176.

Cirillo J, Lavender AP, Ridding MC & Semmler JG. (2009). Motor cortex plasticity induced by paired associative stimulation is enhanced in physically active individuals. *J Physiol* **587,** 5831-5842.

Civardi C, Boccagni C, Vicentini R, Bolamperti L, Tarletti R, Varrasi C, Monaco F & Cantello R. (2001). Cortical excitability and sleep deprivation: a transcranial magnetic stimulation study. *J Neurol Neurosurg Psychiatry* **71,** 809-812.

Clark BC, Collier SR, Manini TM & Ploutz-Snyder LL. (2005). Sex differences in muscle fatigability and activation patterns of the human quadriceps femoris. *Eur J Appl Physiol* **94,** 196-206.

d'Arsonval A. (1896). Dispositifs pour la mesure des courants alternatifs de toutes fréquences. *C R Biol Soc* **3,** 450-451.

Daanen HA, van Ling S & Tan TK. (2013). Subjective ratings and performance in the heat and after sleep deprivation. *Aviat Space Environ Med* **84,** 701-707.

Dalsgaard MK, Ott P, Dela F, Juul A, Pedersen BK, Warberg J, Fahrenkrug J & Secher NH. (2004). The CSF and arterial to internal jugular venous hormonal differences during exercise in humans. *Exp Physiol* **89,** 271-277.

Darling WG, Wolf SL & Butler AJ. (2006). Variability of motor potentials evoked by transcranial magnetic stimulation depends on muscle activation. *Exp Brain Res* **174,** 376-385.

Davey NJ, Romaiguere P, Maskill DW & Ellaway PH. (1994). Suppression of voluntary motor activity revealed using transcranial magnetic stimulation of the motor cortex in man. *J Physiol* **477,** 223-235.

Davies CT & Thompson MW. (1986). Physiological responses to prolonged exercise in ultramarathon athletes. *J Appl Physiol* **61,** 611-617.

Davranche K, Audiffren M & Denjean A. (2006a). A distributional analysis of the effect of physical exercise on a choice reaction time task. *J Sports Sci* **24,** 323-329.

Davranche K, Burle B, Audiffren M & Hasbroucq T. (2005). Information processing during physical exercise: a chronometric and electromyographic study. *Exp Brain Res* **165,** 532-540.

Davranche K, Burle B, Audiffren M & Hasbroucq T. (2006b). Physical exercise facilitates motor processes in simple reaction time performance: an electromyographic analysis. *Neurosci Lett* **396,** 54-56.

References

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

De Gennaro L, Marzano C, Veniero D, Moroni F, Fratello F, Curcio G, Ferrara M, Ferlazzo F, Novelli L, Concetta Pellicciari M, Bertini M & Rossini PM. (2007). Neurophysiological correlates of sleepiness: a combined TMS and EEG study. *Neuroimage* **36,** 1277-1287.

de Graaf TA, Goebel R & Sack AT. (2012). Feedforward and quick recurrent processes in early visual cortex revealed by TMS? *Neuroimage* **61,** 651-659.

de Haan A, Gerrits KH & de Ruiter CJ. (2009). Counterpoint: the interpolated twitch does not provide a valid measure of the voluntary activation of muscle. *J Appl Physiol* **107,** 355-357; discussion 357-358.

de Noordhout AM, Rapisarda G, Bogacz D, Gerard P, De Pasqua V, Pennisi G & Delwaide PJ. (1999). Corticomotoneuronal synaptic connections in normal man: an electrophysiological study. *Brain* **122** 1327-1340.

Decorte N, Bachasson D, Guinot M, Flore P, Levy P, Verges S & Wuyam B. (2013). Impact of salbutamol on neuromuscular function in endurance athletes. *Med Sci Sports Exerc* **45,** 1925-1932.

Decorte N, Lafaix PA, Millet GY, Wuyam B & Verges S. (2012). Central and peripheral fatigue kinetics during exhaustive constant-load cycling. *Scand J Med Sci Sports* **22,** 381-391.

del Olmo MF, Reimunde P, Viana O, Acero RM & Cudeiro J. (2006). Chronic neural adaptation induced by long-term resistance training in humans. *Eur J Appl Physiol* **96,** 722-728.

Delignières D, Brisswalter J & Legros P. (1994). Influence of physical exercise on choice reaction time in sport experts: the mediating role of resource allocation. *J Hum Mov Stud* **27,** 173-188.

Devanne H, Lavoie BA & Capaday C. (1997). Input-output properties and gain changes in the human corticospinal pathway. *Exp Brain Res* **114,** 329-338.

Dimitrova NA & Dimitrov GV. (2003). Interpretation of EMG changes with fatigue: facts, pitfalls, and fallacies. *J Electromyogr Kinesiol* **13,** 13-36.

Dinges DF, Pack F, Williams K, Gillen KA, Powell JW, Ott GE, Aptowicz C & Pack AI. (1997). Cumulative sleepiness, mood disturbance, and psychomotor vigilance performance decrements during a week of sleep restricted to 4-5 hours per night. *Sleep* **20,** 267-277.

Doeltgen SH & Ridding MC. (2010). Behavioural exposure and sleep do not modify corticospinal and intracortical excitability in the human motor system. *Clin Neurophysiol* **121,** 448-452.

Doran SM, Van Dongen HP & Dinges DF. (2001). Sustained attention performance during sleep deprivation: evidence of state instability. *Arch Ital Biol* **139,** 253-267.

Duclay J, Pasquet B, Martin A & Duchateau J. (2011). Specific modulation of corticospinal and spinal excitabilities during maximal voluntary isometric, shortening and lengthening contractions in synergist muscles. *J Physiol* **589,** 2901-2916.

Easthope CS, Hausswirth C, Louis J, Lepers R, Vercruyssen F & Brisswalter J. (2010). Effects of a trail running competition on muscular performance and efficiency in well-trained young and master athletes. *Eur J Appl Physiol* **110,** 1107-1116.

Farina D, Merletti R & Enoka RM. (2004). The extraction of neural strategies from the surface EMG. *J Appl Physiol* **96,** 1486-1495.

Feasson L, Camdessanche JP, El Mandhi L, Calmels P & Millet GY. (2006). Fatigue et affections neuromusculaires. *Ann Readapt Med Phys* **49,** 289-300, 375-284.

Fernandez-del-Olmo M, Rodriguez FA, Marquez G, Iglesias X, Marina M, Benitez A, Vallejo L & Acero RM. (2013). Isometric knee extensor fatigue following a Wingate test: peripheral and central mechanisms. *Scand J Med Sci Sports* **23,** 57-65.

Fisher BE, Wu AD, Salem GJ, Song J, Lin CH, Yip J, Cen S, Gordon J, Jakowec M & Petzinger G. (2008). The effect of exercise training in improving motor performance and corticomotor excitability in people with early Parkinson's disease. *Arch Phys Med Rehabil* **89,** 1221-1229.

Fitts RH. (2011). Cellular, molecular, and metabolic basis of muscle fatigue. *Compr Physiol* **Supplement 29,** 1151-1183.

Fitzgerald PB, Brown TL & Daskalakis ZJ. (2002). The application of transcranial magnetic stimulation in psychiatry and neurosciences research. *Acta Psychiatr Scand* **105,** 324-340.

Forestier N & Nougier V. (1998). The effects of muscular fatigue on the coordination of a multijoint movement in human. *Neurosci Lett* **252,** 187-190.

Forrester LW, Hanley DF & Macko RF. (2006). Effects of treadmill exercise on transcranial magnetic stimulation-induced excitability to quadriceps after stroke. *Arch Phys Med Rehabil* **87,** 229-234.

Forsberg A, Tesch P & Karlsson J. (1979). Effect of prolonged exercise on muscle strength performance. In *Biomechanics VI-A*, ed. Asmussen E & Jorgensen K, pp. 62-67. University Park Press, Baltimore, USA.

Fourchet F, Millet GP, Tomazin K, Guex K, Nosaka K, Edouard P, Degache F & Millet GY. (2012). Effects of a 5-h hilly running on ankle plantar and dorsal flexor force and fatigability. *Eur J Appl Physiol* **112,** 2645-2652.

Froyd C, Millet GY & Noakes TD. (2013). The development of peripheral fatigue and short-term recovery during self-paced high-intensity exercise. *J Physiol* **591,** 1339-1346.

Gandevia SC. (2001). Spinal and supraspinal factors in human muscle fatigue. *Physiol Rev* **81,** 1725-1789.

Gandevia SC, Allen GM, Butler JE & Taylor JL. (1996). Supraspinal factors in human muscle fatigue: evidence for suboptimal output from the motor cortex. *J Physiol* **490,** 529-536.

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

Garland SJ, Enoka RM, Serrano LP & Robinson GA. (1994). Behavior of motor units in human biceps brachii during a submaximal fatiguing contraction. *J Appl Physiol* **76,** 2411-2419.

Garrandes F, Colson SS, Pensini M, Seynnes O & Legros P. (2007). Neuromuscular fatigue profile in endurance-trained and power-trained athletes. *Med Sci Sports Exerc* **39,** 149-158.

Ge W & Khalsa PS. (2003). Encoding of compressive stress during indentation by group III and IV muscle mechano-nociceptors in rat gracilis muscle. *J Neurophysiol* **89,** 785-792.

Gibbons CE, Pietrosimone BG, Hart JM, Saliba SA & Ingersoll CD. (2010). Transcranial magnetic stimulation and volitional quadriceps activation. *J Athl Train* **45,** 570-579.

Giesebrecht S, Martin PG, Gandevia SC & Taylor JL. (2011). Altered corticospinal transmission to the hand after maximum voluntary efforts. *Muscle Nerve* **43,** 679-687.

Gimenez P, Kerherve H, Messonnier LA, Feasson L & Millet GY. (2013). Changes in the energy cost of running during a 24-h treadmill exercise. *Med Sci Sports Exerc* **45,** 1807-1813.

Girard O, Bishop DJ & Racinais S. (2013). Neuromuscular adjustments of the quadriceps muscle after repeated cycling sprints. *PLoS One* **8,** e61793.

Glace BW, Kremenic IJ & McHugh MP. (2013). Sex differences in central and peripheral mechanisms of fatigue in cyclists. *Eur J Appl Physiol* **113,** 1091-1098.

Glace BW, McHugh MP & Gleim GW. (1998). Effects of a 2-hour run on metabolic economy and lower extremity strength in men and women. *J Orthop Sports Phys Ther* **27,** 189-196.

Gonzalez-Alonso J, Dalsgaard MK, Osada T, Volianitis S, Dawson EA, Yoshiga CC & Secher NH. (2004). Brain and central haemodynamics and oxygenation during maximal exercise in humans. *J Physiol* **557,** 331-342.

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

Goodall S, Romer LM & Ross EZ. (2009). Voluntary activation of human knee extensors measured using transcranial magnetic stimulation. *Exp Physiol* **94,** 995-1004.

Goodall S, Ross EZ & Romer LM. (2010). Effect of graded hypoxia on supraspinal contributions to fatigue with unilateral knee-extensor contractions. *J Appl Physiol* **109,** 1842-1851.

Goodman J, Radomski M, Hart L, Plyley M & Shephard RJ. (1989). Maximal aerobic exercise following prolonged sleep deprivation. *Int J Sports Med* **10,** 419-423.

Grego F, Vallier JM, Collardeau M, Rousseu C, Cremieux J & Brisswalter J. (2005). Influence of exercise duration and hydration status on cognitive function during prolonged cycling exercise. *Int J Sports Med* **26,** 27-33.

Griffin L & Cafarelli E. (2007). Transcranial magnetic stimulation during resistance training of the tibialis anterior muscle. *J Electromyogr Kinesiol* **17,** 446-452.

Groppa S, Oliviero A, Eisen A, Quartarone A, Cohen LG, Mall V, Kaelin-Lang A, Mima T, Rossi S, Thickbroom GW, Rossini PM, Ziemann U, Valls-Sole J & Siebner HR. (2012). A practical guide to diagnostic transcranial magnetic stimulation: report of an IFCN committee. *Clin Neurophysiol* **123,** 858-882.

Gruet M, Temesi J, Rupp T, Levy P, Millet GY & Verges S. (2013a). Stimulation of the motor cortex and corticospinal tract to assess human muscle fatigue. *Neuroscience* **231,** 384-399.

Gruet M, Temesi J, Rupp T, Millet GY & Verges S. (2013b). Effect of different approaches to target force on transcranial magnetic stimulation responses. *Muscle Nerve* **48,** 430-432.

Guezennec CY, Abdelmalki A, Serrurier B, Merino D, Bigard X, Berthelot M, Pierard C & Peres M. (1998). Effects of prolonged exercise on brain ammonia and amino acids. *Int J Sports Med* **19,** 323-327.

Hakkinen K. (1994). Neuromuscular fatigue in males and females during strenuous heavy resistance loading. *Electromyogr Clin Neurophysiol* **34,** 205-214.

Harrison Y & Horne JA. (1998). Sleep loss impairs short and novel language tasks having a prefrontal focus. *J Sleep Res* **7,** 95-100.

Hasegawa H, Piacentini MF, Sarre S, Michotte Y, Ishiwata T & Meeusen R. (2008). Influence of brain catecholamines on the development of fatigue in exercising rats in the heat. *J Physiol* **586,** 141-149.

Hill AV. (1924). Muscular activity and carbohydrate metabolism. *Science* **60,** 505-514.

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

Hoffman BW, Oya T, Carroll TJ & Cresswell AG. (2009). Increases in corticospinal responsiveness during a sustained submaximal plantar flexion. *J Appl Physiol* **107,** 112-120.

Hoheisel U, Unger T & Mense S. (2005). Excitatory and modulatory effects of inflammatory cytokines and neurotrophins on mechanosensitive group IV muscle afferents in the rat. *Pain* **114,** 168-176.

Holland GJ. (1968). Effects of limited sleep deprivation on performance of selected motor tasks. *Res Q* **39,** 285-294.

Hollge J, Kunkel M, Ziemann U, Tergau F, Geese R & Reimers CD. (1997). Central fatigue in sports and daily exercises. A magnetic stimulation study. *Int J Sports Med* **18,** 614-617.

Horne JA & Pettitt AN. (1984). Sleep deprivation and the physiological response to exercise under steady-state conditions in untrained subjects. *Sleep* **7,** 168-179.

Hosono Y, Urushihara R, Harada M, Morita N, Murase N, Kunikane Y, Shimazu H, Asanuma K, Uguisu H & Kaji R. (2008). Comparison of monophasic versus biphasic stimulation in rTMS over premotor cortex: SEP and SPECT studies. *Clin Neurophysiol* **119,** 2538-2545.

Houlden DA, Schwartz ML, Tator CH, Ashby P & MacKay WA. (1999). Spinal cord-evoked potentials and muscle responses evoked by transcranial magnetic stimulation in 10 awake human subjects. *J Neurosci* **19,** 1855-1862.

Hovey C & Jalinous R. (2006). *The guide to magnetic stimulation.* The Magstim Co., Ltd., Whitland, UK.

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 & Enoka RM. (2001). Sex differences in the fatigability of arm muscles depends on absolute force during isometric contractions. *J Appl Physiol* **91,** 2686-2694.

Hunter SK, Todd G, Butler JE, Gandevia SC & Taylor JL. (2008). Recovery from supraspinal fatigue is slowed in old adults after fatiguing maximal isometric contractions. *J Appl Physiol* **105,** 1199-1209.

Ide K, Horn A & Secher NH. (1999). Cerebral metabolic response to submaximal exercise. *J Appl Physiol* **87,** 1604-1608.

Iglesias C, Lourenco G & Marchand-Pauvert V. (2012). Weak motor cortex contribution to the quadriceps activity during human walking. *Gait Posture* **35,** 360-366.

Iguchi M & Shields RK. (2012). Cortical and segmental excitability during fatiguing contractions of the soleus muscle in humans. *Clin Neurophysiol* **123,** 335-343.

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

Jensen JL, Marstrand PC & Nielsen JB. (2005). Motor skill training and strength training are associated with different plastic changes in the central nervous system. *J Appl Physiol* **99,** 1558-1568.

Kalmar JM & Cafarelli E. (2006). Central excitability does not limit postfatigue voluntary activation of quadriceps femoris. *J Appl Physiol* **100,** 1757-1764.

Kamibayashi K, Nakajima T, Takahashi M, Akai M & Nakazawa K. (2009). Facilitation of corticospinal excitability in the tibialis anterior muscle during robot-assisted passive stepping in humans. *Eur J Neurosci* **30,** 100-109.

Kammer T, Beck S, Thielscher A, Laubis-Herrmann U & Topka H. (2001). Motor thresholds in humans: a transcranial magnetic stimulation study comparing different pulse waveforms, current directions and stimulator types. *Clin Neurophysiol* **112,** 250-258.

Kastrup A, Kruger G, Neumann-Haefelin T, Glover GH & Moseley ME. (2002). Changes of cerebral blood flow, oxygenation, and oxidative metabolism during graded motor activation. *Neuroimage* **15,** 74-82.

Keller ML, Pruse J, Yoon T, Schlinder-Delap B, Harkins A & Hunter SK. (2011). Supraspinal fatigue is similar in men and women for a low-force fatiguing contraction. *Med Sci Sports Exerc* **43,** 1873-1883.

Khedr EM, Galal O, Said A, Abd-elsameea M & Rothwell JC. (2007). Lack of post-exercise depression of corticospinal excitability in patients with Parkinson's disease. *Eur J Neurol* **14,** 793-796.

Kidgell DJ & Pearce AJ. (2010). Corticospinal properties following short-term strength training of an intrinsic hand muscle. *Hum Mov Sci* **29,** 631-641.

Kidgell DJ & Pearce AJ. (2011). What has transcranial magnetic stimulation taught us about neural adaptations to strength training? A brief review. *J Strength Cond Res* **25,** 3208-3217.

Kidgell DJ, Stokes MA, Castricum TJ & Pearce AJ. (2010). Neurophysiological responses after short-term strength training of the biceps brachii muscle. *J Strength Cond Res* **24,** 3123-3132.

Kiers L, Cros D, Chiappa KH & Fang J. (1993). Variability of motor potentials evoked by transcranial magnetic stimulation. *Electroencephalogr Clin Neurophysiol* **89,** 415-423.

Killgore WD. (2010). Effects of sleep deprivation on cognition. *Prog Brain Res* **185,** 105-129.

Kimiskidis VK, Papagiannopoulos S, Sotirakoglou K, Kazis DA, Kazis A & Mills KR. (2005). Silent period to transcranial magnetic stimulation: construction and properties of stimulus-response curves in healthy volunteers. *Exp Brain Res* **163,** 21-31.

Klass M, Levenez M, Enoka RM & Duchateau J. (2008). Spinal mechanisms contribute to differences in the time to failure of submaximal fatiguing contractions performed with different loads. *J Neurophysiol* **99,** 1096-1104.

Klass M, Roelands B, Levenez M, Fontenelle V, Pattyn N, Meeusen R & Duchateau J. (2012). Effects of noradrenaline and dopamine on supraspinal fatigue in well-trained men. *Med Sci Sports Exerc* **44,** 2299-2308.

Konishi M, Takahashi M, Endo N, Numao S, Takagi S, Miyashita M, Midorikawa T, Suzuki K & Sakamoto S. (2012). Effects of sleep deprivation on autonomic and endocrine functions throughout the day and on exercise tolerance in the evening. *J Sports Sci* **31,** 248-255.

Kremenic IJ, Glace BW, Ben-Avi SS, Nicholas SJ & McHugh MP. (2009). Central fatigue after cycling evaluated using peripheral magnetic stimulation. *Med Sci Sports Exerc* **41,** 1461-1466.

Kreuzer P, Langguth B, Popp R, Raster R, Busch V, Frank E, Hajak G & Landgrebe M. (2011). Reduced intra-cortical inhibition after sleep deprivation: a transcranial magnetic stimulation study. *Neurosci Lett* **493,** 63-66.

Krishnan C & Dhaher Y. (2012). Corticospinal responses of quadriceps are abnormally coupled with hip adductors in chronic stroke survivors. *Exp Neurol* **233,** 400-407.

Kujirai T, Caramia MD, Rothwell JC, Day BL, Thompson PD, Ferbert A, Wroe S, Asselman P & Marsden CD. (1993). Corticocortical inhibition in human motor cortex. *J Physiol* **471,** 501-519.

Kukulka CG & Clamann HP. (1981). Comparison of the recruitment and discharge properties of motor units in human brachial biceps and adductor pollicis during isometric contractions. *Brain Res* **219,** 45-55.

Lagerquist O, Mang CS & Collins DF. (2012). Changes in spinal but not cortical excitability following combined electrical stimulation of the tibial nerve and voluntary plantar-flexion. *Exp Brain Res* **222,** 41-53.

LeDuc PA, Caldwell JA, Jr. & Ruyak PS. (2000). The effects of exercise as a countermeasure for fatigue in sleep-deprived aviators. *Mil Psychol* **12,** 249-266.

Lee M & Carroll TJ. (2005). The amplitude of Mmax in human wrist flexors varies during different muscle contractions despite constant posture. *J Neurosci Methods* **149,** 95-100.

Lee M, Gandevia SC & Carroll TJ. (2008). Cortical voluntary activation can be reliably measured in human wrist extensors using transcranial magnetic stimulation. *Clin Neurophysiol* **119,** 1130-1138.

Lefaucheur JP. (2005). Motor cortex dysfunction revealed by cortical excitability studies in Parkinson's disease: influence of antiparkinsonian treatment and cortical stimulation. *Clin Neurophysiol* **116,** 244-253.

Lefaucheur JP, Andre-Obadia N, Poulet E, Devanne H, Haffen E, Londero A, Cretin B, Leroi AM, Radtchenko A, Saba G, Thai-Van H, Litre CF, Vercueil L, Bouhassira D, Ayache SS, Farhat WH, Zouari HG, Mylius V, Nicolier M & Garcia-Larrea L. (2011). Recommandations franc¸aises sur l'utilisation de la stimulation magnétique transcrânienne répétitive (rTMS) : règles de sécurité et indications thérapeutiques. *Neurophysiol Clin* **41,** 221-295.

Legramante JM, Raimondi G, Adreani CM, Sacco S, Iellamo F, Peruzzi G & Kaufman MP. (2000). Group III muscle afferents evoke reflex depressor responses to repetitive muscle contractions in rabbits. *Am J Physiol Heart Circ Physiol* **278,** H871-877.

Lentz M & Nielsen JF. (2002). Post-exercise facilitation and depression of M wave and motor evoked potentials in healthy subjects. *Clin Neurophysiol* **113,** 1092-1098.

Lepers R, Maffiuletti NA, Rochette L, Brugniaux J & Millet GY. (2002). Neuromuscular fatigue during a long-duration cycling exercise. *J Appl Physiol* **92,** 1487-1493.

Levenez M, Garland SJ, Klass M & Duchateau J. (2008). Cortical and spinal modulation of antagonist coactivation during a submaximal fatiguing contraction in humans. *J Neurophysiol* **99,** 554-563.

Liu JZ, Shan ZY, Zhang LD, Sahgal V, Brown RW & Yue GH. (2003). Human brain activation during sustained and intermittent submaximal fatigue muscle contractions: an FMRI study. *J Neurophysiol* **90,** 300-312.

Ljubisavljevic M, Milanovic S, Radovanovic S, Vukcevic I, Kostic V & Anastasijevic R. (1996). Central changes in muscle fatigue during sustained submaximal isometric voluntary contraction as revealed by transcranial magnetic stimulation. *Electroencephalogr Clin Neurophysiol* **101,** 281-288.

Lo JC, Groeger JA, Santhi N, Arbon EL, Lazar AS, Hasan S, von Schantz M, Archer SN & Dijk DJ. (2012). Effects of partial and acute total sleep deprivation on performance across cognitive domains, individuals and circadian phase. *PLoS One* **7,** e45987.

Lucas SJ, Anson JG, Palmer CD, Hellemans IJ & Cotter JD. (2009). The impact of 100 hours of exercise and sleep deprivation on cognitive function and physical capacities. *J Sports Sci* **27,** 719-728.

Mador MJ, Kufel TJ, Pineda LA, Steinwald A, Aggarwal A, Upadhyay AM & Khan MA. (2001). Effect of pulmonary rehabilitation on quadriceps fatiguability during exercise. *Am J Respir Crit Care Med* **163,** 930-935.

Madsen PL, Sperling BK, Warming T, Schmidt JF, Secher NH, Wildschiodtz G, Holm S & Lassen NA. (1993). Middle cerebral artery blood velocity and cerebral blood flow and O2 uptake during dynamic exercise. *J Appl Physiol* **74,** 245-250.

Mang CS, Clair JM & Collins DF. (2011). Neuromuscular electrical stimulation has a global effect on corticospinal excitability for leg muscles and a focused effect for hand muscles. *Exp Brain Res* **209,** 355-363.

Manganotti P, Bongiovanni LG, Fuggetta G, Zanette G & Fiaschi A. (2006). Effects of sleep deprivation on cortical excitability in patients affected by juvenile myoclonic epilepsy: a combined transcranial magnetic stimulation and EEG study. *J Neurol Neurosurg Psychiatry* **77,** 56-60.

Manganotti P, Palermo A, Patuzzo S, Zanette G & Fiaschi A. (2001). Decrease in motor cortical excitability in human subjects after sleep deprivation. *Neurosci Lett* **304,** 153-156.

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

Martin B & Haney R. (1982). Self-selected exercise intensity is unchanged by sleep loss. *Eur J Appl Physiol Occup Physiol* **49,** 79-86.

Martin BJ. (1981). Effect of sleep deprivation on tolerance of prolonged exercise. *Eur J Appl Physiol Occup Physiol* **47,** 345-354.

Martin BJ, Bender PR & Chen H. (1986). Stress hormonal response to exercise after sleep loss. *Eur J Appl Physiol Occup Physiol* **55,** 210-214.

Martin BJ & Chen HI. (1984). Sleep loss and the sympathoadrenal response to exercise. *Med Sci Sports Exerc* **16,** 56-59.

Martin BJ & Gaddis GM. (1981). Exercise after sleep deprivation. *Med Sci Sports Exerc* **13,** 220-223.

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

Martin PG & Rattey J. (2007). Central fatigue explains sex differences in muscle fatigue and contralateral cross-over effects of maximal contractions. *Pflugers Arch* **454,** 957-969.

Martin V, Kerherve H, Messonnier LA, Banfi JC, Geyssant A, Bonnefoy R, Feasson L & Millet GY. (2010). Central and peripheral contributions to neuromuscular fatigue induced by a 24-h treadmill run. *J Appl Physiol* **108,** 1224-1233.

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

McCombe Waller S, Forrester L, Villagra F & Whitall J. (2008). Intracortical inhibition and facilitation with unilateral dominant, unilateral nondominant and bilateral movement tasks in left- and right-handed adults. *J Neurol Sci* **269,** 96-104.

McDonnell MN, Orekhov Y & Ziemann U. (2006). The role of GABA(B) receptors in intracortical inhibition in the human motor cortex. *Exp Brain Res* **173,** 86-93.

McKay WB, Stokic DS, Sherwood AM, Vrbova G & Dimitrijevic MR. (1996). Effect of fatiguing maximal voluntary contraction on excitatory and inhibitory responses elicited by transcranial magnetic motor cortex stimulation. *Muscle Nerve* **19,** 1017-1024.

McKay WB, Tuel SM, Sherwood AM, Stokic DS & Dimitrijevic MR. (1995). Focal depression of cortical excitability induced by fatiguing muscle contraction: a transcranial magnetic stimulation study. *Exp Brain Res* **105,** 276-282.

McMorris T & Graydon J. (2000). The effect of incremental exercise on cognitive performance. *Int J Sport Psychol* **31,** 66–81.

McMurray RG & Brown CF. (1984). The effect of sleep loss on high intensity exercise and recovery. *Aviat Space Environ Med* **55,** 1031-1035.

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

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

McNeil CJ, Giesebrecht S, Khan SI, Gandevia SC & Taylor JL. (2011b). The reduction in human motoneurone responsiveness during muscle fatigue is not prevented by increased muscle spindle discharge. *J Physiol* **589,** 3731-3738.

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

Mehta JP, Verber MD, Wieser JA, Schmit BD & Schindler-Ivens SM. (2009). A novel technique for examining human brain activity associated with pedaling using fMRI. *J Neurosci Methods* **179,** 230-239.

Meney I, Waterhouse J, Atkinson G, Reilly T & Davenne D. (1998). The effect of one night's sleep deprivation on temperature, mood, and physical performance in subjects with different amounts of habitual physical activity. *Chronobiol Int* **15,** 349-363.

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

Milanovic S, Filipovic SR, Blesic S, Ilic TV, Dhanasekaran S & Ljubisavljevic M. (2011). Paired-associative stimulation can modulate muscle fatigue induced motor cortex excitability changes. *Behav Brain Res* **223,** 30-35.

Mileva KN, Bowtell JL & Kossev AR. (2009). Effects of low-frequency whole-body vibration on motor-evoked potentials in healthy men. *Exp Physiol* **94,** 103-116.

References

Mileva KN, Sumners DP & Bowtell JL. (2012). Decline in voluntary activation contributes to reduced maximal performance of fatigued human lower limb muscles. *Eur J Appl Physiol* **112,** 3959-3970.

Millet GP & Millet GY. (2012). Ultramarathon is an outstanding model for the study of adaptive responses to extreme load and stress. *BMC Med* **10,** 77.

Millet GY. (2011). Can neuromuscular fatigue explain running strategies and performance in ultra-marathons?: the flush model. *Sports Med* **41,** 489-506.

Millet GY, Banfi JC, Kerherve H, Morin JB, Vincent L, Estrade C, Geyssant A & Feasson L. (2011a). Physiological and biological factors associated with a 24 h treadmill ultra-marathon performance. *Scand J Med Sci Sports* **21,** 54-61.

Millet GY & Lepers R. (2004). Alterations of neuromuscular function after prolonged running, cycling and skiing exercises. *Sports Med* **34,** 105-116.

Millet GY, Lepers R, Maffiuletti NA, Babault N, Martin V & Lattier G. (2002). Alterations of neuromuscular function after an ultramarathon. *J Appl Physiol* **92,** 486-492.

Millet GY, Martin V, Lattier G & Ballay Y. (2003a). Mechanisms contributing to knee extensor strength loss after prolonged running exercise. *J Appl Physiol* **94,** 193-198.

Millet GY, Martin V, Maffiuletti NA & Martin A. (2003b). Neuromuscular fatigue after a ski skating marathon. *Can J Appl Physiol* **28,** 434-445.

Millet GY, Martin V, Martin A & Verges S. (2011b). Electrical stimulation for testing neuromuscular function: from sport to pathology. *Eur J Appl Physiol* **111,** 2489-2500.

Millet GY, Millet GP, Lattier G, Maffiuletti NA & Candau R. (2003c). Alteration of neuromuscular function after a prolonged road cycling race. *Int J Sports Med* **24,** 190-194.

Millet GY, Morin JB, Degache F, Edouard P, Feasson L, Verney J & Oullion R. (2009). Running from Paris to Beijing: biomechanical and physiological consequences. *Eur J Appl Physiol* **107,** 731-738.

Millet GY, Tomazin K, Verges S, Vincent C, Bonnefoy R, Boisson RC, Gergele L, Feasson L & Martin V. (2011c). Neuromuscular consequences of an extreme mountain ultra-marathon. *PLoS One* **6,** e17059.

Mills KR, Boniface SJ & Schubert M. (1992). Magnetic brain stimulation with a double coil: the importance of coil orientation. *Electroencephalogr Clin Neurophysiol* **85,** 17-21.

Mills KR & Thomson CC. (1995). Human muscle fatigue investigated by transcranial magnetic stimulation. *Neuroreport* **6,** 1966-1968.

Morin JB, Tomazin K, Edouard P & Millet GY. (2011). Changes in running mechanics and spring-mass behavior induced by a mountain ultra-marathon race. *J Biomech* **44,** 1104-1107.

Mosso A. (1904). *Fatigue*. Swan Sonnenschein, London.

Myles WS. (1985). Sleep deprivation, physical fatigue, and the perception of exercise intensity. *Med Sci Sports Exerc* **17,** 580-584.

Neely G, Ljunggren G, Sylven C & Borg G. (1992). Comparison between the Visual Analogue Scale (VAS) and the Category Ratio Scale (CR-10) for the evaluation of leg exertion. *Int J Sports Med* **13,** 133-136.

Neubauer O, Konig D & Wagner KH. (2008). Recovery after an Ironman triathlon: sustained inflammatory responses and muscular stress. *Eur J Appl Physiol* **104,** 417-426.

Nicol C, Komi PV & Marconnet P. (1991). Fatigue effects of marathon running on neuromuscular performance. . *Scand J Med Sci Sports* **1,** 18-24.

Nordlund MM, Thorstensson A & Cresswell AG. (2004). Central and peripheral contributions to fatigue in relation to level of activation during repeated maximal voluntary isometric plantar flexions. *J Appl Physiol* **96,** 218-225.

Nudo RJ, Milliken GW, Jenkins WM & Merzenich MM. (1996). Use-dependent alterations of movement representations in primary motor cortex of adult squirrel monkeys. *J Neurosci* **16,** 785-807.

Nybo L, Dalsgaard MK, Steensberg A, Moller K & Secher NH. (2005). Cerebral ammonia uptake and accumulation during prolonged exercise in humans. *J Physiol* **563,** 285-290.

Nybo L & Nielsen B. (2001). Hyperthermia and central fatigue during prolonged exercise in humans. *J Appl Physiol* **91,** 1055-1060.

Oberg PA. (1973). Magnetic stimulation of nerve tissue. *Med Biol Eng* **11,** 55-64.

Oliver SJ, Costa RJ, Laing SJ, Bilzon JL & Walsh NP. (2009). One night of sleep deprivation decreases treadmill endurance performance. *Eur J Appl Physiol* **107,** 155-161.

Paiva WS, Fonoff ET, Marcolin MA, Cabrera HN & Teixeira MJ. (2012). Cortical mapping with navigated transcranial magnetic stimulation in low-grade glioma surgery. *Neuropsychiatr Dis Treat* **8,** 197-201.

Papeo L, Pascual-Leone A & Caramazza A. (2013). Disrupting the brain to validate hypotheses on the neurobiology of language. *Front Hum Neurosci* **7,** 148.

Patrick GTW & Gilbert JA. (1896). Studies from the psychological laboratory of the University of Iowa: on the effects of loss of sleep. *Psychol Rev* **3,** 469-483.

Pearce AJ, Hendy A, Bowen WA & Kidgell DJ. (2012). Corticospinal adaptations and strength maintenance in the immobilized arm following 3 weeks unilateral strength training. *Scand J Med Sci Sports*. DOI: 10.1111/j.1600-0838.2012.01453.x.

Penfield W & Rasmussen T. (1950). *The cerebral cortex of man.* Macmillan, New York.

Petersen NT, Butler JE, Marchand-Pauvert V, Fisher R, Ledebt A, Pyndt HS, Hansen NL & Nielsen JB. (2001). Suppression of EMG activity by transcranial magnetic stimulation in human subjects during walking. *J Physiol* **537,** 651-656.

Petersen NT, Pyndt HS & Nielsen JB. (2003). Investigating human motor control by transcranial magnetic stimulation. *Exp Brain Res* **152,** 1-16.

Pickett GF & Morris AF. (1975). Effects of acute sleep and food deprivation on total body response time and cardiovascular performance. *J Sports Med Phys Fitness* **15,** 49-56.

Pires FO, Noakes TD, Lima-Silva AE, Bertuzzi R, Ugrinowitsch C, Lira FS & Kiss MA. (2011). Cardiopulmonary, blood metabolite and rating of perceived exertion responses to constant exercises performed at different intensities until exhaustion. *Br J Sports Med* **45,** 1119-1125.

Place N, Lepers R, Deley G & Millet GY. (2004). Time course of neuromuscular alterations during a prolonged running exercise. *Med Sci Sports Exerc* **36,** 1347-1356.

Plyley MJ, Shephard RJ, Davis GM & Goode RC. (1987). Sleep deprivation and cardiorespiratory function. Influence of intermittent submaximal exercise. *Eur J Appl Physiol Occup Physiol* **56,** 338-344.

Polson MJ, Barker AT & Freeston IL. (1982). Stimulation of nerve trunks with time-varying magnetic fields. *Med Biol Eng Comput* **20,** 243-244.

Presland JD, Dowson MN & Cairns SP. (2005). Changes of motor drive, cortical arousal and perceived exertion following prolonged cycling to exhaustion. *Eur J Appl Physiol* **95,** 42-51.

Race Across America website [Internet]. Boulder, USA: RAAM; [cited 07 March 2013]. Available from: www.raceacrossamerica.org.

Racinais S & Girard O. (2012). Neuromuscular failure is unlikely to explain the early exercise cessation in hot ambient conditions. *Psychophysiology* **49,** 853-865.

Racinais S, Hue O, Blonc S & Le Gallais D. (2004). Effect of sleep deprivation on shuttle run score in middle-aged amateur athletes. Influence of initial score. *J Sports Med Phys Fitness* **44,** 246-248.

Rasmussen P, Dawson EA, Nybo L, van Lieshout JJ, Secher NH & Gjedde A. (2007). Capillary-oxygenation-level-dependent near-infrared spectrometry in frontal lobe of humans. *J Cereb Blood Flow Metab* **27,** 1082-1093.

Rasmussen P, Nielsen J, Overgaard M, Krogh-Madsen R, Gjedde A, Secher NH & Petersen NC. (2010). Reduced muscle activation during exercise related to brain oxygenation and metabolism in humans. *J Physiol* **588,** 1985-1995.

Reid A, Chiappa K & Cros D. (2002). Motor threshold, facilitation and the silent period in cortical magnetic stimulation. In *Handbook of TMS*, ed. Pascual-Leone A, et al, pp. 97-111. Oxford University Press, New York.

Reid C. (1928). The mechanism of voluntary muscular fatigue. *Exp Physiol* **19,** 17-42.

Reis J, Swayne OB, Vandermeeren Y, Camus M, Dimyan MA, Harris-Love M, Perez MA, Ragert P, Rothwell JC & Cohen LG. (2008). Contribution of transcranial magnetic stimulation to the understanding of cortical mechanisms involved in motor control. *J Physiol* **586,** 325-351.

Rodgers CD, Paterson DH, Cunningham DA, Noble EG, Pettigrew FP, Myles WS & Taylor AW. (1995). Sleep deprivation: effects on work capacity, self-paced walking, contractile properties and perceived exertion. *Sleep* **18,** 30-38.

Rosler KM, Hess CW, Heckmann R & Ludin HP. (1989). Significance of shape and size of the stimulating coil in magnetic stimulation of the human motor cortex. *Neurosci Lett* **100,** 347-352.

Ross EZ, Cotter JD, Wilson L, Fan JL, Lucas SJ & Ainslie PN. (2012). Cerebrovascular and corticomotor function during progressive passive hyperthermia in humans. *J Appl Physiol* **112,** 748-758.

Ross EZ, Goodall S, Stevens A & Harris I. (2010a). Time course of neuromuscular changes during running in well-trained subjects. *Med Sci Sports Exerc* **42,** 1184-1190.

Ross EZ, Gregson W, Williams K, Robertson C & George K. (2010b). Muscle contractile function and neural control after repetitive endurance cycling. *Med Sci Sports Exerc* **42,** 206-212.

Ross EZ, Middleton N, Shave R, George K & Nowicky A. (2007). Corticomotor excitability contributes to neuromuscular fatigue following marathon running in man. *Exp Physiol* **92,** 417-426.

Rossini PM, Barker AT, Berardelli A, Caramia MD, Caruso G, Cracco RQ, Dimitrijevic MR, Hallett M, Katayama Y, Lucking CH & et al. (1994). Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. *Electroencephalogr Clin Neurophysiol* **91,** 79-92.

Rupp T, Jubeau M, Wuyam B, Perrey S, Levy P, Millet GY & Verges S. (2012). Time-dependent effect of acute hypoxia on corticospinal excitability in healthy humans. *J Neurophysiol* **108,** 1270-1277.

Rupp T & Perrey S. (2008). Prefrontal cortex oxygenation and neuromuscular responses to exhaustive exercise. *Eur J Appl Physiol* **102,** 153-163.

Russ DW & Kent-Braun JA. (2003). Sex differences in human skeletal muscle fatigue are eliminated under ischemic conditions. *J Appl Physiol* **94,** 2414-2422.

Sacco P, Hope PA, Thickbroom GW, Byrnes ML & Mastaglia FL. (1999). Corticomotor excitability and perception of effort during sustained exercise in the chronic fatigue syndrome. *Clin Neurophysiol* **110,** 1883-1891.

Sagaspe P, Sanchez-Ortuno M, Charles A, Taillard J, Valtat C, Bioulac B & Philip P. (2006). Effects of sleep deprivation on Color-Word, Emotional, and Specific Stroop interference and on self-reported anxiety. *Brain Cogn* **60,** 76-87.

Saisanen L, Pirinen E, Teitti S, Kononen M, Julkunen P, Maatta S & Karhu J. (2008). Factors influencing cortical silent period: optimized stimulus location, intensity and muscle contraction. *J Neurosci Methods* **169,** 231-238.

Sakai K, Ugawa Y, Terao Y, Hanajima R, Furubayashi T & Kanazawa I. (1997). Preferential activation of different I waves by transcranial magnetic stimulation with a figure-of-eight-shaped coil. *Exp Brain Res* **113,** 24-32.

Sale MV, Ridding MC & Nordstrom MA. (2008). Cortisol inhibits neuroplasticity induction in human motor cortex. *J Neurosci* **28,** 8285-8293.

Sammut R, Thickbroom GW, Wilson SA & Mastaglia FL. (1995). The origin of the soleus late response evoked by magnetic stimulation of human motor cortex. *Electroencephalogr Clin Neurophysiol* **97,** 164-168.

Sander M, Macefield VG & Henderson LA. (2010). Cortical and brain stem changes in neural activity during static handgrip and postexercise ischemia in humans. *J Appl Physiol* **108,** 1691-1700.

Saugy J, Place N, Millet GY, Degache F, Schena F & Millet GP. (2013). Alterations of neuromuscular function after the world's most challenging mountain ultra-marathon. *PLoS One* **8,** e65596.

Scalise A, Desiato MT, Gigli GL, Romigi A, Tombini M, Marciani MG, Izzi F & Placidi F. (2006). Increasing cortical excitability: a possible explanation for the proconvulsant role of sleep deprivation. *Sleep* **29,** 1595-1598.

Schneider S, Rouffet DM, Billaut F & Struder HK. (2013). Cortical current density oscillations in the motor cortex are correlated with muscular activity during pedaling exercise. *Neuroscience* **228,** 309-314.

Schomburg ED, Steffens H, Dibaj P & Sears TA. (2012). Major contribution of Aδ-fibres to increased reflex transmission in the feline spinal cord during acute muscle inflammation. *Neurosci Res* **72,** 155-162.

Schubert M, Curt A, Colombo G, Berger W & Dietz V. (1999). Voluntary control of human gait: conditioning of magnetically evoked motor responses in a precision stepping task. *Exp Brain Res* **126,** 583-588.

Scott JP & McNaughton LR. (2004). Sleep deprivation, energy expenditure and cardiorespiratory function. *Int J Sports Med* **25,** 421-426.

Scott JP, McNaughton LR & Polman RC. (2006). Effects of sleep deprivation and exercise on cognitive, motor performance and mood. *Physiol Behav* **87,** 396-408.

Secher NH, Seifert T & Van Lieshout JJ. (2008). Cerebral blood flow and metabolism during exercise: implications for fatigue. *J Appl Physiol* **104,** 306-314.

Seifert T & Petersen NC. (2010). Changes in presumed motor cortical activity during fatiguing muscle contraction in humans. *Acta Physiol (Oxf)* **199,** 317-326.

Sidhu SK, Bentley DJ & Carroll TJ. (2009a). Cortical voluntary activation of the human knee extensors can be reliably estimated using transcranial magnetic stimulation. *Muscle Nerve* **39,** 186-196.

Sidhu SK, Bentley DJ & Carroll TJ. (2009b). Locomotor exercise induces long-lasting impairments in the capacity of the human motor cortex to voluntarily activate knee extensor muscles. *J Appl Physiol* **106,** 556-565.

Sidhu SK, Cresswell AG & Carroll TJ. (2012a). Motor cortex excitability does not increase during sustained cycling exercise to volitional exhaustion. *J Appl Physiol* **113,** 401-409.

Sidhu SK, Cresswell AG & Carroll TJ. (2013a). Corticospinal Responses to Sustained Locomotor Exercises: Moving Beyond Single-Joint Studies of Central Fatigue. *Sports Med* **43,** 437-449.

Sidhu SK, Hoffman BW, Cresswell AG & Carroll TJ. (2012b). Corticospinal contributions to lower limb muscle activity during cycling in humans. *J Neurophysiol* **107,** 306-314.

Sidhu SK, Lauber B, Cresswell AG & Carroll TJ. (2013b). Sustained cycling exercise increases intracortical inhibition. *Med Sci Sports Exerc* **45,** 654-662.

Simon JR. (1990). The effects of an irrelevant directional cue on human information processing. In *Stimulus-response compatibility: an integrated perspective*, ed. Proctor RW & Reeve TG, pp. 31-86. North-Holland, Amsterdam.

Skein M, Duffield R, Edge J, Short MJ & Mundel T. (2011). Intermittent-sprint performance and muscle glycogen after 30 h of sleep deprivation. *Med Sci Sports Exerc* **43,** 1301-1311.

Skein M, Duffield R, Minett GM, Snape A & Murphy A. (2013). The effect of overnight sleep deprivation following competitive rugby league matches on post-match physiological and perceptual recovery. *Int J Sports Physiol Perform* **8,** 556-564.

Smith JL, Martin PG, Gandevia SC & Taylor JL. (2007). Sustained contraction at very low forces produces prominent supraspinal fatigue in human elbow flexor muscles. *J Appl Physiol* **103,** 560-568.

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

Sommer M, Alfaro A, Rummel M, Speck S, Lang N, Tings T & Paulus W. (2006). Half sine, monophasic and biphasic transcranial magnetic stimulation of the human motor cortex. *Clin Neurophysiol* **117,** 838-844.

Sommer M, Lang N, Tergau F & Paulus W. (2002). Neuronal tissue polarization induced by repetitive transcranial magnetic stimulation? *Neuroreport* **13,** 809-811.

Stefan K, Kunesch E, Cohen LG, Benecke R & Classen J. (2000). Induction of plasticity in the human motor cortex by paired associative stimulation. *Brain* **123,** 572-584.

Stevens-Lapsley JE, Thomas AC, Hedgecock JB & Kluger BM. (2013). Corticospinal and intracortical excitability of the quadriceps in active older and younger healthy adults. *Arch Gerontol Geriatr* **56,** 279-284.

Symons JD, Bell DG, Pope J, VanHelder T & Myles WS. (1988a). Electro-mechanical response times and muscle strength after sleep deprivation. *Can J Sport Sci* **13,** 225-230.

Symons JD, VanHelder T & Myles WS. (1988b). Physical performance and physiological responses following 60 hours of sleep deprivation. *Med Sci Sports Exerc* **20,** 374-380.

Szubski C, Burtscher M & Loscher WN. (2007). Neuromuscular fatigue during sustained contractions performed in short-term hypoxia. *Med Sci Sports Exerc* **39,** 948-954.

Tallent J, Goodall S, Hortobagyi T, St Clair Gibson A, French DN & Howatson G. (2012). Repeatability of corticospinal and spinal measures during lengthening and shortening contractions in the human tibialis anterior muscle. *PLoS One* **7,** e35930.

Tallent J, Goodall S, Hortobagyi T, St Clair Gibson A & Howatson G. (2013). Corticospinal responses of resistance-trained and un-trained males during dynamic muscle contractions. *J Electromyogr Kinesiol* **23,** 1075-1081.

Tarkka IM, McKay WB, Sherwood AM & Dimitrijevic MR. (1995). Early and late motor evoked potentials reflect preset agonist-antagonist organization in lower limb muscles. *Muscle Nerve* **18,** 276-282.

Taylor JL. (2006). Stimulation at the cervicomedullary junction in human subjects. *J Electromyogr Kinesiol* **16,** 215-223.

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

Taylor JL, Allen GM, Butler JE & Gandevia SC. (1997). Effect of contraction strength on responses in biceps brachii and adductor pollicis to transcranial magnetic stimulation. *Exp Brain Res* **117,** 472-478.

Taylor JL, Allen GM, Butler JE & Gandevia SC. (2000). Supraspinal fatigue during intermittent maximal voluntary contractions of the human elbow flexors. *J Appl Physiol* **89,** 305-313.

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

Taylor JL & Gandevia SC. (2001). Transcranial magnetic stimulation and human muscle fatigue. *Muscle Nerve* **24,** 18-29.

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, Arnal PJ, Davranche K, Bonnefoy R, Levy P, Verges S & Millet GY. (2013). Does central fatigue explain reduced cycling after complete sleep deprivation? *Med Sci Sports Exerc*. DOI: 10.1249/MSS.0b013e31829ce379.

Terao Y, Ugawa Y, Hanajima R, Machii K, Furubayashi T, Mochizuki H, Enomoto H, Shiio Y, Uesugi H, Iwata NK & Kanazawa I. (2000). Predominant activation of I1-waves from the leg motor area by transcranial magnetic stimulation. *Brain Res* **859,** 137-146.

Magstim website [Internet]. Whitland, UK: The Magstim Co., Ltd.; [cited 03 April 2013]. Available from: www.magstim.com.

Thompson SP. (1910). A physiological effect of an alternating magnetic field. *Proc R Soc Lond B* **82,** 396-398.

Timinkul A, Kato M, Omori T, Deocaris CC, Ito A, Kizuka T, Sakairi Y, Nishijima T, Asada T & Soya H. (2008). Enhancing effect of cerebral blood volume by mild exercise in healthy young men: a near-infrared spectroscopy study. *Neurosci Res* **61,** 242-248.

Todd G, Butler JE, Taylor JL & Gandevia SC. (2005). Hyperthermia: a failure of the motor cortex and the muscle. *J Physiol* **563,** 621-631.

Todd G, Flavel SC & Ridding MC. (2006). Low-intensity repetitive transcranial magnetic stimulation decreases motor cortical excitability in humans. *J Appl Physiol* **101,** 500-505.

Todd G, Taylor JL & Gandevia SC. (2003). Measurement of voluntary activation of fresh and fatigued human muscles using transcranial magnetic stimulation. *J Physiol* **551,** 661-671.

Tomazin K, Verges S, Decorte N, Oulerich A, Maffiuletti NA & Millet GY. (2011). Fat tissue alters quadriceps response to femoral nerve magnetic stimulation. *Clin Neurophysiol* **122,** 842-847.

Tsai LL, Young HY, Hsieh S & Lee CS. (2005). Impairment of error monitoring following sleep deprivation. *Sleep* **28,** 707-713.

Ugawa Y, Terao Y, Hanajima R, Sakai K & Kanazawa I. (1995). Facilitatory effect of tonic voluntary contraction on responses to motor cortex stimulation. *Electroencephalogr Clin Neurophysiol* **97,** 451-454.

Valls-Sole J, Pascual-Leone A, Wassermann EM & Hallett M. (1992). Human motor evoked responses to paired transcranial magnetic stimuli. *Electroencephalogr Clin Neurophysiol* **85,** 355-364.

Verges S, Maffiuletti NA, Kerherve H, Decorte N, Wuyam B & Millet GY. (2009). Comparison of electrical and magnetic stimulations to assess quadriceps muscle function. *J Appl Physiol* **106,** 701-710.

Verges S, Rupp T, Jubeau M, Wuyam B, Esteve F, Levy P, Perrey S & Millet GY. (2012). Cerebral perturbations during exercise in hypoxia. *Am J Physiol Regul Integr Comp Physiol* **302,** R903-916.

Verin E, Ross E, Demoule A, Hopkinson N, Nickol A, Fauroux B, Moxham J, Similowski T & Polkey MI. (2004). Effects of exhaustive incremental treadmill exercise on diaphragm and quadriceps motor potentials evoked by transcranial magnetic stimulation. *J Appl Physiol* **96,** 253-259.

Vesia M, Yan X, Henriques DY, Sergio LE & Crawford JD. (2008). Transcranial magnetic stimulation over human dorsal-lateral posterior parietal cortex disrupts integration of hand position signals into the reach plan. *J Neurophysiol* **100,** 2005-2014.

Viitasalo JT, Komi PV, Jacobs I & Karlsson J. (1982). Effects of prolonged cross-country skiing on neuromuscular performance. In *Exercise and Sport Biology*, ed. Komi PV, pp. 191-198. Human Kinetics, Champaign, USA.

Vila-Cha C, Falla D & Farina D. (2010). Motor unit behavior during submaximal contractions following six weeks of either endurance or strength training. *J Appl Physiol* **109,** 1455-1466.

Villamar MF, Santos Portilla A, Fregni F & Zafonte R. (2012). Noninvasive brain stimulation to modulate neuroplasticity in traumatic brain injury. *Neuromodulation* **15,** 326-338.

Volianitis S, Fabricius-Bjerre A, Overgaard A, Stromstad M, Bjarrum M, Carlson C, Petersen NT, Rasmussen P, Secher NH & Nielsen HB. (2008). The cerebral metabolic ratio is not affected by oxygen availability during maximal exercise in humans. *J Physiol* **586,** 107-112.

Watanabe K, Kouzaki M & Moritani T. (2013). Region-specific myoelectric manifestations of fatigue in human rectus femoris muscle. *Muscle Nerve* **48,** 226-234.

Weier AT, Pearce AJ & Kidgell DJ. (2012). Strength training reduces intracortical inhibition. *Acta Physiol* **206,** 109-119.

Werhahn KJ, Fong JK, Meyer BU, Priori A, Rothwell JC, Day BL & Thompson PD. (1994). The effect of magnetic coil orientation on the latency of surface EMG and single motor unit responses in the first dorsal interosseous muscle. *Electroencephalogr Clin Neurophysiol* **93,** 138-146.

Yagi Y, Coburn KL, Estes KM & Arruda JE. (1999). Effects of aerobic exercise and gender on visual and auditory P300, reaction time, and accuracy. *Eur J Appl Physiol Occup Physiol* **80,** 402-408.

Yang YR, Chen IH, Liao KK, Huang CC & Wang RY. (2010). Cortical reorganization induced by body weight-supported treadmill training in patients with hemiparesis of different stroke durations. *Arch Phys Med Rehabil* **91,** 513-518.

Yoon T, Keller ML, De-Lap BS, Harkins A, Lepers R & Hunter SK. (2009). Sex differences in response to cognitive stress during a fatiguing contraction. *J Appl Physiol* **107,** 1486-1496.

Yoon T, Schlinder-Delap B & Hunter SK. (2013). Fatigability and recovery of arm muscles with advanced age for dynamic and isometric contractions. *Exp Gerontol* **48,** 259-268.

Yoon T, Schlinder-Delap B, Keller ML & Hunter SK. (2012). Supraspinal fatigue impedes recovery from a low-intensity sustained contraction in old adults. *J Appl Physiol* **112,** 849-858.

APPENDIX – Associated Publications

Associated Publication 1

Gruet M, **Temesi J**, Rupp T, Levy P, Millet GY & Vergès S. (2013). Stimulation of the motor cortex and corticospinal tract to assess human muscle fatigue. *Neuroscience* **231**, 384-399.

Associated Publication 2

Bachasson D, **Temesi J**, Bankole C, Lagrange E, Boutte C, Millet GY, Vergès S, Levy P, Féasson L & Wuyam B. (2013). Assessment of quadriceps strength, endurance and fatigue in FSHD and CMT: benefits and limits of femoral nerve magnetic stimulation. *Clin Neurophysiol*. DOI: 10.1016/j.clinph.2013.08.001.

Associated Publication 3

Millet GY, Bachasson D, **Temesi J**, Wuyam B, Féasson L, Vergès S & Levy P. (2012). Potential interests and limits of magnetic and electrical stimulation techniques to assess neuromuscular fatigue. *Neuromuscul Disord* **22**, S181-186.

Neuroscience 231 (2013) 384–399

REVIEW

STIMULATION OF THE MOTOR CORTEX AND CORTICOSPINAL TRACT TO ASSESS HUMAN MUSCLE FATIGUE

M. GRUET, [a,b] **J. TEMESI,** [c] **T. RUPP,** [a,b] **P. LEVY,** [a,b,d] **G. Y. MILLET** [a,c] **AND S. VERGES** [a,b,d]*

[a] *INSERM U1042, Grenoble F-38000, France*

[b] *Laboratoire HP2, Université Joseph Fourier, Grenoble F-38000, France*

[c] *Université de Lyon, F-42023 Saint-Etienne, France*

[d] *Pole Locomotion, Rééducation & Physiologie, Clinique Physiologie, Sommeil et Exercice, CHU Grenoble, F-38000 Grenoble, France*

Abstract—This review aims to characterize fatigue-related changes in corticospinal excitability and inhibition in healthy subjects. Transcranial magnetic stimulation (TMS) has been extensively used in recent years to investigate modifications within the brain during and after fatiguing exercise. Single-pulse TMS reveals reduction in motor-evoked potentials (MEP) when measured in relaxed muscle following sustained fatiguing contractions. This modulation of corticospinal excitability observed in relaxed muscle is probably not specific to the fatigue induced by the motor task. During maximal and submaximal fatiguing contractions, voluntary activation measured by TMS decreases, suggesting the presence of supraspinal fatigue. The demonstration of supraspinal fatigue does not eliminate the possibility of spinal contribution to central fatigue. Concomitant measurement of TMS-induced MEP and cervicomedullary MEP in the contracting muscle, appropriately normalized to maximal muscle compound action potential, is necessary to determine the relative contribution of cortical and spinal mechanisms in the development of central fatigue. Recent studies comparing electromyographic (EMG) responses to paired-pulse stimuli at the cortical and subcortical levels suggest that impaired motoneuron responsiveness rather than intracortical inhibition may contribute to the development of central fatigue. This review examines the mechanical and EMG responses elicited by TMS (single- and paired-pulse) and cervicomedullary stimulation both during and after a fatiguing exercise. Particular attention is given to the muscle state and the type of fatiguing exercise when assessing and interpreting fatigue-induced changes in these parameters. Methodological concerns and future research interests are also considered.

Key words: cervicomedullary stimulation, corticospinal excitability, fatiguing muscular contractions, motoneurons, motor cortex, transcranial magnetic stimulation.

Contents

INTRODUCTION

Fatigue is a common non-specific symptom experienced by many people and associated with many health conditions. Often described as a feeling of weariness or lack of energy, it relates to the difficulty in performing voluntary tasks. Fatigue can be classified as mental, referring to the cognitive or perceptual aspects of fatigue, or physical, referring to the performance of the motor system. Muscle fatigue can be defined as an exercise-induced reduction in the ability of a muscle or muscle group to generate maximal force or power (Gandevia, 2001). It can originate at different levels of the motor pathway and is usually divided into central and peripheral components. Peripheral fatigue is produced by changes at or distal to the neuromuscular junction. It can be demonstrated by a reduction in twitch

*Correspondence to: S. Verges, Laboratoire HP2 (U1042 INSERM), UF Recherche sur l'Exercice Hôpital Sud, Avenue Kimberley, 38 434 Echirolles, France. Tel: +33-6-70-39-57-73; fax: +33-4-76-76-56-17.
E-mail addresses: gruetma@gmail.com (M. Gruet), sverges@chu-grenoble.fr (S. Verges).

Abbreviations: c*M*max, concomitant *M*max; CMEP, cervicomedullary motor-evoked potential; EMG, electromyographic; LICI, long-interval intracortical inhibition; MEP, motor-evoked potentials; *M*max, maximal muscle compound action potential; MSR, moderate sustainable rate; $MSR_{/2}$, rate half that of MSR; MVC, maximal voluntary contraction; MVR, maximal voluntary rate; SICI, short-interval intracortical inhibition; SIT, superimposed twitch; SP, silent period; TMS, transcranial magnetic stimulation; VA, voluntary activation.

0306-4522/12 $36.00 © 2012 IBRO. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neuroscience.2012.10.058

or tetanic force elicited by peripheral nerve stimulation in the relaxed muscle. Mechanisms related to peripheral fatigue are often insufficient to explain the entire fatigue-related decrease in maximal voluntary force (Millet and Lepers, 2004; Taylor and Gandevia, 2008), thus, some fatigue must be related to modifications within the central nervous system. Central fatigue is defined as a progressive failure to voluntarily activate the muscle and it can originate at both the spinal and supraspinal levels. The peripheral and central components of muscle fatigue are intrinsically related since the recruitment of motoneurons depends on the descending drive from supraspinal sites and the central drive is controlled through a combination of excitatory and inhibitory reflex inputs from muscles, joints, tendons and cutaneous afferents (Millet, 2011). Maximal voluntary activation (VA), as estimated using twitch interpolation (Merton, 1954) is the most conventional technique to assess central fatigue during exercise (Gandevia, 2001; Millet et al., 2003). It involves artificially generating action potentials to propagate along the axons of lower motoneurons by supramaximal stimulation of the peripheral nerve during a maximal voluntary contraction (MVC). If lower motoneurons are not recruited during the MVC or are not firing fast enough, then the stimulus will evoke additional force production, termed superimposed twitch (SIT). The ratio between SIT and a potentiated twitch elicited in the relaxed muscle allows the quantification of VA. A decrease in VA during or after sustained contraction suggests that the failure to drive the muscle occurs at or above the stimulation site on the axons of the lower motoneurons (i.e. central fatigue) (Allen et al., 1998). However, twitch interpolation does not quantify the descending drive to the lower motoneurons nor does it take into account the source of this drive. The part of central fatigue resulting from deficient motor cortical output (i.e. supraspinal fatigue) is thus unknown. Other neurostimulation techniques are required to investigate the corticospinal component of fatigue.

Transcranial magnetic stimulation (TMS) is a non-invasive, pain-less and safe technique to investigate the human motor cortex (Ridding and Rothwell, 2007). TMS is often used to characterize alterations in central motor pathways in neurological diseases (e.g. multiple sclerosis, stroke, chronic fatigue syndrome) and to attempt to link self-reported fatigue to neuromuscular deficiencies in these pathologies (Sacco et al., 1999; Liepert et al., 2005; Knorr et al., 2011). During brief contractions, TMS over the motor cortex can elicit additional force production, i.e. SIT, despite maximal volitional effort (Gandevia et al., 1996). This implies that motor cortical output is suboptimal and therefore insufficient to fully activate all motor units and generate maximal muscular force. Thus, an increase in SIT elicited by TMS during a sustained fatiguing contraction indicates that some fatigue is related to supraspinal mechanisms (Gandevia et al., 1996). However, the presence of supraspinal fatigue does not eliminate the possibility of spinal contribution to central fatigue. Recent studies using TMS investigated modulation in cortical excitability with fatigue. Single-pulse TMS reveals changes in motor-evoked potential (MEP) characteristics during and after a fatiguing exercise. MEP changes can originate at different levels of the motor pathway and appropriate normalization to spinal and peripheral indices is needed to determine the contributions of each. The concomitant use of different neurostimulation techniques is thus required. The main techniques utilized and their resultant evoked parameters are summarized in Table 1. One difficulty is to decide whether these changes are associated with central fatigue and which alterations actually contribute to the decrease in voluntary force. Another is that exercise-related changes in corticospinal excitability may depend on the intensity of the fatiguing task (i.e. maximal vs. submaximal), the functional characteristics of the investigated muscle (i.e. extensor vs. flexor) and the muscle state at the moment of stimulation (i.e. contracted vs. relaxed). Thus, changes in corticospinal excitability during and after a fatiguing exercise must be delineated according to these specific conditions.

This review considers the fatigue-related changes of parameters usually measured with TMS in healthy subjects and analyzes the relationship between cortical excitability and central fatigue. Our approach examines changes in mechanical and electromyographic (EMG) parameters elicited by single-pulse and paired-pulse TMS during and after different fatiguing tasks (i.e. submaximal and maximal) involving different muscles (i.e. of the upper and lower limbs). Studies examining

Table 1. Main neurostimulation techniques used to evaluate muscle fatigue

Stimulation Techniques		Variables	Parameters measured
Transcranial magnetic stimulation	Single pulse	MEP	Corticospinal excitability
		SP	Duration of intracortical $GABA_B$-mediated inhibition
		SIT	Supraspinal deficit
		ERT	Estimated resting twitch used to quantify cortical VA
		Cortical VA	Cortical maximal voluntary activation
	Paired pulses	SICI	Magnitude of intracortical $GABA_A$-mediated inhibition
		LICI	Magnitude of intracortical $GABA_B$-mediated inhibition
Cervicomedullary stimulation		CMEP	Motoneuronal excitability
Peripheral nerve stimulation		*M*max	Sarcolemmal excitability
		Twitch	Skeletal muscle contractility
		VA	Maximal voluntary activation

CMEP, cervicomedullary motor-evoked potentials; ERT, estimated resting twitch; LICI, long-interval intracortical inhibition; MEP, motor-evoked potential; *M*max, maximal muscle compound action potential; SICI, short-interval intracortical inhibition; SIT, superimposed twitch; SP, silent period; VA, voluntary activation.

EMG responses evoked in the relaxed muscle and during brief contractions are separated as the muscular state appears to be a crucial factor in explaining discrepancies between studies (Gandevia and Taylor, 2006; McNeil et al., 2009). With the aim to differentiate between spinal and supraspinal mechanisms, changes in cervicomedullary stimulation-induced EMG responses with fatigue are also incorporated. Some important methodological concerns, including methods of normalization and quantification of EMG and mechanical parameters elicited by TMS, are also considered.

SINGLE-PULSE TMS

Measurements in relaxed muscle

MEP changes after fatiguing exercise were first observed by Brasil-Neto et al. (1993) following an exhaustive exercise of the wrist. A reduction in MEP amplitude relative to the baseline in relaxed muscle, termed post-exercise depression, was found. This result was not associated with changes in maximal muscle compound action potential (*M*max) or *H*-reflex (i.e. an index of α-motoneuron excitability and/or modulation of its pre-synaptic inhibition) amplitudes and was thus interpreted as decreased cortical excitability or efficiency in the generation of the motor command. This MEP depression was confirmed in subsequent studies following single-joint maximal (Pitcher et al., 2005) and submaximal (Khedr et al., 2007; Milanovic et al., 2011) isometric muscular contractions and whole-body exercise such as running (Hollge et al., 1997) and rowing (Fulton et al., 2002). This depression generally peaks within 5 min after exercise cessation and recovers several minutes thereafter (McKay et al., 1995; Zanette et al., 1995). Probably influenced by the lack of diminution of *M*max and *H*-reflex amplitudes with exercise as observed by Brasil-Neto et al. (1993), the aforementioned studies often attributed changes in MEP amplitude to a cortical mechanism without appropriately addressing the question of peripheral and spinal fatigue. Indeed, many studies did not report any measure of peripheral signal transmission (Brasil-Neto et al., 1994; Samii et al., 1996; Lou et al., 2003; Humphry et al., 2004; Perretti et al., 2004; Thickbroom et al., 2008) whereas others reported *M*max characteristics from a separate experiment (Lentz and Nielsen, 2002), a different muscle (Hollge et al., 1997) or in only a subgroup of subjects (Cerri et al., 2010). Significant MEP depression associated with non-significant decrease in *M*max can lead to unchanged MEP/*M*max ratios. Thus, to account for activity-dependent changes in peripheral signal conduction, it is essential to systematically normalize MEP to concomitant *M*max (c*M*max, i.e. elicited nearby in time) (Kalmar and Cafarelli, 2004). A study found a reduced MEP/c*M*max ratio following exercise (Gandevia et al., 1999) whereas another one did not (Zijdewind et al., 2000) (Table 2).

MEP depression without significant changes in *F*-wave (i.e. an index of spinal excitability) or *H*-reflex amplitudes has been reported after fatiguing exercise (Brasil-Neto et al., 1993; Zanette et al., 1995), suggesting an absence of lower motoneuron involvement. To further investigate the role of spinal excitability in MEP changes, electrical stimulation at the cervicomedullary junction (between the mastoids) can be used to evoke single excitatory corticospinal volleys (cervicomedullary motor-evoked potential – CMEP) (Ugawa et al., 1991b). Unlike the *H* reflex, CMEP are thought to be unaffected by presynaptic inhibition (Nielsen and Petersen, 1994), thus making transmastoid stimulation a direct approach to evaluate motoneuron excitability. Although cervicomedullary stimulation primarily activates axons in the corticospinal tract, caution is necessary when interpreting CMEP studies as the stimulus may also activate other structures. Antidromic volleys in Ia afferents (Taylor et al., 2001), vestibular afferents (Watson and Colebatch, 1998) and the cerebellum (Ugawa et al., 1991a) may have minor influences on CMEP responses evoked by electrical transmastoid stimulation (Taylor and Gandevia, 2004; Taylor, 2006). Gandevia et al. (1999) observed a simultaneous reduction of CMEP/c*M*max and MEP/c*M*max areas in the relaxed muscle following a sustained MVC of the elbow flexors, suggesting that diminished motoneuron excitability may contribute to the MEP depression. Thus, it is likely that peripheral and/or spinal limitation contributed to the MEP depression in some of the aforementioned studies, leading to overestimation of cortical deficit. Table 3 summarizes studies that have measured MEP in the relaxed muscle and reported indices of peripheral or spinal transmission before and after a fatiguing exercise.

The MEP depression observed in the relaxed muscle may not only be the consequence of mechanisms originating within the motor cortex, it may also be

Table 2. Summary of changes in EMG and mechanical parameters elicited by single-pulse TMS during and after fatiguing exercise from the current literature

Variables	Kinetics during exercise (from start to task failure)		After exercise (relative to pre-exercise)	
	Maximal	Submaximal	Relaxed muscle	Contracted muscle
MEP/c*M*max	Increase	Progressive linear increase	Depressed or unchanged	Lack of consensus[a]
SP	Rapid increase and then plateau	Progressive increase	N/A	Facilitated
SIT	Increase	Progressive increase	N/A	Facilitated
Cortical VA	Decrease	Progressive decrease	N/A	Depressed

c*M*max, concomitant maximal muscle compound action potential; MEP, motor-evoked potential; SIT, superimposed twitch; SP, silent period; VA, voluntary activation.
[a] MEP/c*M*max changes after exercise are largely dependent on how and when post-exercise measurements are conducted (see text for details).

Table 3. MEP measured in the relaxed muscle after a fatiguing exercise

References	*n*	Fatiguing task characteristics	Task duration	Main muscle	MEP responses	Peripheral and/or spinal indices
Gandevia et al. (1999)	7	Isometric MVC of elbow flexors	2 min	Biceps brachii	MEP/c*M*max area depression that did not recover for over 12 min PE[a]	CMEP/c*M*max area depression that recovered within 2 min PE
Brasil-Neto et al. (1993)	6	Wrist flexion–extension until exhaustion while holding a 3.4 kg dumbbell weight	Unknown	Flexor carpi radialis	MEP depression of ~60% during the first 1 min PE[a]	Unchanged *M*max or *H* reflex
Zanette et al. (1995)	11	Abduction–adduction of the thumb against the little finger at maximal rate	1 min	Thenar eminence	MEP depression of ~55% (maximal at ~5 min PE) that recovered by 35 min PE	Unchanged *M*max or *F* wave
Zijdewind et al. (2000)	10	Isometric abduction of the index finger at 50% MVC	~2 min	First dorsal interosseous	Unchanged MEP/c*M*max	Unchanged *M*max
Pitcher and Miles (2002)	12	Isometric abduction of the first dorsal interosseous: (1) MVC, (2) supramaximal motor point stimulation	2 min	First dorsal interosseous	(1) MEP depression of ~50% immediately PE, and ~25% at 20 min PE (2) MEP facilitation during the first 10 min PE, followed by a MEP depression of ~25% at 20 min PE that recovered by 50 min PE	Reduced *M*max immediately PE for both tasks (recovery by 20 min PE), Unchanged *F* wave/*M*max ratio
Khedr et al. (2007)	10	Opening a binder clip by 10 mm (mean force required: 30 N) using index finger and thumb	10 min	First dorsal interosseous	MEP depression immediately PE that recovered by 20 min	Reduced *M*max immediately PE that recovered by 20 min
Kluger et al. (2012)	20	(1) Isometric handgrip at force above 75% MVC as long as possible and then MVC until force decreases below 40% MVC, (2) Finger tapping (thumb against index) at maximal rate	(1) 116 ± 46 s, (2) 10 min	First dorsal interosseous	(1) Unchanged MEP at 0, 2 and 4 min PE (2) MEP depression of 24% only at 2 min PE	Unchanged *M*max for both tasks
McKay et al. (1995)	5	Isometric MVC of ankle dorsiflexors, until force decreased to 50% MVC	80 ± 7 s	Tibialis anterior	Mean MEP depression of 51% over the 30-min PE period	Unchanged *M*max or *H* reflex
Lentz and Nielsen (2002)	20	Isometric ankle dorsilexion: (1) from 100% to 75% MVC, (2) from 100% to 50% MVC, (3) from 100% to 25% MVC, (4) from 50% to 25% MVC	(1) 21 ± 6 s, (2) 57 ± 19 s, (3) 147 ± 49 s, (4) 204 ± 53 s	Tibialis anterior	MEP depression of a similar range for the four tasks that did not recover for over 10-min PE	Reduced *M*max area only for the 50–25% MVC task

CMEP, cervicomedullary motor-evoked potentials; c*M*max, concomitant *M*max; MEP, motor-evoked potential; *M*max, maximal muscle compound action potential; MVC, maximal voluntary contraction; *n*, number of subjects (for the main part of the experiment); PE, post-exercise. When not specified, amplitude values are reported in the last two columns.

[a] No information about statistical significance.

caused by afferent input from the contracting muscle. Pitcher and Miles (2002) measured MEP changes before and after two fatiguing tasks of the first dorsal interosseous: a sustained 2-min MVC and 2 min of low-frequency electrical stimulation evoked at the motor point. MEP and *M*max depression were induced by both voluntary and electrically-evoked contractions and MEP recovery occurred before that of *M*max. *F* wave and *M*max changed in parallel after both protocols so that the *F*-wave/*M*max ratio remained unchanged, indicating that MEP depression may not be mediated by a decrease in lower motoneuron excitability. However, *F* waves present limitations in evaluating changes in lower motoneuron excitability (Espiritu et al., 2003). For instance, it is unclear whether changes in *F* waves reflect lower motoneuron responses to synaptic input (Hultborn and Nielsen, 1995) because they only test a fraction of the motoneuron pool. The depression in CMEP/c*M*max area observed by Gandevia et al. (1999) in the relaxed elbow flexors following a 2-min MVC recovered to baseline values within 2 min whereas MEP/c*M*max remained depressed for more than 10 min. Thus, it is likely that reduced spinal excitability contributes to the early phase of MEP/c*M*max depression (i.e. first 2 min post-exercise) and has little to no influence thereafter. Therefore, the post-exercise MEP depression observed in the relaxed muscle may be, at least in part, induced by afferent discharges from the exercising muscle that alter cortical excitability and possibly motoneuron excitability. The nature of afferent contribution to MEP depression in the relaxed muscle remains unclear. Muscles are innervated by many small-diameter unmyelinated afferents, called group III and IV afferents, that respond to various chemical (e.g. lactic acid, extracellular ion concentrations) and mechanical (e.g. distension of the peripheral vascular bed) changes in the muscle (Rotto and Kaufman, 1988; Haouzi et al., 1999). The maintenance of muscle ischemia is an effective technique to analyze the reflex effects of group III and IV afferents (Kaufman et al., 1984). To determine whether MEP depression in the relaxed muscle could be maintained by the continued firing of group III and IV afferents, (Taylor et al., 2000c) measured MEP responses in elbow flexors that were held ischemic after a 2-min MVC. To compare ischemic changes in response to motor cortical stimulation to those under non-ischemic conditions, the authors used control data from the same subjects obtained from another study separated by approximately one week (Gandevia et al., 1999). The time course of the MEP following exercise (i.e. gradual decrease over 2 min and depression maintained for more than 10 min) was unaffected by post-contraction ischemia, indicating that group III and IV muscle afferents do not mediate post-exercise MEP depression. As suggested by Taylor et al. (2000b), Golgi tendon organs and non-spindle group II afferents might act at the cortical level during a sustained fatiguing contraction and regulate the descending drive in response to changes in muscular force output. In that case, the firing of such afferents may contribute to the MEP/c*M*max depression observed in relaxed muscle after voluntary contraction (Taylor et al., 2000c) and to the MEP depression after electrically-induced fatiguing contractions (Pitcher and Miles, 2002).

Another crucial question is whether MEP depression in the relaxed muscle is specific to the fatiguing aspect of the motor task. Teo et al. (2012) recently recorded MEP amplitude from the first dorsal interosseus muscle following 10 s of index finger flexion–extension performed at three different rates: maximal voluntary rate (MVR), moderate sustainable rate (MSR) and a rate half that of MSR ($MSR_{/2}$) (Fig. 1). They found significant MEP facilitation after MVR and significant MEP depression after all tasks with greater and longer-lasting reductions after MSR and $MSR_{/2}$ tasks. Although these results are difficult to explain, they suggest that MEP changes following exercise may not only be specific to the fatiguing aspect of the motor task and can also reflect central plastic changes associated with repetitive movements. Kluger et al. (2012) measured MEP changes in the relaxed first dorsal interosseous muscle before and after an imagined hand-grip task. The subjects were asked to imagine squeezing something with their dominant hand as hard as possible for a 2-min period. Surface EMG was used to ensure that the muscle was fully relaxed. Significant MEP depression of ~20% was observed, suggesting that central initiation of motor programs can also induce post-exercise decreases in cortical excitability in the absence of motor fatigue.

In summary, MEP depression can occur in the relaxed muscle following a fatiguing exercise. Its origins may involve afferent input from the fatigued muscle. However, this modulation in cortical excitability is not necessarily related to the fatiguing aspect of the motor task. It is also important to note that in the studies in which TMS is delivered to the relaxed muscle, central fatigue cannot be directly proved as no mechanical measurements (i.e. SIT and VA, see below) are reported. Consequently, it is unknown whether central fatigue was implicated in these studies, and thus whether MEP changes were associated with insufficient cortical drive to the muscle.

Measurements in contracting muscle

SIT and cortical VA. During a sustained isometric MVC, the SIT evoked by motor nerve stimulation increases, suggesting the development of central fatigue (Table 2) (Gandevia et al., 1996; Taylor and Gandevia, 2008). Similarly, the SIT elicited by TMS also increases, indicating that some fatigue is related to supraspinal mechanisms (Gandevia et al., 1996). Initially demonstrated during a 2-min MVC of the elbow flexors (Gandevia et al., 1996), the increase in SIT produced by TMS during maximal contractions was confirmed in various muscle groups (i.e. of the upper and lower limbs) and several exercise paradigms, i.e. continuous and intermittent maximal and submaximal contractions (Todd et al., 2005; Sogaard et al., 2006; Smith et al.,

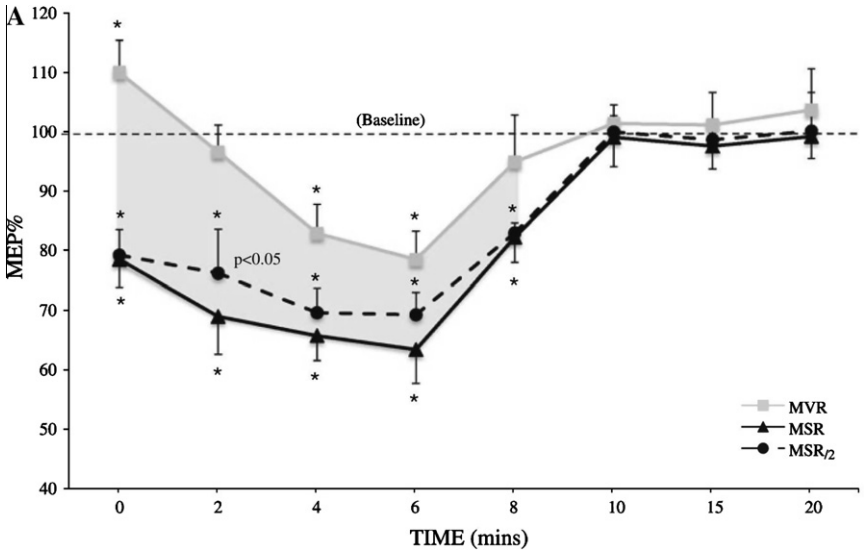

Fig. 1. Change in first dorsal interosseus MEP (expressed as % of baseline values) after each movement task. Comparison of MEP% following a 10-s index finger flexion–extension task at maximal voluntary rate (MVR), moderate sustainable rate (MSR) and a rate half that of MSR ($MSR_{/2}$). * Indicates significant difference ($P < 0.05$) from the baseline and the gray region indicates significant difference between MVR and the submaximal tasks (MSR and $MSR_{/2}$). From Teo et al. (2012).

2007; Hunter et al., 2008; Lee et al., 2008; Sidhu et al., 2009a; Mileva et al., 2012). Cortical VA decreases during sustained maximal (Hunter et al., 2006; Szubski et al., 2007) and submaximal (Smith et al., 2007) isometric fatiguing contractions (Table 2), indicating the progressive development of supraspinal fatigue. Cortical VA is also reduced compared to baseline values following whole-body fatiguing exercise (Ross et al., 2007; Sidhu et al., 2009b; Fernandez-Del-Olmo et al., 2011; Goodall et al., 2012).

The assessment of cortical VA using TMS is more complicated than standard twitch interpolation with peripheral stimulation (Todd et al., 2003). First, it is inappropriate to normalize the SIT elicited during voluntary contractions to that evoked in the relaxed muscle because the motoneuron output evoked by TMS cannot be compared between resting and contracting muscular conditions. This is due to the large increase in corticospinal excitability during the transition from rest to voluntary muscular contraction (Di Lazzaro et al., 1998). Todd et al. (2003) proposed to extrapolate the linear relationship between SIT and voluntary force between 50% and 100% MVC to estimate the size of the resting twitch that would be produced by TMS under comparable conditions of corticospinal excitability. Originally applied in the elbow flexors (Todd et al., 2003), the validity and reliability of extrapolating the relationship between TMS-evoked SIT and voluntary forces at 50%, 75% and 100% MVC has been confirmed in other muscle groups (Lee et al., 2008; Goodall et al., 2009; Sidhu et al., 2009a; Mileva et al., 2012). It is accepted that this method can quantify cortical VA in fresh and fatigued muscles although some methodological concerns remain. The regression of voluntary torque and the SIT is almost always linear in control (i.e. without fatigue) conditions, allowing the estimation of resting twitch amplitude and thus cortical VA (Todd et al., 2003; Hunter et al., 2006; Cahill et al., 2011). However, this relation is frequently non-linear ($r < 0.9$) (e.g. 33% in Hunter et al. (2006)) during or after a fatigue protocol, preventing the estimation of the resting twitch in some subjects (del Olmo et al., 2006; Hunter et al., 2006). To obtain a valid linear extrapolation, it is essential that the stimuli activate most of the motoneurons, which is possible at high levels of force (i.e. >50% MVC) (Goodall et al., 2009). Indeed, TMS is less effective at activating motoneurons at lower contraction levels because of the reduction in corticospinal excitability (Todd et al., 2003). This is characterized by a curvilinear relationship between SIT and voluntary torque when using contraction strengths below 50% MVC (del Olmo et al., 2006; Lee et al., 2008). It may also be impossible to obtain a SIT at high-contraction intensities (>75% MVC) (del Olmo et al., 2006). Therefore, if a SIT can be evoked at high-contraction intensities and if the relationship between SIT and force (50–100% MVC) appears to be linear ($r \geqslant 0.9$), then it is appropriate to estimate resting twitch amplitude and calculate cortical VA.

The decline in cortical VA indicates supraspinal fatigue but does not eliminate the possibility of spinal contribution to central fatigue. The investigation of central excitability and inhibitory parameters measured in contracting muscle before, during and after a fatiguing motor task may help in understanding the origins of central fatigue as characterized by SIT and cortical VA.

MEP. Several recent studies delivered TMS during brief contractions at different levels of force before, during and following exhaustive exercise to assess MEP changes (Szubski et al., 2007; Iguchi and Shields, 2011; Mileva et al., 2012; Sidhu et al., 2012). This methodology has several advantages over assessing

MEP changes in relaxed muscle. First, it has been shown that MEP variability is lower during contractions than in relaxed muscle (Darling et al., 2006), leading to a more reliable estimate of MEP changes with fatigue. Second, analysis of the behavior of the motor cortex during a fatiguing contraction with TMS delivered in relaxed muscle (i.e. during the ''off phase'' of an intermittent exercise) is questionable because motor cortical excitability is greatly modified when muscle is in the contracted state. Thus, it would be more appropriate to analyze MEP kinetics throughout a fatiguing contraction with TMS delivered at intervals during the contraction, i.e. in the condition where central fatigue may occur. Although the majority of recent studies report MEP/c*M*max (Sogaard et al., 2006; Smith et al., 2007; Klass et al., 2008, 2012; Levenez et al., 2008; Hoffman et al., 2009), this is not always the case (Endoh et al., 2005; Hunter et al., 2008; Iguchi and Shields, 2011), leading to problems interpreting MEP changes with fatigue.

MEP kinetics during submaximal fatiguing contractions. During sustained submaximal isometric contractions, MEP/c*M*max increases in the upper- (e.g. elbow flexors) (Sogaard et al., 2006; Smith et al., 2007; Klass et al., 2008; Levenez et al., 2008) and lower-limb (e.g. plantar flexors) (Hoffman et al., 2009) muscles (Table 2). The simultaneous progressive increase in volitional EMG activity is generally interpreted as an augmentation of the central drive to the lower motoneuron pool in order to maintain a constant level of force despite the development of peripheral fatigue (Sogaard et al., 2006; Smith et al., 2007). These observations are consistent with increased corticospinal excitability in submaximal fatiguing contractions. To assess whether these effects were mediated at the spinal and/or cortical levels, two recent studies compared changes in MEP/c*M*max with changes in CMEP/c*M*max (Levenez et al., 2008; Hoffman et al., 2009). Hoffman et al. (2009) observed a large increase in MEP/c*M*max and only a slight increase in CMEP/c*M*max during a sustained 30% MVC of the plantar flexors. This result suggests a small contribution of spinal factors to the increase in corticospinal excitability during submaximal fatiguing contractions. Conversely, during a 50% MVC of the elbow flexors to task failure, Levenez et al. (2008) found similar MEP/c*M*max and CMEP/c*M*max kinetics (i.e. increasing over the first 40% of the task to a plateau), indicating that central changes almost entirely occurred at the spinal level. These disparities in corticospinal responses to fatigue may be due to differences in neural control mechanisms between upper- and some lower-limb muscles. Indeed, the corticospinal projections onto soleus are probably weaker than those to many other muscles including biceps brachii, hand muscles and tibialis anterior (de Noordhout et al., 1999; Petersen et al., 2003; Martin et al., 2008). Martin et al. (2008) demonstrated that it was not possible to evoke large MEP in the soleus, even with high-intensity electrical stimulation over the thoracic spine. In this study, thoracic MEP were evoked in tibialis anterior in 75% of the subjects whereas only 38% had responses in the soleus. These findings may explain the absence of increased CMEP/c*M*max in the soleus during submaximal sustained contractions of the plantar flexors (Hoffman et al., 2009) and emphasize differences in neural control between muscles.

McNeil et al. (2011a) used a different paradigm to investigate corticospinal modulation during a submaximal fatiguing contraction. MEP/c*M*max and CMEP/c*M*max areas were investigated during a 10-min sustained contraction of the elbow flexors at 25% of the maximal EMG signal, i.e. at iso-EMG level. MEP/c*M*max did not change with exercise, whereas CMEP/c*M*max area decreased and was smaller than baseline values at 8 and 10 min of exercise. These results are in contrast with the responses elicited during constant torque contractions (Levenez et al., 2008; Hoffman et al., 2009). Because volitional EMG increased progressively during tasks performed at constant force level in the aforementioned studies while in McNeil et al. (2011a) EMG remains constant (i.e. force decreases), it appears that changes in evoked corticospinal responses should be interpreted in relation to changes in volitional EMG that may intrinsically influence the evoked EMG responses.

Sidhu et al. (2012) were the first to publish changes in corticospinal excitability during submaximal whole-body exercise. They measured MEP/c*M*max and CMEP/c*M*max responses from the knee extensors (i.e. vastus lateralis and rectus femoris) every 3 min during 30 min of cycling at 75% maximum aerobic workload and every minute during subsequent exercise at 105% maximum aerobic workload until exhaustion. Neither MEP/c*M*max nor CMEP/c*M*max changed significantly during exercise. However, when normalized to volitional EMG during cycling, the CMEP remained unchanged whereas the MEP were reduced from 10 min to task failure. These results suggest a tendency toward reduced cortical excitability, both during steady-state exercise at 75% maximal workload and at exhaustion. These changes are in contrast with findings from submaximal single-joint isometric contractions (Levenez et al., 2008; Hoffman et al., 2009). The higher cardiorespiratory and metabolic demands during whole-body exercise in comparison to single-joint exercise may lead factors such as temperature regulation, glucose availability, catecholamine concentration and cerebral oxygenation to have a greater influence on the responses of cells in the motor cortex and within the corticospinal tract (Todd et al., 2005; Hasegawa et al., 2008; Secher et al., 2008; Rupp et al., 2012; Verges et al., 2012).

MEP kinetics during maximal fatiguing contractions. During a sustained MVC, MEP has been reported to increase during the first seconds and then level off (Taylor et al., 2000a; Hunter et al., 2006, 2008), increase linearly (Szubski et al., 2007) or remain stable (Iguchi and Shields, 2011), depending on the protocol used (i.e. continuous vs. intermittent) and the muscle investigated (Table 4). Unlike during submaximal contractions, the high level of ongoing EMG activity during fatiguing maximal contractions may induce

Table 4. MEP kinetics during maximal fatiguing contractions

References	*n*	Fatiguing task characteristics	TMS timing	Task duration	Main muscle	MEP responses	Peripheral and/or spinal indices
Taylor et al. (1996)	7	Isometric MVC of elbow flexors	Every 10–15 s	2 min	Biceps brachii	Increase in MEP area (156% of control values, essentially over the first 30 s, before reaching a plateau)	Unchanged CMEP area
Taylor et al. (1999)	8	Isometric MVC of elbow flexors	Every 10 s	2 min	Biceps brachii	Increase in MEP area (153% of control values, greater than the increase in *M*max area), essentially over the first 20–40 s and maintained over the remainder of the 2-min	Increase in *M*max area (87% of control values), essentially over the first 20–40s and maintained over the remainder of the 2-min
Taylor et al. (2000a)	9	Intermittent isometric MVC of elbow flexors: (1): 5-s on 5-s off, (2) 15-s on 5-s off, (3) 15-s on 10-s off, (4) 30-s on 5-s off	(1): Every 30 s, (2), (3) and (4): 2 s after the start and 2 s before the end of each MVC	(1) 7 min 30 s, (2) 4 min, (3) 5 min, (4) 3 min 30 s	Biceps brachii	Increase in maximal MEP area by > 50% in all protocols[a]	N/A
Hunter et al. (2008)	13	Six intermittent isometric MVC (22-s on 10-s off) of elbow flexors	2 s after the start and 2 s before the end of each 22-s MVC	192 s	Biceps brachii	Increase in MEP area from 58% (first MVC) to 118% of baseline *M*max (last MVC)	N/A
Szubski et al. (2007)	12	Isometric MVC of index-finger abductors	Every 20 s	90 s	First dorsal interosseous	Increase in MEP/c*M*max (241% of pre-fatigue values)	N/A
Iguchi and Shields (2011)	10	45 Intermittent isometric MVC (9 × 5 MVC: 7-s on 3-s off) of plantar flexors. After the 5th MVC of each epoch: 10 s at 10% MVC	On the 3rd MVC of each epoch and at the end of the 10 s at 10% MVC	About 9 min	Soleus	Increase in MEP at 10% MVC (253% of pre-fatigue values) and no change in MEP at the 3rd MVC of each epoch	Decrease in *H*-reflex to 66% of pre-fatigue values after the first epoch and no further changes.
Mileva et al. (2012)	11	Intermittent isometric MVC of ankle dorsiflexors (2-s on 1-s off) until voluntary force decreased to 50% of the initial MVC or below	On the 1st MVC and each 10th MVC	368 ± 51 s	Tibialis anterior	Increase in MEP of 49% at the end of exercise	N/A
McKay et al. (1996)	6	Isometric MVC of ankle dorsiflexors	2 s After the onset of the MVC and each 15 s	2 min	Tibialis anterior	Trend toward an increase in MEP ($P < 0.13$)	Unchanged *M*max

CMEP,cervicomedullary motor-evoked potentials; c*M*max, concomitant *M*max; MEP, motor-evoked potential; *M*max, maximal muscle compound action potential; MVC, maximal voluntary contraction; n, number of subjects (for the main part of the experiment); N/A, not applicable. When not specified, amplitude values are reported in the last two columns.

[a] No information about statistical significance.

variability in MEP recordings, contributing in part to the substantial differences between studies. Also, concomitant reporting of MEP changes with indices of peripheral transmission is essential as *M*max amplitude and area can increase, decrease or remain unchanged during a sustained MVC (Mills and Thomson, 1995; McKay et al., 1996; Taylor et al., 1999; Taylor and Gandevia, 2001). Increasing MEP/c*M*max during a sustained MVC has been observed in the biceps brachii (Taylor et al., 1999) and first dorsal interosseus (Szubski et al., 2007) (Table 2). In contrast, CMEP/c*M*max decreased in the final 30 s of a sustained 2-min MVC of the elbow flexors (Butler et al., 2003). A decrease in spinal excitability was also recently observed during intermittent isometric MVC of plantar flexors (Iguchi and Shields, 2011) although the underlying mechanisms remain disputed. Following a 2-min MVC of the elbow flexors, CMEP/c*M*max recovered within 15 s even when the discharge of group III and IV afferents was maintained by holding the elbow flexors ischemic after the contraction (Butler et al., 2003). Conversely, a 2-min MVC of the elbow extensors induced CMEP/c*M*max reduction that persisted throughout 2 min of maintained ischemic (Martin et al., 2006), indicating that inputs from group III and IV afferents may contribute to lower motoneuron inhibition during fatigue of this muscle group. It is important to determine whether the differential effect of these afferents on lower motoneurons of extensor and flexor muscles is similar in other muscles groups (e.g. extensors and flexors of the knee and ankle) in humans. It is also important to note that unlike sustained submaximal exercise during which a constant force output is maintained and volitional EMG increases, both EMG activity and force decline during a sustained MVC (Gandevia, 2001; Iguchi and Shields, 2011). Thus, the progressive decline of CMEP/c*M*max during a sustained MVC may also be due to the concomitant decrease in EMG activity.

MEP responses in contracting muscle after fatiguing contractions. MEP measured during brief contractions after fatiguing exercise have also been reported and compared with baseline MEP. Post-exercise MEP are usually recorded immediately following a sustained fatiguing isometric contraction. Thus, they must be interpreted in conjunction with the MEP kinetics during the fatiguing contraction. As previously described, MEP and MEP/c*M*max generally increase during a sustained contraction and are thus larger at task failure than at the baseline (Smith et al., 2007; Szubski et al., 2007; Taylor and Gandevia, 2008). MEP/c*M*max measured immediately after exercise is also increased and progressively returns to baseline values within several minutes (Sogaard et al., 2006; Smith et al., 2007; Szubski et al., 2007; Klass et al., 2008). However, in contrast to the MEP or MEP/c*M*max depression observed in relaxed muscle (Table 3), the MEP measured during a voluntary contraction remains above the baseline values (Sogaard et al., 2006; Smith et al., 2007; Iguchi and Shields, 2011; Keller et al., 2011). It is likely that the voluntary effort required to perform a contraction transiently overcomes the decreased motor cortical excitability that commonly leads to MEP depression in relaxed muscle.

Following whole-body endurance or high-intensity exercise, MEP size mainly depends on the delay between the end of the task and the beginning of post-exercise measurements. Ross et al. (2007) reported depressed MEP in the tibialis anterior following a marathon. The fact that post-marathon measurements occurred up to 20 min post-exercise and that decreased MEP amplitude was associated with a non-significant decrease in *M*max does not allow the drawing of clear conclusions on MEP/c*M*max changes. Unchanged MEP/c*M*max has been reported in the rectus femoris following eight 5-min bouts of cycling at 80% of maximum workload (Sidhu et al., 2009b) and in the vastus lateralis following a constant load cycling trial at ~80% of maximal work rate performed to exhaustion (Goodall et al., 2012). In contrast, Fernandez-Del-Olmo et al. (2011) reported an increase in MEP/c*M*max area in the vastus lateralis after two Wingate tests. The differences between this study and the former two might reflect specific central adaptations to submaximal and maximal exercise (Taylor and Gandevia, 2008). A more likely explanation is that the submaximal isometric contractions in Fernandez-Del-Olmo et al. (2011) were performed at the same absolute force across the experimental session (i.e. based on percentages of the baseline MVC). Thus, the increase in MEP observed in this study may be interpreted as a compensatory mechanism to generate the required motor output and overcome the reduced peripheral force production (Fernandez-Del-Olmo et al., 2011). Conversely, the unchanged MEP areas observed by Sidhu et al. (2009b) and Goodall et al. (2012) may be related to their being measured at the same relative strength levels (i.e. taking into account the post-exercise MVC reduction).

Silent period. When single-pulse TMS is delivered during a voluntary contraction, the elicited MEP is followed by a period of near-silence in the EMG signal, termed silent period (SP). This period of EMG suppression is believed to be mediated by the activation of long-lasting $GABA_B$ receptors (McDonnell et al., 2006). It is acknowledged that spinal mechanisms contribute to the early part of the SP (Inghilleri et al., 1993). Since the EMG interruption continues beyond the recovery of motoneuron excitability, the later part of the SP is thought to be mediated through intracortical inhibitory mechanisms (Inghilleri et al., 1993).

The SP lengthens during a fatiguing contraction and the time to recover increases with increasing task duration (Taylor et al., 2000a; Sogaard et al., 2006; Smith et al., 2007; Taylor and Gandevia, 2008). An increase in SP during sustained contraction has been found in a range of muscles, including hand (Szubski et al., 2007), upper-limb (Hunter et al., 2006; Levenez et al., 2008) and lower-limb (McKay et al., 1996; Iguchi and Shields, 2011) muscles. Overall, the SP lengthens gradually during submaximal contraction whereas it

increases rapidly over the first seconds of a sustained MVC with no further change until task failure (Table 2) (Taylor et al., 1996; Todd et al., 2005; Sogaard et al., 2006; Smith et al., 2007; Levenez et al., 2008; Iguchi and Shields, 2011). As SP prolongation following cervicomedullary stimulation-induced CMEP is less than that occurring after MEP, the increase in SP following MEP likely includes additional inhibition at the supraspinal level (Taylor et al., 1996; Levenez et al., 2008).

Hilty et al. (2011b) observed an increase in SP following a fatiguing exercise of knee extensors. However, the post-exercise SP remained unchanged when firing of group III–IV muscle afferents was attenuated via intrathecal fentanyl injection. This result suggests that central projections of group III–IV muscle afferents may facilitate the fatigue-induced increase in SP in knee extensors. In contrast, change in SP after fatiguing contractions of the elbow flexors has been shown to be independent of ischemia-induced increase in firing of these afferents (Gandevia et al., 1996; Taylor et al., 2000c). Similar to the differential influence of group III–IV afferents on the lower motoneurons innervating extensor and flexor muscles (Butler et al., 2003; Martin et al., 2006), it is possible that the role of these afferents on fatigue-induced increases in intracortical inhibition depends on the investigated muscle group.

Many factors can induce variability in SP. First, the subject instructions greatly influence SP and these were not reported in many studies. Mathis et al. (1998) demonstrated that SP was unpredictable when the subjects were left without precise post-TMS instructions. They also found significantly longer SP when the subjects were instructed to relax quickly than when they were instructed to quickly regain the target force. Second, the SP has large inter-examiner variability (Reid et al., 2002). The low level of EMG present during the SP (due to spinal reflex facilitation by muscle spindle afferents) (Butler et al., 2012) and the immediate post SP increase in EMG activity, termed burst (Chin et al., 2012) may also confound the determination of the SP. With the aim to overcome these difficulties, Saisanen et al. (2008) recently provided guidelines to obtain a more stable SP. These recommendations notably include a TMS intensity of 110–120% of resting motor threshold delivered during contractions at 40–60% MVC.

Relationship between cortical excitability/inhibition and VA changes with fatigue. In his seminal review published in 2001, Gandevia raised some arguments to suggest that progressive development of activation deficit (i.e. increase in SIT) may not necessitate altered motor cortical excitability (Gandevia, 2001). When the elbow flexors are held ischemic near the end of or after a sustained MVC, activation deficit remains present while EMG responses to TMS (i.e. MEP and SP) begin to recover (Gandevia et al., 1996; Taylor et al., 1999, 2000a,c). Taylor et al. (2000a) observed that different types of fatiguing exercise induced similar central activation deficit but different patterns of SP lengthening. Although these findings appear to challenge the link between central fatigue and cortical excitability, they must be interpreted with caution. First, there is high inter-individual variability in exercise-induced SP increase (Cerri et al., 2010). Second, subjects in the aforementioned studies performed sustained isometric contractions and activation deficit was derived indirectly from the increment in force produced by TMS relative to the ongoing force. This method leads to an overestimation of activation failure (Gandevia et al., 1996), possibly partially accounting for its slow recovery compared to indices of corticospinal excitability. Recent studies reported exercise-induced reduction in cortical VA, either with simultaneous increases in MEP and SP during sustained isometric contractions (Hunter et al., 2008; Keller et al., 2011; Mileva et al., 2012) or with unchanged MEP after whole-body exercise (Sidhu et al., 2009b; Goodall et al., 2012). These opposing results suggest a complex relationship between central fatigue and cortical excitability.

Kalmar and Cafarelli (2006) used an original approach to examine the relationship between MEP and central fatigue. The authors demonstrated that caffeine ingestion (6 mg/kg body mass 1 h before the measurements) induced MEP facilitation (TMS delivered during 3% MVC contraction) early in the fatigue protocol (i.e. submaximal intermittent contractions of knee extensors) and eliminated the MEP depression observed at task failure in the placebo trial (Fig. 2). This increase in central excitability did not reduce the fatigue-related decrease in VA or voluntary force. It is difficult to explain this finding as the same voluntary output (i.e. VA) was found in two conditions (i.e. placebo and caffeine trial) despite different outputs (i.e. MEP) elicited by the same input (TMS). Several reasons may explain this phenomenon. First, VA was not determined by TMS, leading to limited information about corticospinal pathway involvement. Furthermore, the modulation of corticospinal excitability following caffeine ingestion was demonstrated with TMS delivered during low-force contractions. It is possible that MEP elicited during weak contractions are unrelated to responses during maximal voluntary force production and that caffeine-induced increases in corticospinal excitability do not apply at high levels of force (Gandevia and Taylor, 2006). Further studies are needed to replicate the findings of Kalmar and Cafarelli (2006) with accepted means of evaluating cortical VA (Todd et al., 2003) and then to establish whether this ability to manipulate cortical VA and/or cortical excitability occurs with different motor tasks and at higher contraction intensities.

PAIRED-PULSE TMS

Single-pulse TMS studies have suggested intracortical inhibition as a potential contributor to the development of muscle fatigue. Until recently, its evaluation had been limited to changes in SP and associated limitations (see SP section). Different levels of intracortical inhibition can also be explored using paired-pulse TMS. At intensities

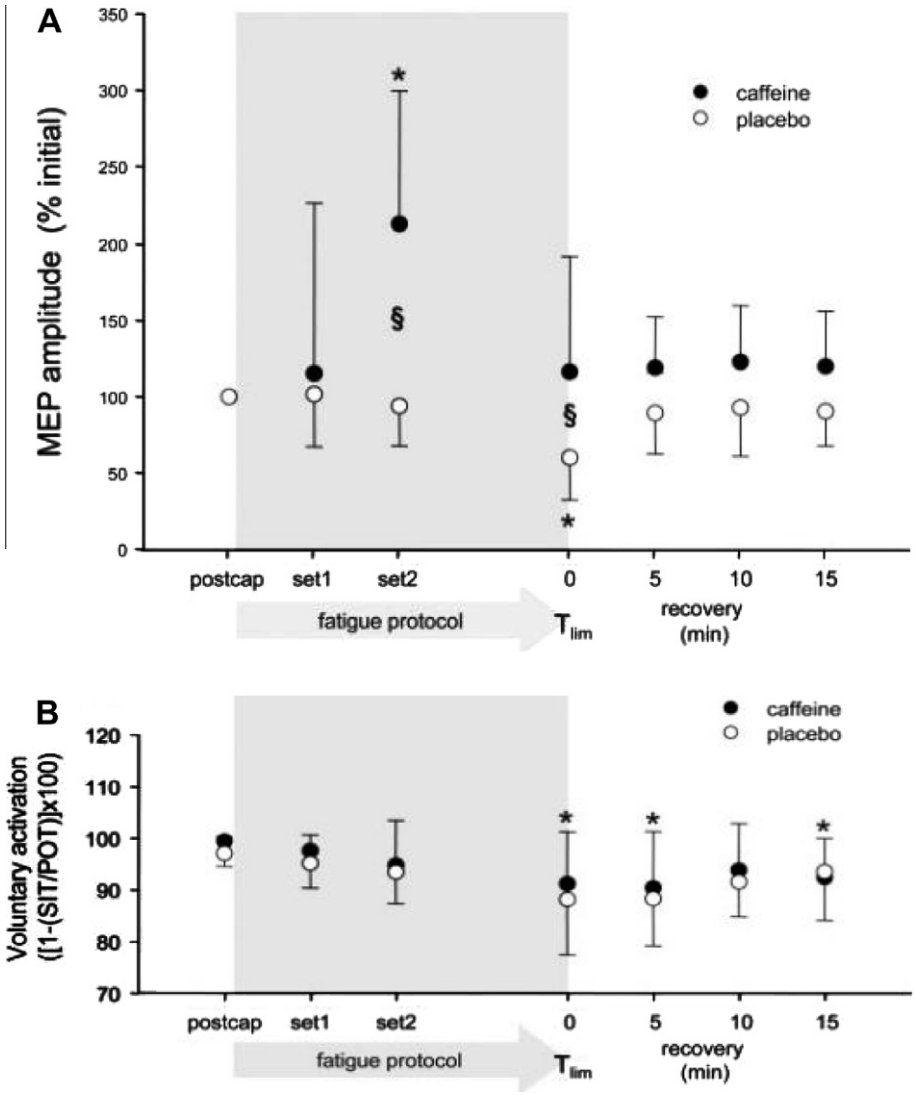

Fig. 2. Changes in MEP amplitude and maximal voluntary activation (nerve stimulation) during fatigue and recovery. The MEP amplitude (A) and percent voluntary activation (B) are expressed as a percentage of the prefatigue (postcapsule) value in the caffeine trial (●) and placebo trial (○). (A) *Significant difference from the postcapsule value within a drug treatment, $P < 0.05$; §Significant difference between drug treatments, $P < 0.016$. (B) *Significant difference from the postcapsule value for the pooled caffeine and placebo data, $P < 0.05$. Adapted from Kalmar and Cafarelli (2006).

above the motor threshold, a conditioning pulse and a test pulse delivered to the motor cortex through the same coil induce a facilitation of the test response at interstimulus intervals of 25–50 ms while the response is inhibited at 50–200 ms intervals (long-interval inhibition, LICI) (Valls-Sole et al., 1992). Pharmacological studies indicate that LICI, similar to SP, is mediated by the activation of $GABA_B$ inhibitory networks (McDonnell et al., 2006). However, they may respond differentially to $GABA_B$ activity enhancement (McDonnell et al., 2006), suggesting that different processes may underlie LICI and SP. SP refers to the duration of the inhibition whereas LICI should be considered as an estimate of the magnitude of inhibition. It is also possible to measure short-interval intracortical inhibition (SICI) by applying, at short interstimulus intervals (i.e. 2–5 ms), a subthreshold conditioning pulse followed by a suprathreshold test pulse (Kujirai et al., 1993). The interaction between conditioning and test pulses is thought to occur at the cortical level and be directly related to $GABA_A$ intracortical inhibitory activity (Ziemann et al., 1996; Di Lazzaro et al., 2005).

In an attempt to overcome the limitations of single-pulse TMS and notably to better understand the role of different intracortical inhibitory circuits in the development of central fatigue, recent studies investigated EMG responses to sustained volitional contractions with paired TMS pulses. However, studies that investigated SICI and LICI modulation with fatigue are often difficult to interpret for several reasons. First, some studies measured paired-pulse parameters only before and following exercise or commenced post-testing beyond the time at which acute changes might be detected (Tergau et al., 2000; Liepert et al., 2005). Furthermore, in the majority of studies, SICI and LICI measurements were made in relaxed muscle (Tergau et al., 2000; Benwell et al.,

2006, 2007; Boerio et al., 2012) and thus may not be representative of motor cortical behavior during fatiguing contractions.

Benwell et al. (2006) delineated the time course of SICI during 10 min of intermittent maximal contractions of the first dorsal interosseus muscle with paired TMS pulses delivered in the relaxed muscle. Despite an increase at the onset of the exercise, SICI decreased progressively thereafter in parallel with force. In a subsequent study, the same authors observed similar

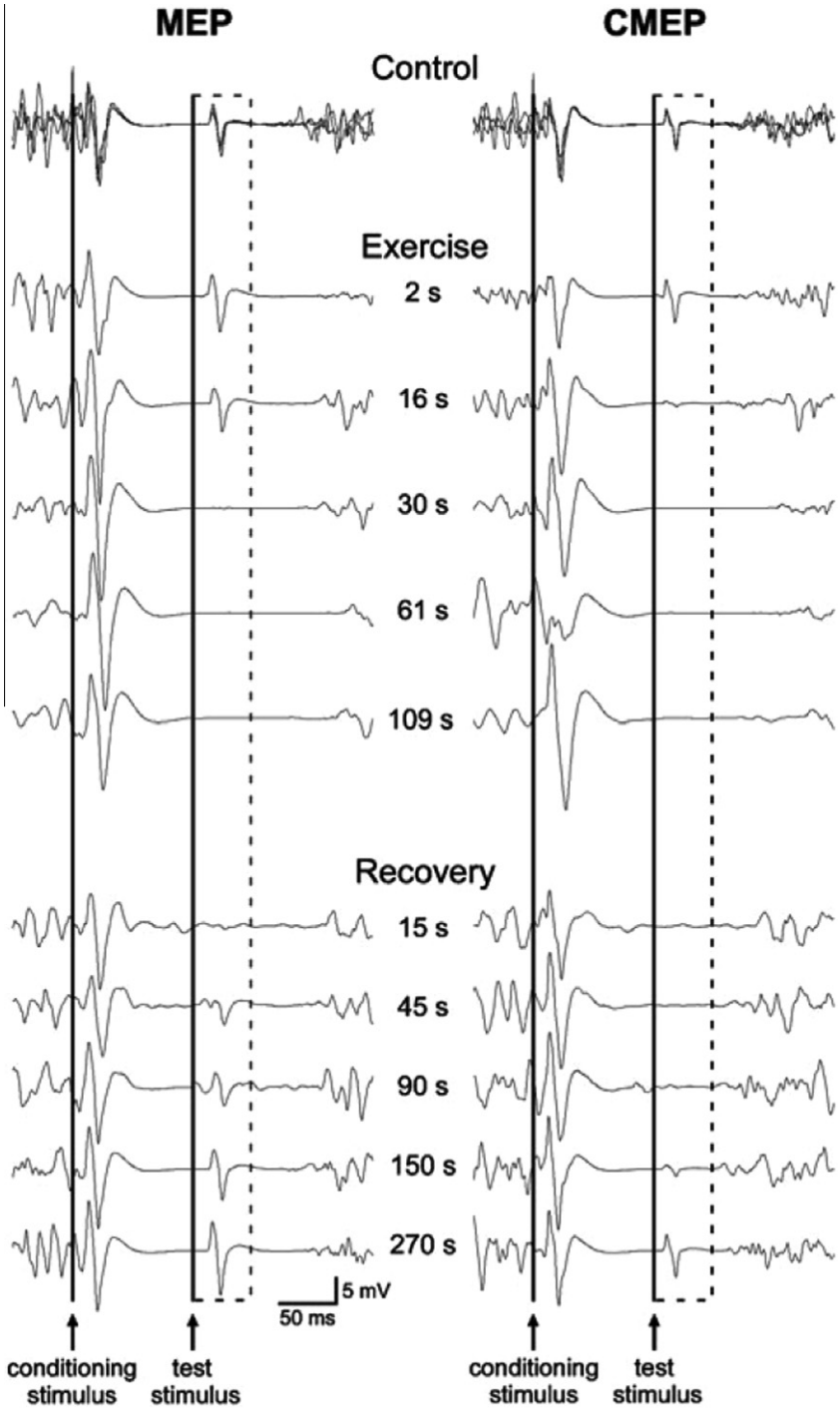

Fig. 3. Individual traces of biceps brachii EMG recorded from a single subject in brief control of maximal voluntary contractions (MVC) and a sustained 2-min MVC. Responses obtained during the three brief control MVC with paired conditioning-test stimulation are overlaid. The time course of stimulation during the 2-min MVC and the recovery period is indicated between the two sets of traces. The dashed box surrounds the conditioned test MEP (left) and CMEP (right) evoked in the silent period following the conditioning TMS stimulus. The continuous vertical lines indicate the timing of the conditioning and test stimuli. For this subject, conditioned MEP and CMEP are completely abolished at 30 s into the sustained contraction. From McNeil et al. (2009).

changes in LICI (Benwell et al., 2007). In both studies, these decreases (suggesting reduced intracortical inhibition) were associated with increased SP. The authors interpreted the dissociation between these measures of long-lasting inhibition by arguing that SP and LICI probably reflect processes occurring in different neuron pools (Benwell et al., 2007). A major concern with this interpretation is that the SP was measured during MVC whereas LICI was measured in the relaxed muscle.

McNeil et al. (2009) recently investigated changes in LICI during a 2-min MVC of elbow flexors. In an attempt to investigate the role of spinal mechanisms, the same protocol was repeated with the TMS test pulse replaced by cervicomedullary stimulation. Both conditioned MEP and CMEP decreased rapidly with fatigue (i.e. indicating increased LICI) and were almost completely suppressed within 30 s (Fig. 3). Moreover, the recovery was slower for conditioned MEP and CMEP (i.e. 90 s) in comparison to unconditioned responses (i.e. 30 s). The simultaneous abolishment of conditioned MEP and CMEP indicates that there is a major spinal component in LICI changes. Two possible mechanisms may contribute to the decrease in lower motoneuron excitability: a disfacilitation caused by a decline in muscle spindle firing rates and changes in intrinsic motoneuron properties (Butler et al., 2003). McNeil et al. (2011b) recently investigated whether the former could explain the reduction in conditioned CMEP during fatiguing contractions. They tested whether excitatory input from muscle spindles produced by tendon vibration affected the conditioned CMEP during a sustained 2-min MVC of the elbow flexors. The conditioned CMEP decreased rapidly with fatigue but was unaffected by tendon vibration. This result suggests that CMEP depression during a sustained maximal contraction does not depend on altered descending drive (as it is transiently suppressed in the SP) and is minimally affected by reduced input from muscle spindle discharge. Thus, changes in intrinsic motoneuron properties are the most likely explanation for reduced CMEP during sustained maximal contractions. It is of note that the increase in LICI observed by McNeil et al. (2009) and thought to be mediated by a spinal component was associated with a concomitant increase in SP. This finding suggests that SP may not be a specific index of intracortical inhibition as frequently claimed, rather reflecting the inhibition of upper motoneuron activity in the spinal cord. This remains to be confirmed by comparing the kinetics of SP and LICI during different fatiguing tasks and in other muscle groups.

McNeil et al. (2011a) also found a similar reduction of conditioned MEP and CMEP during a sustained submaximal contraction of the elbow flexors, confirming that impaired spinal mechanisms rather than intracortical inhibition account for the fatigue-related changes in LICI. The authors compared MEP and CMEP responses to low-intensity (i.e. intensity to evoke conditioned CMEP of ~15% *M*max) and high-intensity (i.e. ~50% *M*max) stimuli. The high-intensity test stimuli resulted in less inhibition of both conditioned MEP and CMEP, suggesting that high-threshold upper motoneurons are less affected by sustained submaximal contractions. It would be beneficial to determine whether similar reductions in conditioned MEP and CMEP could be observed in a muscle in which spinal factors are thought to contribute little to the increase in corticospinal responses during fatiguing contractions (e.g. plantar flexors (Hoffman et al., 2009)).

CONCLUSION

In isolation, TMS is useful to detect the supraspinal component of muscle fatigue. To determine the relative contribution of cortical and spinal mechanisms in central nervous system changes during fatiguing exercise, this method must be combined with other neurostimulation techniques (i.e. cervicomedullary and peripheral nerve stimulation). Significant changes in corticospinal excitability can be observed during sustained fatiguing contractions. The role of these changes in relation to central activation deficit remains to be elucidated. Pharmacological interventions aimed at modifying cortical excitability (e.g. caffeine) or manipulating central projection of muscle afferents (e.g. fentanyl) used in conjunction with TMS may aid in clarifying the relationship between changes in corticospinal excitability and central fatigue.

TMS over the motor cortex only provides information on transmission along the corticomotor tract. When considering the complexity of the phenomena preceding the execution of the motor command and the subsequent activation of motor cortical cells (Tanaka and Watanabe, 2012), it is likely that additional mechanisms upstream from the motor cortex contribute to central fatigue. Using techniques such as corticomuscular coherence and functional magnetic resonance imaging, recent evidence suggests that different neural systems may exchange information and increase and synchronize their activities during fatiguing contractions (e.g. Ushiyama et al., 2011; Hilty et al., 2011a). Motor cortical functioning is thus probably influenced by other brain regions (e.g. prefrontal cortex, somatosensory cortex) that may contribute to the development of central fatigue during exercise. Integration of neuroimaging and corticomuscular coherence methods (e.g. EEG–EMG) with neurostimulation techniques may allow better identification of specific sites associated with supraspinal failure during exercise. Future research must overcome the methodological difficulties in coupling these techniques during a given muscular exercise and acknowledge the limits imposed by motor task specificity in fatigue-induced changes in brain activity.

CONTRIBUTORS

MG, JT, TR, GM and SV wrote the manuscript and approved the final version of the manuscript. PL helped to review several aspects of the literature and approved the final version of the manuscript.

Acknowledgements—M. Gruet was supported by a grant from the "Fonds de Dotation Recherche en Santé Respiratoire, appel d'offres 2011" for research on the brain during exercise. J. Temesi was supported by a doctoral research Grant from the Rhône-Alpes Region.

REFERENCES

Allen GM, McKenzie DK, Gandevia SC (1998) Twitch interpolation of the elbow flexor muscles at high forces. Muscle Nerve 21:318–328.

Benwell NM, Sacco P, Hammond GR, Byrnes ML, Mastaglia FL, Thickbroom GW (2006) Short-interval cortical inhibition and corticomotor excitability with fatiguing hand exercise: a central adaptation to fatigue? Exp Brain Res 170:191–198.

Benwell NM, Mastaglia FL, Thickbroom GW (2007) Differential changes in long-interval intracortical inhibition and silent period duration during fatiguing hand exercise. Exp Brain Res 179:255–262.

Boerio D, Lefaucheur JP, Bassez G, Hogrel JY (2012) Central and peripheral components of exercise-related fatigability in myotonic dystrophy type 1. Acta Neurol Scand 125:38–46.

Brasil-Neto JP, Pascual-Leone A, Valls-Sole J, Cammarota A, Cohen LG, Hallett M (1993) Postexercise depression of motor evoked potentials: a measure of central nervous system fatigue. Exp Brain Res 93:181–184.

Brasil-Neto JP, Cohen LG, Hallett M (1994) Central fatigue as revealed by postexercise decrement of motor evoked potentials. Muscle Nerve 17:713–719.

Butler JE, Taylor JL, Gandevia SC (2003) Responses of human motoneurons to corticospinal stimulation during maximal voluntary contractions and ischemia. J Neurosci 23:10224–10230.

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

Cahill F, Kalmar JM, Pretorius T, Gardiner PF, Giesbrecht GG (2011) Whole-body hypothermia has central and peripheral influences on elbow flexor performance. Exp Physiol 96:528–538.

Cerri G, Cocchi CA, Montagna M, Zuin M, Podda M, Cavallari P, Selmi C (2010) Patients with primary biliary cirrhosis do not show post-exercise depression of cortical excitability. Clin Neurophysiol 121:1321–1328.

Chin O, Cash RF, Thickbroom GW (2012) Electromyographic bursting following the cortical silent period induced by transcranial magnetic stimulation. Brain Res 1446:40–45.

Darling WG, Wolf SL, Butler AJ (2006) Variability of motor potentials evoked by transcranial magnetic stimulation depends on muscle activation. Exp Brain Res 174:376–385.

de Noordhout AM, Rapisarda G, Bogacz D, Gerard P, De Pasqua V, Pennisi G, Delwaide PJ (1999) Corticomotoneuronal synaptic connections in normal man: an electrophysiological study. Brain 122(Pt 7):1327–1340.

del Olmo MF, Reimunde P, Viana O, Acero RM, Cudeiro J (2006) Chronic neural adaptation induced by long-term resistance training in humans. Eur J Appl Physiol 96:722–728.

Di Lazzaro V, Restuccia D, Oliviero A, Profice P, Ferrara L, Insola A, Mazzone P, Tonali P, Rothwell JC (1998) Effects of voluntary contraction on descending volleys evoked by transcranial stimulation in conscious humans. J Physiol 508(Pt 2):625–633.

Di Lazzaro V, Oliviero A, Saturno E, Dileone M, Pilato F, Nardone R, Ranieri F, Musumeci G, Fiorilla T, Tonali P (2005) Effects of lorazepam on short latency afferent inhibition and short latency intracortical inhibition in humans. J Physiol 564:661–668.

Endoh T, Nakajima T, Sakamoto M, Komiyama T (2005) Effects of muscle damage induced by eccentric exercise on muscle fatigue. Med Sci Sports Exerc 37:1151–1156.

Espiritu MG, Lin CS, Burke D (2003) Motoneuron excitability and the F wave. Muscle Nerve 27:720–727.

Fernandez-Del-Olmo M, Rodriguez FA, Marquez G, Iglesias X, Marina M, Benitez A, Vallejo L, Acero RM (2011) Isometric knee extensor fatigue following a Wingate test: peripheral and central mechanisms. Scand J Med Sci Sports http://dx.doi.org/10.1111/j.1600-0838.2011.01355.x. [Epub ahead of print].

Fulton RC, Strutton PH, McGregor AH, Davey NJ (2002) Fatigue-induced change in corticospinal drive to back muscles in elite rowers. Exp Physiol 87:593–600.

Gandevia SC (2001) Spinal and supraspinal factors in human muscle fatigue. Physiol Rev 81:1725–1789.

Gandevia SC, Taylor JL (2006) Supraspinal fatigue: the effects of caffeine on human muscle performance. J Appl Physiol 100:1749–1750.

Gandevia SC, Allen GM, Butler JE, Taylor JL (1996) Supraspinal factors in human muscle fatigue: evidence for suboptimal output from the motor cortex. J Physiol 490(Pt 2):529–536.

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

Goodall S, Romer LM, Ross EZ (2009) Voluntary activation of human knee extensors measured using transcranial magnetic stimulation. Exp Physiol 94:995–1004.

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

Haouzi P, Hill JM, Lewis BK, Kaufman MP (1999) Responses of group III and IV muscle afferents to distension of the peripheral vascular bed. J Appl Physiol 87:545–553.

Hasegawa H, Piacentini MF, Sarre S, Michotte Y, Ishiwata T, Meeusen R (2008) Influence of brain catecholamines on the development of fatigue in exercising rats in the heat. J Physiol 586:141–149.

Hilty L, Langer N, Pascual-Marqui R, Boutellier U, Lutz K (2011a) Fatigue-induced increase in intracortical communication between mid/anterior insular and motor cortex during cycling exercise. Eur J Neurosci 34:2035–2042.

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

Hoffman BW, Oya T, Carroll TJ, Cresswell AG (2009) Increases in corticospinal responsiveness during a sustained submaximal plantar flexion. J Appl Physiol 107:112–120.

Hollge J, Kunkel M, Ziemann U, Tergau F, Geese R, Reimers CD (1997) Central fatigue in sports and daily exercises. A magnetic stimulation study. Int J Sports Med 18:614–617.

Hultborn H, Nielsen JB (1995) H-reflexes and F-responses are not equally sensitive to changes in motoneuronal excitability. Muscle Nerve 18:1471–1474.

Humphry AT, Lloyd-Davies EJ, Teare RJ, Williams KE, Strutton PH, Davey NJ (2004) Specificity and functional impact of post-exercise depression of cortically evoked motor potentials in man. Eur J Appl Physiol 92:211–218.

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, Todd G, Butler JE, Gandevia SC, Taylor JL (2008) Recovery from supraspinal fatigue is slowed in old adults after fatiguing maximal isometric contractions. J Appl Physiol 105:1199–1209.

Iguchi M, Shields RK (2011) Cortical and segmental excitability during fatiguing contractions of the soleus muscle in humans. Clin Neurophysiol 123:335–343.

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

Kalmar JM, Cafarelli E (2004) Central fatigue and transcranial magnetic stimulation: effect of caffeine and the confound of peripheral transmission failure. J Neurosci Methods 138:15–26.

Kalmar JM, Cafarelli E (2006) Central excitability does not limit postfatigue voluntary activation of quadriceps femoris. J Appl Physiol 100:1757–1764.

Kaufman MP, Rybicki KJ, Waldrop TG, Ordway GA (1984) Effect of ischemia on responses of group III and IV afferents to contraction. J Appl Physiol 57:644–650.

Keller ML, Pruse J, Yoon T, Schlinder-Delap B, Harkins A, Hunter SK (2011) Supraspinal fatigue is similar in men and women for a low-force fatiguing contraction. Med Sci Sports Exerc 43:1873–1883.

Khedr EM, Galal O, Said A, Abd-elsameea M, Rothwell JC (2007) Lack of post-exercise depression of corticospinal excitability in patients with Parkinson's disease. Eur J Neurol 14:793–796.

Klass M, Levenez M, Enoka RM, Duchateau J (2008) Spinal mechanisms contribute to differences in the time to failure of submaximal fatiguing contractions performed with different loads. J Neurophysiol 99:1096–1104.

Klass M, Roelands B, Levenez M, Fontenelle V, Pattyn N, Meeusen R, Duchateau J (2012) Effects of noradrenaline and dopamine on supraspinal fatigue in well-trained men. Med Sci Sports Exerc 44:2299–2308.

Kluger BM, Palmer C, Shattuck JT, Triggs WJ (2012) Motor evoked potential depression following repetitive central motor initiation. Exp Brain Res 216:585–590.

Knorr S, Ivanova TD, Doherty TJ, Campbell JA, Garland SJ (2011) The origins of neuromuscular fatigue post-stroke. Exp Brain Res 214:303–315.

Kujirai T, Caramia MD, Rothwell JC, Day BL, Thompson PD, Ferbert A, Wroe S, Asselman P, Marsden CD (1993) Corticocortical inhibition in human motor cortex. J Physiol 471:501–519.

Lee M, Gandevia SC, Carroll TJ (2008) Cortical voluntary activation can be reliably measured in human wrist extensors using transcranial magnetic stimulation. Clin Neurophysiol 119:1130–1138.

Lentz M, Nielsen JF (2002) Post-exercise facilitation and depression of M wave and motor evoked potentials in healthy subjects. Clin Neurophysiol 113:1092–1098.

Levenez M, Garland SJ, Klass M, Duchateau J (2008) Cortical and spinal modulation of antagonist coactivation during a submaximal fatiguing contraction in humans. J Neurophysiol 99:554–563.

Liepert J, Mingers D, Heesen C, Baumer T, Weiller C (2005) Motor cortex excitability and fatigue in multiple sclerosis: a transcranial magnetic stimulation study. Mult Scler 11:316–321.

Lou JS, Benice T, Kearns G, Sexton G, Nutt J (2003) Levodopa normalizes exercise related cortico-motoneuron excitability abnormalities in Parkinson's disease. Clin Neurophysiol 114: 930–937.

Martin PG, Smith JL, Butler JE, Gandevia SC, Taylor JL (2006) Fatigue-sensitive afferents inhibit extensor but not flexor motoneurons in humans. J Neurosci 26:4796–4802.

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

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

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

McKay WB, Tuel SM, Sherwood AM, Stokic DS, Dimitrijevic MR (1995) Focal depression of cortical excitability induced by fatiguing muscle contraction: a transcranial magnetic stimulation study. Exp Brain Res 105:276–282.

McKay WB, Stokic DS, Sherwood AM, Vrbova G, Dimitrijevic MR (1996) Effect of fatiguing maximal voluntary contraction on excitatory and inhibitory responses elicited by transcranial magnetic motor cortex stimulation. Muscle Nerve 19: 1017–1024.

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

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

McNeil CJ, Giesebrecht S, Khan SI, Gandevia SC, Taylor JL (2011b) The reduction in human motoneurone responsiveness during muscle fatigue is not prevented by increased muscle spindle discharge. J Physiol 589:3731–3738.

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

Milanovic S, Filipovic SR, Blesic S, Ilic TV, Dhanasekaran S, Ljubisavljevic M (2011) Paired-associative stimulation can modulate muscle fatigue induced motor cortex excitability changes. Behav Brain Res 223:30–35.

Mileva KN, Sumners DP, Bowtell JL (2012) Decline in voluntary activation contributes to reduced maximal performance of fatigued human lower limb muscles. Eur J Appl Physiol 112:3959–3970.

Millet GY (2011) Can neuromuscular fatigue explain running strategies and performance in ultra-marathons?: the flush model. Sports Med 41:489–506.

Millet GY, Lepers R (2004) Alterations of neuromuscular function after prolonged running, cycling and skiing exercises. Sports Med 34:105–116.

Millet GY, Martin V, Lattier G, Ballay Y (2003) Mechanisms contributing to knee extensor strength loss after prolonged running exercise. J Appl Physiol 94:193–198.

Mills KR, Thomson CC (1995) Human muscle fatigue investigated by transcranial magnetic stimulation. Neuroreport 6:1966–1968.

Nielsen J, Petersen N (1994) Is presynaptic inhibition distributed to corticospinal fibres in man? J Physiol 477(Pt 1):47–58.

Perretti A, Balbi P, Orefice G, Trojano L, Marcantonio L, Brescia-Morra V, Ascione S, Manganelli F, Conte G, Santoro L (2004) Post-exercise facilitation and depression of motor evoked potentials to transcranial magnetic stimulation: a study in multiple sclerosis. Clin Neurophysiol 115:2128–2133.

Petersen NT, Pyndt HS, Nielsen JB (2003) Investigating human motor control by transcranial magnetic stimulation. Exp Brain Res 152:1–16.

Pitcher JB, Miles TS (2002) Alterations in corticospinal excitability with imposed vs. voluntary fatigue in human hand muscles. J Appl Physiol 92:2131–2138.

Pitcher JB, Robertson AL, Clover EC, Jaberzadeh S (2005) Facilitation of cortically evoked potentials with motor imagery during post-exercise depression of corticospinal excitability. Exp Brain Res 160:409–417.

Reid A, Chiappa K (2002) Motor threshold, facilitation and the silent period in cortical magnetic stimulation. In: Pascual-Leone A et al., editors. Handbook of TMS. New York: Oxford University Press. p. 97-111.

Ridding MC, Rothwell JC (2007) Is there a future for therapeutic use of transcranial magnetic stimulation? Nat Rev Neurosci 8:559–567.

Ross EZ, Middleton N, Shave R, George K, Nowicky A (2007) Corticomotor excitability contributes to neuromuscular fatigue following marathon running in man. Exp Physiol 92:417–426.

Rotto DM, Kaufman MP (1988) Effect of metabolic products of muscular contraction on discharge of group III and IV afferents. J Appl Physiol 64:2306–2313.

Rupp T, Jubeau M, Wuyam B, Perrey S, Levy P, Millet GY, Verges S (2012) Time-dependant effect of acute hypoxia on corticospinal excitability in healthy humans. J Neurophysiol 108:1270–1277.

Sacco P, Hope PA, Thickbroom GW, Byrnes ML, Mastaglia FL (1999) Corticomotor excitability and perception of effort during sustained exercise in the chronic fatigue syndrome. Clin Neurophysiol 110:1883–1891.

Saisanen L, Pirinen E, Teitti S, Kononen M, Julkunen P, Maatta S, Karhu J (2008) Factors influencing cortical silent period: optimized stimulus location, intensity and muscle contraction. J Neurosci Methods 169:231–238.

Samii A, Wassermann EM, Ikoma K, Mercuri B, George MS, O'Fallon A, Dale JK, Straus SE, Hallett M (1996) Decreased postexercise

facilitation of motor evoked potentials in patients with chronic fatigue syndrome or depression. Neurology 47:1410–1414.
Secher NH, Seifert T, Van Lieshout JJ (2008) Cerebral blood flow and metabolism during exercise: implications for fatigue. J Appl Physiol 104:306–314.
Sidhu SK, Bentley DJ, Carroll TJ (2009a) Cortical voluntary activation of the human knee extensors can be reliably estimated using transcranial magnetic stimulation. Muscle Nerve 39:186–196.
Sidhu SK, Bentley DJ, Carroll TJ (2009b) Locomotor exercise induces long-lasting impairments in the capacity of the human motor cortex to voluntarily activate knee extensor muscles. J Appl Physiol 106:556–565.
Sidhu SK, Cresswell AG, Carroll TJ (2012) Motor cortex excitability does not increase during sustained cycling exercise to volitional exhaustion. J Appl Physiol 113:401–409.
Smith JL, Martin PG, Gandevia SC, Taylor JL (2007) Sustained contraction at very low forces produces prominent supraspinal fatigue in human elbow flexor muscles. J Appl Physiol 103:560–568.
Sogaard K, Gandevia SC, Todd G, Petersen NT, Taylor JL (2006) The effect of sustained low-intensity contractions on supraspinal fatigue in human elbow flexor muscles. J Physiol 573:511–523.
Szubski C, Burtscher M, Loscher WN (2007) Neuromuscular fatigue during sustained contractions performed in short-term hypoxia. Med Sci Sports Exerc 39:948–954.
Tanaka M, Watanabe Y (2012) Supraspinal regulation of physical fatigue. Neurosci Biobehav Rev 36:727–734.
Taylor JL (2006) Stimulation at the cervicomedullary junction in human subjects. J Electromyogr Kinesiol 16:215–223.
Taylor JL, Gandevia SC (2001) Transcranial magnetic stimulation and human muscle fatigue. Muscle Nerve 24:18–29.
Taylor JL, Gandevia SC (2004) Noninvasive stimulation of the human corticospinal tract. J Appl Physiol 96:1496–1503.
Taylor JL, Gandevia SC (2008) A comparison of central aspects of fatigue in submaximal and maximal voluntary contractions. J Appl Physiol 104:542–550.
Taylor JL, Butler JE, Allen GM, Gandevia SC (1996) Changes in motor cortical excitability during human muscle fatigue. J Physiol 490(Pt 2):519–528.
Taylor JL, Butler JE, Gandevia SC (1999) Altered responses of human elbow flexors to peripheral-nerve and cortical stimulation during a sustained maximal voluntary contraction. Exp Brain Res 127:108–115.
Taylor JL, Allen GM, Butler JE, Gandevia SC (2000a) Supraspinal fatigue during intermittent maximal voluntary contractions of the human elbow flexors. J Appl Physiol 89:305–313.
Taylor JL, Butler JE, Gandevia SC (2000b) Changes in muscle afferents, motoneurons and motor drive during muscle fatigue. Eur J Appl Physiol 83:106–115.
Taylor JL, Petersen N, Butler JE, Gandevia SC (2000c) Ischaemia after exercise does not reduce responses of human motoneurones to cortical or corticospinal tract stimulation. J Physiol 525(Pt 3):793–801.
Taylor JL, Butler JE, Petersen NT, Gandevia SC (2001) Unexpected reflex response to transmastoid stimulation in human subjects during near-maximal effort. J Physiol 536:305–312.
Teo WP, Rodrigues JP, Mastaglia FL, Thickbroom GW (2012) Post-exercise depression in corticomotor excitability after dynamic movement: a general property of fatiguing and non-fatiguing exercise. Exp Brain Res 216:41–49.
Tergau F, Geese R, Bauer A, Baur S, Paulus W, Reimers CD (2000) Motor cortex fatigue in sports measured by transcranial magnetic double stimulation. Med Sci Sports Exerc 32:1942–1948.
Thickbroom GW, Sacco P, Faulkner DL, Kermode AG, Mastaglia FL (2008) Enhanced corticomotor excitability with dynamic fatiguing exercise of the lower limb in multiple sclerosis. J Neurol 255:1001–1005.
Todd G, Taylor JL, Gandevia SC (2003) Measurement of voluntary activation of fresh and fatigued human muscles using transcranial magnetic stimulation. J Physiol 551:661–671.
Todd G, Butler JE, Taylor JL, Gandevia SC (2005) Hyperthermia: a failure of the motor cortex and the muscle. J Physiol 563:621–631.
Ugawa Y, Day BL, Rothwell JC, Thompson PD, Merton PA, Marsden CD (1991a) Modulation of motor cortical excitability by electrical stimulation over the cerebellum in man. J Physiol 441:57–72.
Ugawa Y, Rothwell JC, Day BL, Thompson PD, Marsden CD (1991b) Percutaneous electrical stimulation of corticospinal pathways at the level of the pyramidal decussation in humans. Ann Neurol 29:418–427.
Ushiyama J, Katsu M, Masakado Y, Kimura A, Liu M, Ushiba J (2011) Muscle fatigue-induced enhancement of corticomuscular coherence following sustained submaximal isometric contraction of the tibialis anterior muscle. J Appl Physiol 110:1233–1240.
Valls-Sole J, Pascual-Leone A, Wassermann EM, Hallett M (1992) Human motor evoked responses to paired transcranial magnetic stimuli. Electroencephalogr Clin Neurophysiol 85:355–364.
Verges S, Rupp T, Jubeau M, Wuyam B, Esteve F, Levy P, Perrey S, Millet GY (2012) Invited Review: Cerebral Perturbations During Exercise in Hypoxia. Am J Physiol Regul Integr Comp Physiol 302:R903–916.
Watson SR, Colebatch JG (1998) Vestibular-evoked electromyographic responses in soleus: a comparison between click and galvanic stimulation. Exp Brain Res 119:504–510.
Zanette G, Bonato C, Polo A, Tinazzi M, Manganotti P, Fiaschi A (1995) Long-lasting depression of motor-evoked potentials to transcranial magnetic stimulation following exercise. Exp Brain Res 107:80–86.
Ziemann U, Lonnecker S, Steinhoff BJ, Paulus W (1996) The effect of lorazepam on the motor cortical excitability in man. Exp Brain Res 109:127–135.
Zijdewind I, Zwarts MJ, Kernell D (2000) Potentiating and fatiguing cortical reactions in a voluntary fatigue test of a human hand muscle. Exp Brain Res 130:529–532.

(Accepted 29 October 2012)
(Available online 3 November 2012)

Clinical Neurophysiology xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Clinical Neurophysiology

journal homepage: www.elsevier.com/locate/clinph

Assessement of quadriceps strength, endurance and fatigue in FSHD and CMT: Benefits and limits of femoral nerve magnetic stimulation

D. Bachasson [a,b,c], J. Temesi [d], C. Bankole [d,f], E. Lagrange [c,e], C. Boutte [c,e], G.Y. Millet [b,d], S. Verges [a,b,*], P. Levy [a,b,c], L. Feasson [d,e,f], B. Wuyam [a,b,c]

[a] Université Joseph Fourier, Laboratoire HP2, F-38000 Grenoble, France
[b] INSERM, U1042, F-38000 Grenoble, France
[c] CHU de Grenoble, Pole Locomotion, Rééducation & Physiologie, Clinique Physiologie, Sommeil et Exercice, F-38000 Grenoble, France
[d] Université de Lyon, Laboratoire de Physiologie de l'Exercice, F-42023 Saint-Etienne, France
[e] Centres Référent Maladies Neuromusculaires Rares Rhône Alpes, CHU de Grenoble, F-38000 Grenoble, France
[f] CHU de St Etienne, Unité de Myologie, F-42055 St. Etienne, France

ARTICLE INFO

Article history:
Accepted 1 August 2013
Available online xxxx

Keywords:
Fascioscapulohumeral dystrophy
Charcot-Marie-Tooth disease
Hereditary motor and sensory neuropathy
Femoral magnetic nerve stimulation
Muscle strength
Muscle fatigue
Muscle endurance
Neuromuscular diseases
Experienced fatigue

HIGHLIGHTS

- Reliable assessment of quadriceps strength, endurance and fatigue can be obtained over a single session in patients with neuromuscular diseases by using the quadriceps intermittent fatigue (QIF) test.
- Femoral nerve magnetic stimulation exhibits limitations due to insufficient stimulation intensity in ~30% of patients with fascioscapulohumeral dystrophy (FSHD) and in all patients with Charcot-Marie-Tooth disease (CMT).
- Patients with FSHD and CMT exhibit similar endurance and neuromuscular fatigue compared to healthy controls during standardized isolated quadriceps contractions.

ABSTRACT

Objectives: To (i) evaluate the feasibility and the reliability of a test assessing quadriceps strength, endurance and fatigue in patients with fascioscapulohumeral dystrophy (FSHD) and Charcot-Marie-Tooth disease (CMT), (ii) compare quadriceps function between patients and healthy controls.
Methods: Controls performed the test once and patients twice on two separate visits. It involved progressive sets of 10 isometric contractions each followed by neuromuscular assessments with FNMS.
Results: Volitional assessment of muscle strength, endurance and fatigue appeared to be reliable in FSHD and CMT patients. Supramaximal FNMS was achieved in ~70% of FSHD patients and in no CMT patients. In FSHD patients, Femoral nerve magnetic stimulation (FNMS) provided reliable assessment of central (typical error as a coefficient of variation (CV_{TE}) < 8% for voluntary activation) and peripheral (CV_{TE} < 10% and intraclass coefficient correlation >0.85 for evoked responses) function. Patients and controls had similar reductions in evoked quadriceps responses, voluntary activation and similar endurance.
Conclusions: This test provides reliable evaluation but FNMS exhibits limitations due to insufficient stimulation intensity particularly in neurogenic conditions. It showed similar central and peripheral quadriceps fatigability in patients and controls.
Significance: This test may be a valuable tool for patient follow-up although further development of magnetic stimulation devices is needed to extend its applicability.

Abbreviations: CMT, Charcot-Marie-Tooth disease; Db_{10}, 10-Hz potentiated doublets; Db_{10}:Db_{100}, peak potentiated 10-Hz doublets/peak potentiated 100-Hz doublets; Db_{100}, 100-Hz potentiated doublets; FNMS, femoral nerve magnetic stimulation; FSHD, fascioscapulohumeral dystrophy; MVC, maximal voluntary contraction; $MVC_{RMS/M}$, root mean squared calculated from vastus lateralis EMG signal normalized to M-wave amplitude during maximal voluntary contraction; SF-36, medical outcomes study short-form; Tw_P, potentiated single twitch; VA, maximal voluntary activation.

* Corresponding author. Address: Laboratoire HP2 (INSERM U 1042), UF Recherche sur l'Exercice, Hôpital Sud, Avenue Kimberley, 38 434 Echirolles, France. Tel.: +33 4 76 76 68 60; fax: +33 4 76 76 89 21.
E-mail address: sverges@chu-grenoble.fr (S. Verges).

1388-2457/$36.00 © 2013 Published by Elsevier Ireland Ltd. on behalf of International Federation of Clinical Neurophysiology.
http://dx.doi.org/10.1016/j.clinph.2013.08.001

1. Introduction

In patients with neuromuscular disorders, muscle weakness leads to severe impairment of functional capacities with negative influence on physical activity and participation. Experienced fatigue (i.e. tiredness, lack of energy and feeling of exhaustion not necessarily induced by exercise) is a common symptom in neuromuscular diseases (Angelini and Tasca, 2012; Chaudhuri and Behan, 2004). In addition to muscle weakness, enhanced subjective muscle fatigability is also reported by patients (Feasson et al., 2006). Availability of reliable and well-tolerated non-invasive evaluation of neuromuscular function (i.e. strength, endurance and fatigue) in patients with neuromuscular disorders is critical to provide relevant outcomes for observational and interventional studies.

Strength production embraces mechanisms within all levels of the motor pathway from the brain to skeletal muscle and are classically classified as central (neural) or peripheral (muscular). Similarly, neuromuscular fatigue (i.e. exercise-induced reduction in voluntary strength (Bigland-Ritchie et al., 1978)) involves peripheral (i.e. alterations in muscle contractility) and central (i.e. reduction in muscle activation during voluntary contractions caused by a decrease in motoneuron output at the spinal or/and supraspinal level (Gandevia, 2001)) mechanisms. Procedures to assess maximal voluntary strength are well-documented in both healthy subjects (Hogrel et al., 2007) and patients (Horemans et al., 2004) but exhibit numerous limitations (*e.g.* effects of patient cooperation/motivation, fear of pain or muscle damage, joint dysfunction and lack of distinction between central and peripheral factors). To overcome these limitations, artificial mechanical and electrophysiological responses evoked *via* muscle or peripheral nerve stimulation can be used to assess muscle contractility and the degree of muscle activation before and throughout a fatiguing task (see Millet et al. (2012) for review). However, the lack of standardized procedures concerning stimulation patterns and fatiguing tasks often makes results difficult to interpret. For instance, Schillings et al. (2007) used muscle electrical stimulation to assess biceps brachii muscle function in patients with myogenic or neurogenic disorders. The authors reported impaired voluntary activation at rest and smaller peripheral fatigue in patients (*i.e.* smaller reduction in evoked muscular responses compared to healthy controls) following a 2-min sustained isometric maximal voluntary contraction (MVC). In this work, impaired initial activation level leading to lower strength production in patients during the 2-min MVC might explain lower peripheral fatigue in patients compared to controls. In addition, the use of uncomfortable 100-Hz electrical stimulation trains was potentially responsible for submaximal activation in patients and the use of submaximal unpotentiated (rather than supramaximal potentiated) evoked responses while assessing peripheral fatigue (Kufel et al., 2002; Millet et al., 2012) also raised methodological concerns.

We recently developed a new clinical test to assess quadriceps function (Quadriceps Intermittent Fatigue test: QIF) involving intermittent isometric contractions and repetitive neuromuscular assessment *via* femoral nerve magnetic stimulation (FNMS). In healthy subjects, FNMS provides similar results to electrical stimulation as recently shown by our group (Verges et al., 2009) and is better tolerated than electrical stimulation in patients (Szecsi et al., 2010). The design of the QIF test has the advantage of (i) evaluating the changes in central and peripheral fatigue development rather than a final measurement only, (ii) limiting the influence of psychological and motivational confounding factors using progressive loading and multiple assessments, and (iii) limiting the discomfort associated with stimulations by using single and doublets stimulations rather than stimulation trains. We first showed that the QIF test is reliable in healthy subjects (Bachasson et al., 2013a) and then that it is well-tolerated and meaningful in patients with fibromyalgia syndrome (Bachasson et al., 2013b). The reliability of a comprehensive procedure to assess quadriceps strength, endurance and fatigue with the support of FNMS in patients with neuromuscular diseases remains to be evaluated.

Accordingly, we evaluated the feasibility and the reliability of the QIF test in patients with neuromuscular disorders. We studied patients with fascioscapulohumeral dystrophy (FSHD) and patients with Charcot-Marie-Tooth disease (CMT), among the most prevalent genetically-inherited muscular dystrophies and polyneuropathies in adults, respectively. We hypothesized that (i) the QIF test and FNMS are safe and reliable in patients with neuromuscular disorders, (ii) patients with neuromuscular disorders would have larger peripheral and central fatigue during the QIF test compared to a group of healthy controls. To clarify the functional consequences of muscle dysfunction in patients, we also assessed the relationship between quadriceps function, exercise capacity, functional capacities and experienced fatigue assessed by questionnaires.

2. Methods

2.1. Subjects

Nineteen FSHD patients (chromosome 4 linked) and eight CMT (type IA) patients with confirmed genetic diagnosis and twenty-three healthy controls volunteered to participate in this study. Twenty-three healthy subjects were enrolled to build two control groups ($n = 19$ and $n = 8$) matched for age, sex and BMI with the two groups of patients. Main subjects characteristics are presented in Table 1. All patients were able to walk and had neither contra-indication for maximal exercise testing nor severe knee condition. All subjects gave their written informed consent to participate in this study. The study was conducted according to the Declaration of Helsinki with approval from the local Committee on Human Research (*Comité de protection des personnes Sud-EST V*).

2.2. Study design

During the first visit, patients and controls had a clinical examination and answered questionnaires. During the second visit, subjects performed a 6-min walk test and, after one hour of rest, they performed a maximal incremental exercise test on a cycle ergometer. During the third visit, subjects performed a QIF test. Twelve FSHD patients and all CMT patients had a fourth visit to repeat the QIF test in order to assess between-day reliability.

2.3. Anthropometric measurements

Body fat percentage was assessed from four skin folds (Durnin and Womersley, 1974). We estimated quadriceps volume based on a truncated cone calculation using three thigh circumferences and thigh skin fold (Jones and Pearson, 1969).

2.4. Questionnaires

Quality of life was evaluated with the Medical Outcomes Study Short-Form (SF-36) (Aaronson et al., 1992). Experienced fatigue was evaluated with the fatigue severity scale (Krupp et al., 1989).

2.5. Maximal cycling test

Subjects performed a standard maximal incremental exercise test on a computer-controlled electrically braked cycle ergometer (Ergometrics 800, Ergoline, Bitz, Germany) with breath-by-breath gas analysis and electrocardiogram (Medisoft, Dinant, Belgium)

Please cite this article in press as: Bachasson D et al. Assessement of quadriceps strength, endurance and fatigue in FSHD and CMT: Benefits and limits of femoral nerve magnetic stimulation. Clin Neurophysiol (2013), http://dx.doi.org/10.1016/j.clinph.2013.08.001

Table 1
Characteristics of patients with fascioscapulohumeral dystrophy (FSHD), Charcot-Marie-Tooth disease (CMT) and controls.

	FSHD (n = 19)	Controls (n = 19)	P values	CMT (n = 8)	Controls (n = 8)	P values
Subjects characteristics						
Sex (women/men)	5/14	5/14	–	5/3	5/3	–
Age (y)	41 ± 13	39 ± 14	0.64	41 ± 14	41 ± 15	1.0
Height (cm)	176 ± 9	172 ± 9	0.25	167 ± 7	168 ± 5	0.69
Body weight (kg)	74 ± 15	72 ± 13	0.65	69 ± 10	65 ± 13	0.51
BMI (kg m^{-2})	23.8 ± 4.0	24.5 ± 3.0	0.85	24.8 ± 3.4	22.9 ± 2.9	0.26
Body fat percentage (%)	24.5 ± 8.6	23.2 ± 8.9	0.66	31.3 ± 8.7	26.2 ± 2.9	0.17
6-min walking distance (m)	464 ± 147	683 ± 96	<0.001	456 ± 96	652 ± 75	<0.001
Maximal incremental cycling test						
Peak workload (W)	122 ± 72	214 ± 61	<0.001	119 ± 32	184 ± 40	<0.01
$VO_{2,peak}$ (L min^{-1})	1.91 ± 0.66	2.61 ± 0.65	<0.01	1.73 ± 0.38	2.21 ± 0.44	<0.05
$VO_{2,peak}$ (mL min^{-1} kg^{-1})	26 ± 11	38 ± 9	<0.01	26 ± 6	35 ± 5	<0.01
Maximal HR (% predicted)	88 ± 10	94 ± 5	<0.05	95 ± 11	97 ± 3	0.63
$[La]_{max}$ (mmol L^{-1})	7.8 ± 2.9	10.1 ± 2.4	<0.05	7.2 ± 1.6	8.9 ± 1.9	0.10

Mean values ± SD; BMI, body mass index; $VO_{2,peak}$, peak oxygen consumption; $[La]_{max}$, maximal blood lactate at exhaustion; P values, statistical results of comparisons between patients and controls.

(Balady et al., 2010) for the determination of peak workload and peak oxygen consumption. A fingertip blood sample was obtained 3 min after exhaustion and analyzed for lactate concentration (NOVA+, Nova Biomedical Corporation, Waltham MA, USA).

2.6. Quadriceps neuromuscular assessment

2.6.1. Experimental setup

Measurements were conducted on the right limb in controls and on the strongest limb in patients. Subjects lay supine on a customized chair. The knee was flexed at 90° and the hip angle was 130° to facilitate coil placement in the femoral triangle for FNMS. Voluntary strength and evoked responses to FNMS were measured with a strain gauge (SBB 200 kg Tempo Technologies, Taipei, Taiwan) connected to an inextensible ankle strap. Compensatory movement of the upper body was limited by two belts across the thorax and abdomen. Subjects were instructed to keep their hands on their abdomen at all times. Visual feedback of both the force produced and the target force levels (see below) was provided to the subjects. Quadriceps surface EMG signal was recorded from the *vastus lateralis* (as a surrogate for the whole quadriceps (Place et al., 2007)) as described in detail previously (Verges et al., 2009). EMG signals were amplified (BioAmp, ADInstruments, Sydney, Australia) with a 5 to 500-Hz filter. EMG and force signals were digitized (Powerlab, ADInstruments) at a sampling frequency of 2000 Hz and recorded (Labchart; ADInstruments).

2.6.2. Femoral Nerve Magnetic Stimulation (FNMS)

FNMS was performed with a 45-mm figure-eight coil powered by two Magstim 200 stimulators (peak magnetic field 2.5 T, stimulation duration 0.1 ms; Magstim, Whitland, United Kingdom) linked by Bistim Module (Magstim), as previously described (Verges et al., 2009). Single (twitch) and paired stimuli (10-Hz and 100-Hz doublets) were delivered at maximal stimulator output. The coil was positioned high in the femoral triangle in front of the femoral nerve. The optimal position to evoke maximal unpotentiated quadriceps peak strength and maximal *vastus lateralis* M-wave amplitude was determined and marked on the skin. After 20 min of rest, stimulus supramaximality was assessed at stimulator power outputs of 100%, 95%, 90%, 85% and 80% (see Fig. 1). FNMS was considered to be supramaximal when the unpotentiated twitch at 80% of maximal power output was greater or equal to 90% of unpotentiated twitch amplitude at 100% of maximal power output. Ninety percent (=100–10%) was used because 10% represents twice the twitch variability in a healthy population (Bachasson et al., 2013a). Supramaximal stimulation is necessary to avoid the confounding effect of nerve hyperpolarization induced by muscle fatigue (Millet et al., 2012).

2.6.3. QIF test

Before starting the initial neuromuscular assessment, subjects performed ten 5-s submaximal isometric quadriceps contractions in order to warm up the quadriceps muscle and to familiarize themselves with both visual feedback and soundtrack instructions (see below). Then subjects performed three MVCs with 1 min of rest between each MVC. Following these MVC, subjects performed four submaximal contractions at 20%, 40%, 60% and 80% of MVC, each with a 100 Hz doublet delivered during contraction in order to evaluate the strength-activation relationship (See Fig. 2). Then the baseline neuromuscular assessment was performed. It consisted of a 5-s MVC superimposed with 100-Hz doublet followed 2 s later (*i.e.* in relaxed muscle) by two potentiated doublets at 100-Hz (Db_{100}) and 10-Hz (Db_{10}) delivered 4 s apart. Fifteen seconds later the subject performed a second MVC followed after 2 s by one potentiated single twitch (Tw_p). During all MVCs, subjects were vigorously encouraged by the experimenter. Potentiated (Kufel et al., 2002) evoked high- and low-frequency paired stimuli allow assessment of both high- and low-frequency peripheral fatigue (Verges et al., 2009) and high-frequency superimposed stimuli provide optimal resolution for central activation assessment (Place et al., 2007).

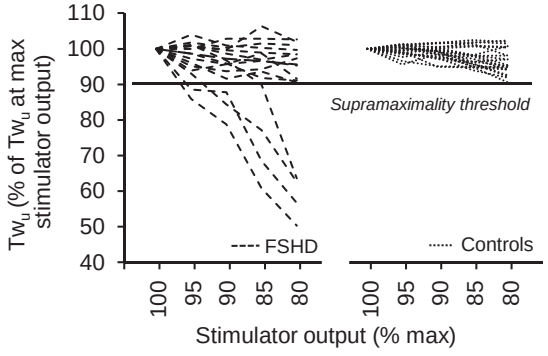

Fig. 1. Unpotentiated twitch amplitude (Tw_u) at different stimulator outputs in patients with fascioscapulohumeral dystrophy (FSHD) and controls. Supramaximal threshold corresponding to twice the Tw_u coefficient of variation is provided. All subjects with Tw_u amplitude below this threshold at 80% of maximal stimulator output were excluded from evoked response analysis.

Please cite this article in press as: Bachasson D et al. Assessement of quadriceps strength, endurance and fatigue in FSHD and CMT: Benefits and limits of femoral nerve magnetic stimulation. Clin Neurophysiol (2013), http://dx.doi.org/10.1016/j.clinph.2013.08.001

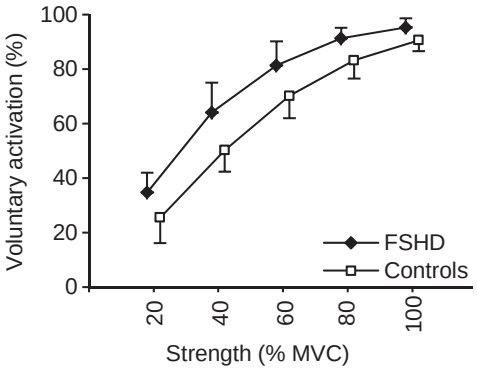

Fig. 2. Voluntary activation at 20, 40, 60, 80 and 100% of maximal voluntary contraction (MVC) in patients with fascioscapulohumeral dystrophy (FSHD) and healthy controls (n = 13 in both groups).

After baseline assessment, sets of 10 intermittent (5-s on/5-s off) isometric contractions at submaximal target forces were performed, starting at 10% MVC for the first set and increasing by 10% MVC each set until task failure. Subjects had visual feedback of the target force level and listened to a soundtrack indicating the contraction-relaxation rhythm. The range used for the target force level was defined as ±2.5% of MVC. Task failure was defined as two consecutive contractions below the target force level for more than 2.5 s. Five seconds after the end of each 10-contraction set and at exhaustion, neuromuscular assessments similar to baseline assessments were performed. In FSHD patients, serum creatine kinase was measured before and 24 h after the test.

2.7. Data analysis

The following parameters were calculated from the mechanical responses to FNMS: peak force for unpotentiated twitch, Tw_p, Db_{100},Db_{10} and the ratio Db_{10}:Db_{100} ($Db_{10:100}$ as an index of low frequency peripheral fatigue) to characterize peripheral mechanisms of neuromuscular function and peak force during superimposed Db_{100} to calculate voluntary activation (characterizing central mechanisms of the neuromuscular function). Peak-to-peak M-wave amplitude, area and latency (from FNMS to first M-wave peak) were calculated from Tw_p to assess possible alterations of action potential propagation (Dimitrova and Dimitrov, 2003). Maximal rates of force development and relaxation and the mechanical latency between FNMS and the beginning of the quadriceps mechanical response were calculated from Tw_p to provide further insights into muscle contractility and action potential propagation. Maximal voluntary activation (VA) during MVC was calculated as follows:

$$VA = [1 - \text{Superimposed } Db_{100}/Db_{100}] \times 100$$

A correction was applied to the original equation when the superimposed stimulation was administrated before or after the maximal MVC force (Strojnik and Komi, 1998). The same equation was used in order to assess voluntary activation at submaximal force levels (Fig. 2). The root mean squared calculated from *vastus lateralis* EMG signal normalized to M-wave amplitude during MVC ($MVC_{RMS/M}$) was also calculated as another index of central activation (Millet et al., 2012). The following parameters were calculated from submaximal contractions: total number of contractions (*i.e.* endurance index) and force–time integral.

2.8. Statistical analysis

All variables are reported as mean ± standard deviation. Normal distribution and homogeneity of variance analysis were confirmed using the Kolmogorov–Smirnov and Skewness test, respectively. Unpaired t-tests were conducted to compare patients and controls for the following variables: subject characteristics, questionnaire scores and neuromuscular function at baseline. To compare changes in variables during the QIF test and differences between groups, we used two-way repeated measures ANOVAs (time - × group) and t-tests with Bonferroni correction for *post hoc* analysis. Pearson's correlations were used to determine relationships between variables. To assess reliability of neuromuscular measurements, we calculated change in the mean values of both sessions with 95% confidence intervals and used paired t-tests for detection of systematic bias (Atkinson and Nevill, 1998). Due to our sample size, we used typical error expressed as a coefficient of variation (CV_{TE}) to study absolute reliability (Hopkins, 2000). Relative reliability was assessed by intraclass correlation coefficient (ICC) with 95% confidence intervals of variation (Hopkins, 2002). ICCs were not calculated for VA due to the ceiling effect associated with these measurements (Clark et al., 2007; Place et al., 2007). The alpha level was set at 0.05 for all tests. All other statistical analyses were performed with a statistical software package (NCSS, Kaysville, Utah USA).

3. Results

3.1. Functional capacities and questionnaires

Data from maximal incremental cycling test are shown in Table 1. During the maximal incremental cycling test, FSHD and CMT patients had lower maximal workload and peak oxygen consumption than controls. Maximal heart rate as a percentage of maximal theoretical value and blood lactate concentration were significantly lower in FSHD patients only compared to controls. 6-min walking distance was also reduced in patients. Scores of fatigue severity scale and SF-36 questionnaires are shown in Table 2. Eleven FSHD patients and five CMT patients reported significant experienced fatigue (*i.e.* >36, (Amato et al., 2001)).

3.2. FNMS supramaximality

FNMS supramaximality data for FSHD patients and all controls are shown in Fig. 1. FNMS was well-tolerated and no adverse effects were reported. In two FSHD patients, we were unable to obtain M-wave or mechanical responses. FNMS supramaximality was not confirmed in four other FSHD patients. These six patients were excluded from further analysis involving FNMS responses. In all other FSHD patients and controls, supramaximal stimulation was achieved and therefore, FNMS data were analyzed in thirteen patients compared to thirteen patient controls. In CMT patients, no reproducible or supramaximal M-wave or mechanical responses could be obtained. Consequently, FNMS data of CMT patients

Table 2
Fatigue severity scale and quality of life in patients with fascioscapulohumeral dystrophy (FSHD) and Charcot-Marie-Tooth disease (CMT).

	FSHD (n = 19)	CMT (n = 8)
Fatigue severity scale	38 ± 12	41 ± 7
SF-36 subscores		
Role physical	69 ± 37	66 ± 20
Physical functioning	63 ± 28	66 ± 20
Bodily pain	62 ± 26	62 ± 26
Role emotional	80 ± 40	96 ± 12
Social functioning	69 ± 31	66 ± 21
Mental health	52 ± 23	60 ± 17
Vitality (Energy/Fatigue)	50 ± 23	54 ± 6
General health perception	51 ± 18	52 ± 23

Mean values ± SD; SF-36, Medical Outcomes Study Short-Form.

Please cite this article in press as: Bachasson D et al. Assessement of quadriceps strength, endurance and fatigue in FSHD and CMT: Benefits and limits of femoral nerve magnetic stimulation. Clin Neurophysiol (2013), http://dx.doi.org/10.1016/j.clinph.2013.08.001

Table 3
Quadriceps function at baseline in patients with fascioscapulohumeral dystrophy (FSHD) and controls.

	FSHD	Controls	*P* values
Estimated quadriceps volume (cm^3)	755 ± 156	867 ± 171	<0.05
Voluntary strength (n = 19)			
MVC (Nm)	114 ± 46	207 ± 68	<0.001
MVC/Estimated quadriceps volume ($Nm\ cm^{-3}$)	0.14 ± 0.05	0.25 ± 0.07	<0.001
Evoked responses (n = 13)			
Potentiated single twitch			
Tw_p (Nm)	35 ± 16	61 ± 15	<0.001
Tw_p/Estimated quadriceps volume ($Nm\ cm^{-3}$)	0.038 ± 0.018	0.073 ± 0.016	<0.001
Tw_p contraction time (ms)	72 ± 20	71 ± 11	0.96
Tw_p latency (ms)	24 ± 3	23 ± 2	0.57
Tw_pMRFD ($Nm\ s^{-1}$)	351 ± 220	619 ± 210	<0.001
Tw_pMRFR ($Nm\ s^{-1}$)	−106 ± 59	−203 ± 82	<0.001
M-wave amplitude (mV)	9.3 ± 4.9	8.3 ± 3.7	0.57
M-wave area (mV ms)	0.084 ± 0.044	0.088 ± 0.029	0.85
M-wave latency (ms)	14.5 ± 1.9	13.8 ± 2.9	0.52
Potentiated doublets			
Db_{100} (Nm)	53 ± 25	92 ± 26	<0.001
Db_{10} (Nm)	45 ± 23	87 ± 24	<0.001
$Db_{10:100}$	0.85 ± 0.14	0.94 ± 0.06	<0.05
Central parameters (n = 13)			
VA (%)	95.6 ± 3.5	90.6 ± 4.0	<0.05
$MVC_{RMS/M}$	0.045 ± 0.020	0.048 ± 0.017	0.71

Mean values ± SD; MVC = maximum voluntary contraction; Db_{100} = peak potentiated 100 Hz doublet; Tw_p = peak potentiated single twitch; MRFD = maximal rate of force development; MRFR = maximal rate of force relaxation; $Db_{10:100}$ = ratio of the peak potentiated 10 Hz doublets/peak potentiated 100 Hz doublets; VA = voluntary activation level; $MVC_{RMS/M}$ = root mean squared calculated from vastus lateralis EMG signal normalized to M-wave amplitude during MVC.

Table 4
Quadriceps function at baseline in patients with Charcot-Marie-Tooth disease (CMT).

	CMT (*n* = 8)	Controls (*n* = 8)	*P* values
Estimated quadriceps volume (cm^3)	731 ± 140	770 ± 197	0.66
Voluntary strength			
MVC (Nm)	94 ± 34	149 ± 40	<0.05
MVC/Estimated quadriceps volume ($Nm\ cm^3$)	0.13 ± 0.04	0.20 ± 0.04	<0.05

Mean values ± SD; See Table 3 for abbreviations.

during the quadriceps test were not analyzed and only mechanical and EMG data during voluntary maneuvers were compared between the eight CMT patients and eight patient controls.

3.3. Quadriceps assessments at baseline

Quadriceps neuromuscular characteristics at baseline in FSHD patients and controls are shown in Table 3. Volitional and evoked strength, both as absolute values and normalized to estimated quadriceps volume, were significantly lower in FSHD patients compared to controls. Higher Tw_p maximal rates of force development and relaxation were observed in controls compared to FSHD patients but these differences disappeared when normalized to the Tw_p amplitude (normalized maximal rate of force development, $P = 0.56$; normalized maximal rate of force relaxation, $P = 0.22$). FSHD patients showed significantly lower $Db_{10:100}$ than controls. M-wave amplitude and area were similar in patients compared to controls. No differences in M-wave and mechanical latencies were found between FSHD patients and controls. Concerning central parameters, FSHD patients had significantly higher VA and similar $MVC_{RMS/M}$ compared to controls. The strength-activation relationship also indicated a tendency for greater voluntary activation at 20%, 40%, 60% and 80% of MVC in FSHD patients compared to controls ($P = 0.06$; Fig. 2). In FSHD patients, significant correlations were found between MVC (in Nm) and VA ($r = -0.41$; $P < 0.05$). Also, MVC per kg of body weight was correlated with 6-min walking distance ($r = 0.77$; $P < 0.001$), peak oxygen consumption per kg of body weight ($r = 0.74$; $P < 0.001$), maximal workload during the cycling test ($r = 0.82$; $P < 0.001$), fatigue severity scale score ($r = -0.65$; $P < 0.05$) and the physical functioning SF-36 subscore ($r = 0.58$; $P < 0.01$).

In CMT patients, volitional and evoked strength, both as absolute values and normalized to estimated quadriceps volume, were significantly lower compared to controls (see Table 4). MVC per kg of body weight correlated with peak oxygen consumption per kg of body weight ($r = 0.71$; $P < 0.05$).

3.4. Quadriceps endurance and fatigue

During the QIF test, the total number of submaximal contractions tended to be smaller in patients compared to controls (FSHD 55 ± 8 *versus* 60 ± 9, respectively, $P = 0.06$; CMT 53 ± 6 *versus* 58 ± 9, $P = 0.13$). Compared to controls, the ratio of the force reached on the last submaximal contraction and the first following MVC at exhaustion was significantly lower in FSHD patients (0.89 ± 0.06 *versus* 0.96 ± 0.11; $P < 0.05$) and was similar in CMT patients (0.88 ± 0.11 *versus* 0.90 ± 0.07; $P = 0.64$).

Changes in MVC in FSHD patients and controls are shown in Fig. 3. Changes in Tw_p, Db_{100}, $Db_{10:100}$ and VA are shown in Fig. 4. No significant differences were found between groups for these variables (all $P > 0.05$). No significant changes over time or between groups were found for $MVC_{RMS/M}$, M-wave amplitude, area and latency or mechanical latency (all $P > 0.05$, data not shown). Change in MVC during the quadriceps fatigue test in CMT patients and controls are shown in Fig. 3. No significant difference between groups was observed ($P = 0.18$).

3.5. Reliability of quadriceps neuromuscular assessments in patients

3.5.1. Endurance and muscular work

Mean number of submaximal contractions was similar in test and re-test sessions for FSHD (54 ± 5 *versus* 56 ± 6; $P = 0.17$) and CMT patients (53 ± 5 *versus* 52 ± 5 in CMT patients; $P = 0.60$). CV_{TE} was 4.4% and ICC 0.95 (95% CI: 0.56–0.95) in FSHD patients. In CMT patients, CV_{TE} was 4.5% and ICC 0.87 (95% CI: 0.60–0.97). Total force–time product was similar between sessions in FSHD patients (10178 ± 4752 *versus* 10595 ± 4660 Nm s; $P = 0.14$) and in CMT patients (8356 ± 2430 *versus* 8176 ± 2010 Nm s; $P = 0.60$).

3.5.2. Neuromuscular assessments

Among the twelve FSHD patients that performed a test–retest, 2 had unsatisfactory FNMS supramaximality and were excluded from analysis involving evoked muscle responses. The reliability of volitional and evoked quadriceps strength at baseline and set 50% are shown in Table 5. No significant differences were observed between test and re-test sessions for any parameters. Serum creatine kinase in FSHD patients was not significantly increased 24 h after the quadriceps test (284 ± 136 *versus* 326 ± 140 $IU\ l^{-1}$; $P = 0.48$). The reliability of volitional strength at baseline and set 50% in CMT patients is shown in Table 6. No significant differences were observed between test and re-test.

Please cite this article in press as: Bachasson D et al. Assessement of quadriceps strength, endurance and fatigue in FSHD and CMT: Benefits and limits of femoral nerve magnetic stimulation. Clin Neurophysiol (2013), http://dx.doi.org/10.1016/j.clinph.2013.08.001

4. Discussion

Our results show that the present test involving incremental isometric intermittent loading and FNMS appears to be safe, feasible and reliable to assess quadriceps strength, fatigue and endurance in patients with FSHD. Supramaximal FNMS was however not achieved in ~30% of FSHD patients. Valid quadriceps mechanical responses evoked by FNMS could not be obtained in CMT patients but fatigue and endurance assessments using volitional manoeuvers appear to be reliable. Contrary to our hypothesis, we observed similar peripheral and central fatigability in patients compared to controls. Quadriceps weakness correlated with functional capacities and perceived fatigue in patients but quadriceps fatigability did not.

4.1. Feasibility and reliability of FNMS and the QIF test in patients

4.4.1. FNMS supramaximality

Supramaximal stimulation was obtained in 68% of FSHD patients and 100% of controls. In two male FSHD patients, we were unable to obtain a distinguishable M-wave or evoked response. One of these patients had the second highest percentage body fat amongst patients (34%) and the other had 24% body fat. Among the four patients (one women and three men) with unsatisfactory supramaximality (see Fig. 1), mean body fat percentage was 31 ± 2%. Increased distance between the coil and the femoral nerve caused by subcutaneous fat interposition can lead to submaximal stimulation as previously reported by our group (Tomazin et al., 2011) and may explain, at least in part, the inability to reach supramaximal FNMS in these patients. We were unable to obtain supramaximal stimulation in any CMT patient, even those with low body fat percentage. Altered nerve excitability properties (*e.g.* higher resting excitability threshold, threshold electrotonus abnormalities) previously reported in CMT disease (Meulstee et al., 1997; Nodera et al., 2004) might partly explain these results but further research is needed to clarify the mechanisms involved. As previously done in healthy subjects (Verges et al., 2009), comparison of electrical and magnetic femoral nerve stimulation in neuromuscular patients could also be useful to better characterize advantages and limits of FNMS, in particular in neurogenic patients.

4.4.2. Feasibility and reliability

Our fatiguing protocol appeared to be safe since serum creatine kinase concentrations before and 24 h after the test were similar and since evoked and volitional strengths were similar between test and re-test sessions in FSHD patients. FNMS was well-tolerated in patients as previously reported in other pathological conditions (*e.g.* in COPD (Polkey et al., 1996), chronic heart failure (Hopkinson et al., 2012), fibromyalgia syndrome (Bachasson et al., 2013b)). Reliability of MVC and evoked muscular responses at baseline were satisfactory (CV_{TE} < 7% and ICC > 0.82). MVC and Tw_p reliability was similar to the between-day reliability previously observed in COPD patients (Saey et al., 2003). Percentage reductions in MVC and evoked muscular responses during the QIF test appeared to be similar between the test and re-test sessions. At set 50%, CV_{TE} were <10% and ICC were >0.85 for both MVC and evoked muscular responses. For VA, CV_{TE} was <5% at baseline and at set 50% but relative reliability was lower as shown by large limits of agreement at baseline, influenced by one outlier that showed a large VA reduction in the second session (−21%). These results are in accordance with previous results showing relatively large VA variability in healthy subjects (Morton et al., 2005; Place et al., 2007) and in patients with neuromuscular disorders (Horemans et al., 2004). $MVC_{RMS/M}$ was less reliable than VA as previously observed (Place et al., 2007). Muscle endurance assessed with the total number of submaximal contraction was reliable (CV_{TE} < 5% and ICC > 0.95). Together, these results indicate that the reliability of neuromuscular assessments in FSHD patients is good and suitable for follow-up or interventional studies. MVC measurements before (Solari et al., 2008) and during the QIF test are suitable to evaluate strength and fatigability in patients with CMT.

4.2. Quadriceps properties at baseline in patients versus controls

4.2.1. Voluntary strength and evoked responses

As expected, FSHD patients had lower MVC and evoked muscular responses compared to controls (−45%). When normalized to estimated quadriceps volume, MVC and evoked muscular responses remained lower in patients. This result may reflect fibrosis and lipid infiltration usually observed in dystrophic muscle (Friedman et al., 2012). Also, changes in myocyte ultrastructure (*e.g.* atrophic myotubes) (Barro et al., 2010) and the loss of tendon-fiber continuity during muscle fiber necrosis and regeneration (Goldstein and McNally, 2010) may contribute to the impaired strength–volume relationship in FSHD patients. More accurate measurements of muscle volume and structure with magnetic resonance imaging (Kan et al., 2009) are needed to confirm that strength production per unit of muscle volume is reduced in FSHD patients. Lower $Db_{10:100}$ may indicate that the force–frequency relationship in dystrophic muscle is influenced by factors such as macroscopic and microscopic muscle abnormalities mentioned

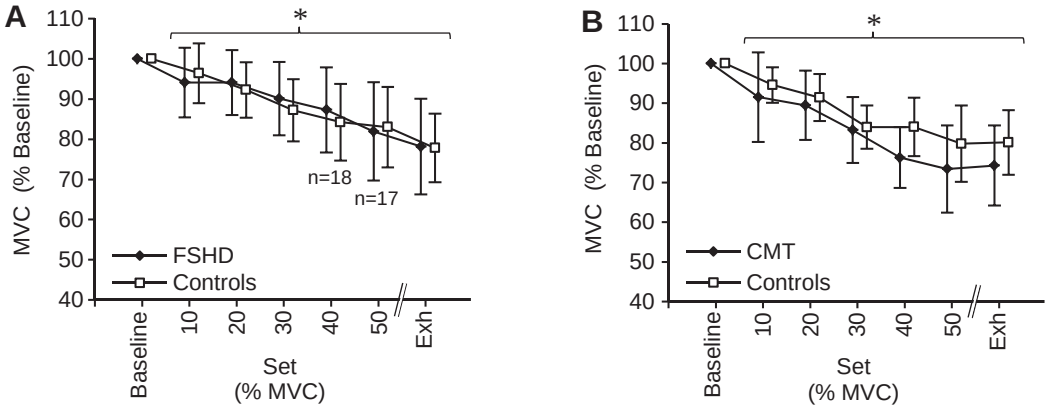

Fig. 3. Maximal voluntary strength (MVC) during the quadriceps fatigue test in patients with fascioscapulohumeral dystrophy (FSHD, $n = 19$, Panel A) and Charcot-Marie-Tooth disease (CMT, $n = 8$, Panel B) compared to healthy controls. Baseline, initial measurement; 10–50, measurements after sets of 10 contractions at 10–50% of MVC; Exh, measurement immediately after exhaustion; *significantly different from baseline ($P < 0.05$).

Please cite this article in press as: Bachasson D et al. Assessement of quadriceps strength, endurance and fatigue in FSHD and CMT: Benefits and limits of femoral nerve magnetic stimulation. Clin Neurophysiol (2013), http://dx.doi.org/10.1016/j.clinph.2013.08.001

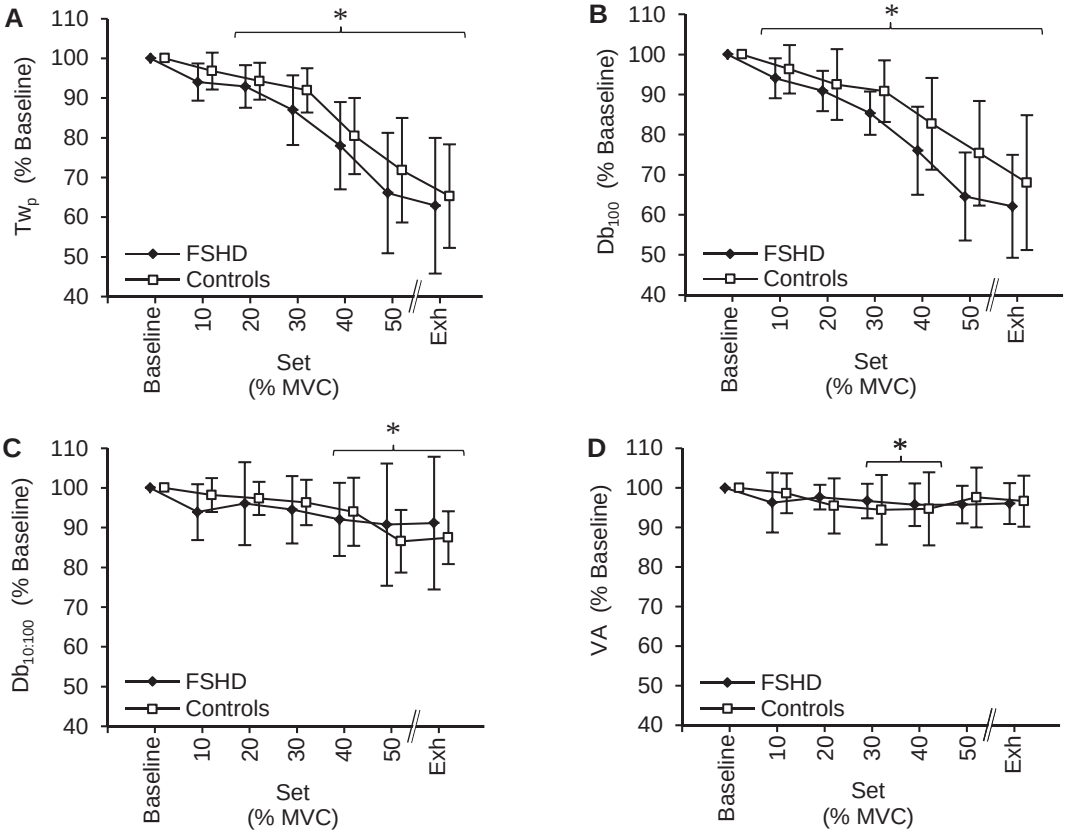

Fig. 4. Potentiated twitch (Tw_p, Panel A) and potentiated 100-Hz doublet (Db_{100}, Panel B) amplitudes evoked *via* magnetic femoral nerve stimulation, ratio of potentiated 10-Hz on potentiated 100-Hz doublets ($Db_{10:100}$, Panel C) and voluntary action (VA, Panel D) during the quadriceps fatigue test in patients with fascioscapulohumeral dystrophy (FSHD) and healthy controls ($n = 13$ in both groups). See Fig. 3 for abbreviations. *significantly different from baseline ($P < 0.05$).

above (Barro et al., 2010; Friedman et al., 2012; Goldstein and McNally, 2010). Conversely, similar M-wave characteristics indicate that nerve conduction and action potential propagation are preserved in FSHD patients. In CMT patients, MVC was significantly reduced compared to controls (−37%) in line with previous reports (Schillings et al., 2007). In CMT, proximal leg compartments usually display less atrophy and fatty infiltration than distal compartments (Gallardo et al., 2006). Estimated quadriceps volume was not significantly reduced in CMT patients and therefore MVC normalized to estimated quadriceps volume was lower compared to controls. As discussed above, accurate measurements of muscle volume are needed and, in the absence of VA measurements, we are unable to discriminate between central and peripheral factors responsible for this weakness in CMT.

4.2.2. Central parameters

One unexpected result was the higher VA at baseline in FSHD patients compared to controls. In both groups, mean VA was >90% which is within the range usually observed in healthy human quadriceps (O'Brien et al., 2008; Place et al., 2007). A tendency for higher activation level at submaximal fraction of MVC was also found in FSHD patients (see Fig. 2). Similar $MVC_{RMS/M}$ in patients and controls do not support a difference in central activation between groups but this parameter may be insufficiently reliable to detect small changes (Place et al., 2007). Higher VA in FSHD patients contrasts with the previous work of Schillings et al. (2007) reporting large activation failure in biceps brachii of FSHD patients. This discrepancy might be partly explained by the use of different stimulation procedures (*e.g.* muscle electrical train stimulation *versus* FNMS) and differences in muscle groups. On the other hand, normal activation has also been reported in other neuromuscular disease such as post-polio syndrome (Allen et al., 1997). Schillings et al. (2007) suggested that lower voluntary activation in patients might reflect a protective mechanism to prevent muscle from further damage. Previous findings however showed that intracortical inhibition assessed with transcranial magnetic stimulation might be reduced in FSHD and may reflect a compensatory phenomenon of the central nervous system to overcome peripheral muscle weakness (Di Lazzaro et al., 2004). This mechanism might underlie the enhanced VA observed in FSHD in the present work although the relationship between central inhibition/excitability and the level of activation assessed at the peripheral level is still to be clarified (Gruet et al., 2013). Furthermore, the weakest FSHD patients may be accustomed to recruiting a greater percentage of their maximal muscle capacity in daily activities, thus accounting for the inverse correlation between quadriceps strength and VA.

4.3. Quadriceps fatigability and endurance in patients versus controls

4.3.1. MVC, peripheral fatigability and endurance

Our results showed similar reductions in MVC and evoked muscular responses in FSHD patients and controls during a standardized fatigue protocol at identical relative intensities (*i.e.* identical % of MVC). Reductions in $Db_{10:100}$ were also similar indicating that the amount of low-frequency fatigue was comparable in both groups. M-wave characteristics did not change during the test meaning that impairment of action potential propagation is not involved in the fatigue induced by this protocol in either FSHD patients or controls. These results contrast with the study of Schillings et al. (2007), which reported smaller reductions in MVC and evoked muscular responses in patients after a 2-min sustained MVC compared to controls. In this study, central activation

Please cite this article in press as: Bachasson D et al. Assessement of quadriceps strength, endurance and fatigue in FSHD and CMT: Benefits and limits of femoral nerve magnetic stimulation. Clin Neurophysiol (2013), http://dx.doi.org/10.1016/j.clinph.2013.08.001

Table 5
Between-day reliability values for volitional ($n = 12$), evoked quadriceps strength and central parameters ($n = 10$) at baseline and at set 50% in patients with fascioscapulohumeral dystrophy.

	Change in mean (95 % CI)	CV_{TE} (95% CI)	ICC (95 % CI)
Baseline			
MVC (Nm)	−0.8 (−3.2–1.5)	2.3 (1.6–3.9)	0.99 (0.99–0.99)
Tw_p (Nm)	−0.6 (−3.6–2.5)	7.7 (5.2–14.8)	0.98 (0.94–0.99)
Db_{100} (Nm)	1.1 (−2.1–4.3)	5.3 (3.6–10.1)	0.99 (0.97–0.99)
$Db_{10:100}$	−0.05 (−0.11–0.01)	6.4 (4.3–12.3)	0.82 (0.40–0.96)
VA (%)	−1.9 (−6.4–3.1)	4.6 (3.3–9.5)	/
$MVC_{RMS/M}$	0.004 (−0.008–0.015)	16 (10.7–26.0)	0.79 (0.49–0.96)
Set 50%			
MVC (% Pre)	−1.6 (−8.2–5.0)	6.0 (3.9–13.3)	0.99 (0.92–0.99)
Tw_p (% Pre)	−1.6 (−8.0–4.7)	9.3 (6.3–17.7)	0.88 (0.57–0.97)
Db_{100} (% Pre)	0.1 (−7.7–7.9)	9.9 (6.7–19.0)	0.85 (0.48–0.96)
$Db_{10:100}$	−0.09 (−0.16–0.02)	7.1 (4.7–14.4)	0.92 (0.66–0.98)
VA (%)	−1.23 (−8.0–5.5)	6.9 (4.6–13.1)	/
$MVC_{RMS/M}$	0.000 (−0.004–0.004)	7.9 (5.2–16.0)	0.91 (0.62–0.98)

95% CI, 95% Confidence interval; ICC, intraclass correlation coefficient; CV_{TE}, typical error expressed as a coefficient of variation; MVC = maximum voluntary contraction; Db_{100}, = peak potentiated 100 Hz doublet; Tw_p = peak potentiated single twitch; $Db_{10:100}$ = ratio of the peak potentiated doublets at 10 over 100 Hz; VA = voluntary activation level; $MVC_{RMS/M}$ = root mean squared calculated from *vastus lateralis* EMG signal normalized to M-wave amplitude during MVC.

Table 6
Between-day reliability values for volitional quadriceps strength at baseline and at set 50% in patients with Charcot-Marie-Tooth disease ($n = 8$).

	Change in mean (95 % CI)	CV_{TE} (95% CI)	ICC (95 % CI)
Baseline			
MVC (Nm)	−1.5 (−8.4–5.7)	5.8 (3.8–9.7)	0.98 (0.94–0.99)
Set 50%			
MVC (% Pre)	−0.6 (−2.9–1.8)	3.0 (2.1–3.8)	0.94 (0.82–0.98)

Mean values ± SD; See Table 4 for abbreviations.

in patients was greatly impaired and therefore lower muscle recruitment may have induced less fatigue. Our results also contrast with the work of Schulte-Mattler et al. (2003), who showed increased contractile fatigue in dorsiflexors induced by intermittent electrical neurostimulation in FSHD patients. However, these results are difficult to compare with the present study because the group of patients studied was particularly heterogeneous involving various neurogenic and myopathic diseases (*e.g.* only four patients with FSHD). FSHD patients showed a tendency to have reduced muscle endurance ($P = 0.06$) as measured by the total number of submaximal contractions. Turki et al. (2012) recently reported increased oxidative stress and impaired mitochondrial function in fifteen patients with FSHD compared to a group of healthy controls. The authors reported that both quadriceps volitional strength and endurance (*i.e.* time to exhaustion during dynamic contractions at 30% of MVC) correlate with these abnormalities. Although quadriceps endurance was much shorter in FSHD, time to exhaustion was highly variable in both groups (384 ± 353 s in patients *versus* 603 ± 357 s in controls). Furthermore, the amount of fatigue induced was not measured. Since peripheral fatigue kinetics were similar in FSHD patients and controls in the present study, the tendency to lower endurance in FSHD patients may be explained, in part, by the significantly lower ratio of the force reached during the last submaximal contraction and the first following MVC at exhaustion in patients, indicating slightly submaximal effort. Lack of motivation, fear of pain and muscle damage frequently observed in patients may also contribute to earlier task-failure in patients. The discrepancy between our results and Turki et al. (2012) might partly rely on the type of contraction since dystrophic muscle might be more sensitive to muscle damage than healthy muscle during dynamic contractions (Dellorusso et al., 2001). In CMT patients, our result showed similar reduction in MVC during the QIF test and non-significant difference in terms of endurance. In previous studies exploring quadriceps, similar observations have been made (Lindeman et al., 1999; Menotti et al., 2012; Schillings et al., 2007) but these results are difficult to compare because sustained maximal or submaximal contractions were used rather than intermittent submaximal contractions as in the present work. As discussed above, measurements of fatigue using maximal force alone do not discriminate between peripheral and central factors so we cannot distinguish peripheral and central (*i.e.* spinal and supraspinal but also at the peripheral nerve trunk level) factors responsible for MVC reduction in CMT patients. We recently showed that MVCs are not able to detect small differences in muscle fatigue between patients and controls and that evoked responses are more sensitive (Bachasson et al., 2013b). At last, we cannot exclude lack of statistical power to detect differences between CMT and controls.

4.3.2. Central fatigability

Since no significant differences were observed in either VA or $MVC_{RMS/M}$ during the quadriceps fatigue test, central fatigue appeared to be similar in FSHD patients and controls in accordance with the previous work of Schillings et al. (2007). Thus, central activation impairments during a fatiguing task (sustained or intermittent) do not seem to be a limiting factor in FSHD patients. As previously mentioned, we cannot address the issue of central fatigability in CMT patients without VA assessment.

4.4. Relation between quadriceps function, functional capacities and subjective fatigue in patients

Impaired exercise capacity during stationary cycling (in all patients) and 6-min walking distance (in FSHD patients only) appeared to be related to quadriceps weakness rather than muscle endurance or fatigue in line with previous findings (Alfano et al., 2013). This weakness also seemed to impact negatively on physical functioning (SF-36 subscore) and perceived fatigue (fatigue severity scale score) in FSHD patients. In the present study, FSHD patients and controls performed the quadriceps fatigue test at the same relative intensity (*i.e.* at the same % of MVC). Patients probably have to work at a higher percentage of MVC compared to healthy subjects due to significant muscle weakness during spontaneous activity. Therefore, they may develop larger amounts of fatigue in their daily lives. This may explain, in part, why neither central nor peripheral fatigue as assessed in the present study (*i.e.* for the same relative workload) were related to impaired functional capacities, physical functioning or subjective fatigue in FSHD patients.

5. Conclusions

We showed that FNMS is feasible and reliable in ~70% of FSHD patients to assess central and peripheral neuromuscular function at rest and during an isolated quadriceps fatiguing task. In CMT patients, FNMS showed a lack of power to achieve optimal stimulation. Meanwhile, the QIF test appears to be safe and reliable to

Please cite this article in press as: Bachasson D et al. Assessement of quadriceps strength, endurance and fatigue in FSHD and CMT: Benefits and limits of femoral nerve magnetic stimulation. Clin Neurophysiol (2013), http://dx.doi.org/10.1016/j.clinph.2013.08.001

assess global fatigue and endurance with volitional measurements in this population. Development of magnetic stimulation devices is required to extend its applicability to all patients. Additional studies are needed to evaluate the feasibility and the relevance of the QIF test in other neuromuscular diseases involving different pathophysiological mechanisms (*e.g.* metabolic myopathies, amyotrophic lateral sclerosis). We reported significant muscle weakness and similar peripheral and central fatigability during intermittent isometric contractions at identical relative force levels in FSHD patients compared to controls. Impairment of functional and subjective physical capacities and experienced fatigue in patients seems to be related to muscle weakness rather than enhanced muscle fatigability or reduced endurance. Further studies must be conducted to assess neuromuscular fatigue induced by functional exercise unrelated to individual MVC (*e.g.* walking, sit-to-stand transfer) in order to clarify the impact of neuromuscular fatigue on patients' daily living activities.

Acknowledgements

We thank the French Association for Muscle Dystrophy for financial support. DB received a Ph.D. fellowship from the French Association for Muscle Dystrophy (AFM). JT was supported by a doctoral research grant from the Rhône-Alpes Region. The Direction de la Recherche Clinique et l'Innovation (DRCI) of the CHU de Grenoble was the promoter of this work.

References

Aaronson NK, Acquadro C, Alonso J, Apolone G, Bucquet D, Bullinger M, et al. International quality of life assessment (IQOLA) project. Qual Life Res 1992;1:349–51.

Alfano LN, Lowes LP, Flanigan KM, Mendell JR. Correlation of knee strength to functional outcomes in becker muscular dystrophy. Muscle Nerve 2013;47:550–4.

Allen GM, Gandevia AS, Middleton J. Quantitative assessments of elbow flexor muscle performance using twitch interpolation in post-polio patients: no evidence for deterioration. Brain 1997;120:663–72.

Amato MP, Ponziani G, Rossi F, Liedl CL, Stefanile C, Rossi L. Quality of life in multiple sclerosis: the impact of depression, fatigue and disability. Mult Scler 2001;7:340–4.

Angelini C, Tasca E. Fatigue in muscular dystrophies. Neuromuscul Disord 2012;22:S214–20.

Atkinson G, Nevill AM. Statistical methods for assessing measurement error (reliability) in variables relevant to sports medicine. Sports Med 1998;26:217–38.

Bachasson D, Millet GY, Decorte N, Wuyam B, Levy P, Verges S. Quadriceps function assessment using an incremental test and magnetic neurostimulation: a reliability study. J Electromyogr Kinesiol 2013a;23:649–58.

Bachasson D, Guinot M, Wuyam B, Favre-Juvin A, Millet GY, Levy P, et al. Neuromuscular fatigue and exercise capacity in fibromyalgia syndrome. Arthritis Care Res (Hoboken) 2013b;65:432–40.

Balady GJ, Arena R, Sietsema K, Myers J, Coke L, Fletcher GF, et al. Clinician's Guide to cardiopulmonary exercise testing in adults: a scientific statement from the American heart association. Circulation 2010;122:191–225.

Barro M, Carnac G, Flavier S, Mercier J, Vassetzky Y, Laoudj-Chenivesse D. Myoblasts from affected and non-affected FSHD muscles exhibit morphological differentiation defects. J Cell Mol Med 2010;14:275–89.

Bigland-Ritchie B, Jones DA, Hosking GP, Edwards RH. Central and peripheral fatigue in sustained maximum voluntary contractions of human quadriceps muscle. Clin Sci Mol Med 1978;54:609–14.

Chaudhuri A, Behan PO. Fatigue in neurological disorders. Lancet 2004;363:978–88.

Clark BC, Cook SB, Ploutz-Snyder LL. Reliability of techniques to assess human neuromuscular function in vivo. J Electromyogr Kinesiol 2007;17:90–101.

Dellorusso C, Crawford RW, Chamberlain JS, Brooks SV. Tibialis anterior muscles in mdx mice are highly susceptible to contraction-induced injury. J Muscle Res Cell Motil 2001;22:467–75.

Di Lazzaro V, Oliviero A, Tonali PA, Felicetti L, De Marco MB, Saturno E, et al. Changes in motor cortex excitability in facioscapulohumeral muscular dystrophy. Neuromuscul Disord 2004;14:39–45.

Dimitrova NA, Dimitrov GV. Interpretation of EMG changes with fatigue: facts, pitfalls, and fallacies. J Electromyogr Kinesiol 2003;13:13–36.

Durnin JV, Womersley J. Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. Br J Nutr 1974;32:77–97.

Feasson L, Camdessanche JP, El Mandhi L, Calmels P, Millet GY. Fatigue and neuromuscular diseases. Ann Readapt Med Phys 2006;49:289–300 [75–84].

Friedman SD, Poliachik SL, Carter GT, Budech CB, Bird TD, Shaw DW. The magnetic resonance imaging spectrum of facioscapulohumeral muscular dystrophy. Muscle Nerve 2012;45:500–6.

Gallardo E, Garcia A, Combarros O, Berciano J. Charcot-Marie-Tooth disease type 1A duplication: spectrum of clinical and magnetic resonance imaging features in leg and foot muscles. Brain 2006;129:426–37.

Gandevia SC. Spinal and supraspinal factors in human muscle fatigue. Physiol Rev 2001;81:1725–89.

Goldstein JA, McNally EM. Mechanisms of muscle weakness in muscular dystrophy. J Gen Physiol 2010;136:29–34.

Gruet M, Temesi J, Rupp T, Levy P, Millet GY, Verges S. Stimulation of the motor cortex and corticospinal tract to assess human muscle fatigue. Neuroscience 2013;231:384–99.

Hogrel JY, Payan CA, Ollivier G, Tanant V, Attarian S, Couillandre A, et al. Development of a French isometric strength normative database for adults using quantitative muscle testing. Arch Phys Med Rehabil 2007;88:1289–97.

Hopkins WG. Measures of reliability in sports medicine and science. Sports Med 2000;30:1–15.

Hopkins WG. Reliability from consecutive pairs of trials (Excel spreadsheet). In: A new view of statistics. Internet Society of Sport Science. Available from URL: <http://www.sportsci.org/resource/stats/xrely.xls> 2002.

Hopkinson NS, Dayer MJ, Antoine-Jonville S, Swallow EB, Porcher R, Vazir A, et al. Central and peripheral quadriceps fatigue in congestive heart failure. Int J Cardiol 2012. http://dx.doi.org/10.1016/j.ijcard.2012.06.064.

Horemans HL, Beelen A, Nollet F, Jones DA, Lankhorst GJ. Reproducibility of maximal quadriceps strength and its relationship to maximal voluntary activation in postpoliomyelitis syndrome. Arch Phys Med Rehabil 2004;85:1273–8.

Jones PR, Pearson J. Anthropometric determination of leg fat and muscle plus bone volumes in young male and female adults. J Physiol 1969;204:6P–63P.

Kan HE, Scheenen TW, Wohlgemuth M, Klomp DW, van Loosbroek-Wagenmans I, Padberg GW, et al. Quantitative MR imaging of individual muscle involvement in facioscapulohumeral muscular dystrophy. Neuromuscul Disord 2009;19:357–62.

Krupp LB, LaRocca NG, Muir-Nash J, Steinberg AD. The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus. Arch Neurol 1989;46:1121–3.

Kufel TJ, Pineda LA, Mador MJ. Comparison of potentiated and unpotentiated twitches as an index of muscle fatigue. Muscle Nerve 2002;25:438–44.

Lindeman E, Spaans F, Reulen JP, Leffers P, Drukker J. Surface EMG of proximal leg muscles in neuromuscular patients and in healthy controls. Relations to force and fatigue. J Electromyogr Kinesiol 1999;9:299–307.

Menotti F, Bazzucchi I, Felici F, Damiani A, Gori MC, Macaluso A. Neuromuscular function after muscle fatigue in Charcot-Marie-Tooth type 1A patients. Muscle Nerve 2012;46:434–9.

Meulstee J, Darbas A, van Doorn PA, van Briemen L, van der Meche FG. Decreased electrical excitability of peripheral nerves in demyelinating polyneuropathies. J Neurol Neurosurg Psychiatry 1997;62:398–400.

Millet GY, Bachasson D, Temesi J, Wuyam B, Feasson L, Verges S, et al. Potential interests and limits of magnetic and electrical stimulation techniques to assess neuromuscular fatigue. Neuromuscul Disord 2012;22:S181–6.

Morton JP, Atkinson G, MacLaren DP, Cable NT, Gilbert G, Broome C, et al. Reliability of maximal muscle force and voluntary activation as markers of exercise-induced muscle damage. Eur J Appl Physiol 2005;94:541–8.

Nodera H, Bostock H, Kuwabara S, Sakamoto T, Asanuma K, Jia-Ying S, et al. Nerve excitability properties in Charcot-Marie-Tooth disease type 1A. Brain 2004;127:203–11.

O'Brien TD, Reeves ND, Baltzopoulos V, Jones DA, Maganaris CN. Assessment of voluntary muscle activation using magnetic stimulation. Eur J Appl Physiol 2008;104:49–55.

Place N, Maffiuletti NA, Martin A, Lepers R. Assessment of the reliability of central and peripheral fatigue after sustained maximal voluntary contraction of the quadriceps muscle. Muscle Nerve 2007;35:486–95.

Polkey MI, Kyroussis D, Hamnegard CH, Mills GH, Green M, Moxham J. Quadriceps strength and fatigue assessed by magnetic stimulation of the femoral nerve in man. Muscle Nerve 1996;19:549–55.

Saey D, Debigare R, LeBlanc P, Mador MJ, Cote CH, Jobin J, et al. Contractile leg fatigue after cycle exercise: a factor limiting exercise in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2003;168:425–30.

Schillings ML, Kalkman JS, Janssen HM, van Engelen BG, Bleijenberg G, Zwarts MJ. Experienced and physiological fatigue in neuromuscular disorders. Clin Neurophysiol 2007;118:292–300.

Schulte-Mattler WJ, Muller T, Deschauer M, Gellerich FN, Iaizzo PA, Zierz S. Increased metabolic muscle fatigue is caused by some but not all mitochondrial mutations. Arch Neurol 2003;60:50–8.

Solari A, Laura M, Salsano E, Radice D, Pareyson D, Group C-TS. Reliability of clinical outcome measures in Charcot-Marie-Tooth disease. Neuromuscul Disord 2008;18:19–26.

Strojnik V, Komi PV. Neuromuscular fatigue after maximal stretch-shortening cycle exercise. J Appl Physiol 1998;84:344–50.

Please cite this article in press as: Bachasson D et al. Assessement of quadriceps strength, endurance and fatigue in FSHD and CMT: Benefits and limits of femoral nerve magnetic stimulation. Clin Neurophysiol (2013), http://dx.doi.org/10.1016/j.clinph.2013.08.001

Szecsi J, Gotz S, Pollmann W, Straube A. Force-pain relationship in functional magnetic and electrical stimulation of subjects with paresis and preserved sensation. Clin Neurophysiol 2010;121:1589–97.

Tomazin K, Verges S, Decorte N, Oulerich A, Maffiuletti NA, Millet GY. Fat tissue alters quadriceps response to femoral nerve magnetic stimulation. Clin Neurophysiol 2011;122:842–7.

Turki A, Hayot M, Carnac G, Pillard F, Passerieux E, Bommart S, et al. Functional muscle impairment in facioscapulohumeral muscular dystrophy is correlated with oxidative stress and mitochondrial dysfunction. Free Radic Biol Med 2012;53:1068–79.

Verges S, Maffiuletti NA, Kerherve H, Decorte N, Wuyam B, Millet GY. Comparison of electrical and magnetic stimulations to assess quadriceps muscle function. J Appl Physiol 2009;106:701–10.

Please cite this article in press as: Bachasson D et al. Assessement of quadriceps strength, endurance and fatigue in FSHD and CMT: Benefits and limits of femoral nerve magnetic stimulation. Clin Neurophysiol (2013), http://dx.doi.org/10.1016/j.clinph.2013.08.001

Available online at www.sciencedirect.com

SciVerse ScienceDirect

Neuromuscular Disorders 22 (2012) S181–S186

www.elsevier.com/locate/nmd

Potential interests and limits of magnetic and electrical stimulation techniques to assess neuromuscular fatigue

G.Y. Millet [a,b,*], D. Bachasson [b,c], J. Temesi [a], B. Wuyam [b,c,d], L. Féasson [a], S. Vergès [b,c], P. Lévy [b,c,d]

[a] *Université de Lyon, F-42023 Saint-Etienne, France*
[b] *Inserm U1042, Grenoble F-38000, France*
[c] *Université Joseph Fourier, Laboratoire HP2, Grenoble, France*
[d] *CHU de Grenoble, UF recherche exercice, UM Sports & Pathologies & Laboratoire EFCR, Clinique de Physiologie Sommeil Exercice, Grenoble, France*

Abstract

Neuromuscular function can change under different conditions such as ageing, training/detraining, long-term spaceflight, environmental conditions (*e.g.* hypoxia, hyperthermia), disease, therapy/retraining programs and also with the appearance of fatigue. Neuromuscular fatigue can be defined as any decrease in maximal voluntary strength or power. There is no standardized method to induce fatigue and various protocols involving different contraction patterns (such as sustained or intermittent submaximal isometric or dynamic contractions on isokinetic or custom chairs) have been used. Probably due to lack of motivation/cooperation, results of fatigue resistance protocols are more variable in patients than in healthy subjects. Magnetic and electrical stimulation techniques allow non-invasive assessment of central and peripheral origins of fatigue. They also allow investigation of different types of muscle fatigue when combining various types of stimulation with force/surface EMG measurements. Since maximal electrical stimuli may be uncomfortable or even sometimes painful, several alternative methods have been recently proposed: submaximal muscle stimulation, low/high-frequency paired pulses instead of tetanic stimuli and the use of magnetic stimulation at the peripheral level.

Keywords: Electrical and magnetic stimulation; Muscle and central fatigue; EMG; M-wave; Evoked forces

1. Introduction

Neuromuscular function, implying that of the muscle and central nervous system, may change with ageing, training/detraining and long-term spaceflight. Neuromuscular function evaluation may be useful in following the history of a disease and evaluating the effect of a therapy/retraining program in patients. In addition to these chronic alterations, changes can occur during acute conditions such as exposure to different environmental conditions (*e.g.* hyperthermia, hypoxia) and fatigue. Neuromuscular fatigue is an exercise-related decrease in the maximal voluntary force or power of a single muscle or muscle group whether or not the task can be sustained. This may involve processes at all levels of the motor pathway from the brain to skeletal muscle. Classically, alterations of neuromuscular function due to fatigue are classified as central (neural) or peripheral (muscular) in origin. It is well-recognized that these are mutually dependent since recruitment of motoneurones depends on the descending drive from supraspinal sites and central drive is controlled through a combination of influences including excitatory and inhibitory reflex inputs from muscles, joints, tendons and cutaneous afferents. By stimulating a contracting or relaxed muscle at various levels of the neuromuscular system with different types of stimulation, and by recording force or electromyographic (EMG) responses, it is possible to non-invasively gain

* Corresponding author. Address: Bâtiment Médecine du Sport, Myologie, Hôpital Bellevue, 42055 Saint-Etienne Cedex 2, France. Tel.: +33 477 120 366; fax: +33 477 127 229.
E-mail address: guillaume.millet@univ-st-etienne.fr (G.Y. Millet).

0960-8966/$ - see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.nmd.2012.10.007

insight into neuromuscular fatigue. Among the various artificial stimulus techniques that can be used to investigate neuromuscular function in clinical and research fields, electrical stimulation (ES) is probably the most widely used. Magnetic stimulation (MS) has been recently introduced at the peripheral level [1]. In particular, femoral nerve MS is well-tolerated and appears more suitable than ES in clinical practice. MS is also used at the cortical level to measure supraspinal fatigue (*e.g.* [2]). The purpose of this review is to address the potential interests and limits of different techniques used to assess neuromuscular fatigue in the field of pathology. It must be recognized that fatigue is not only defined as strength loss or EMG changes but also as a perception. Sensations of fatigue include both homeostatic and psychological (expectation, arousal, motivation, and mood) factors [3]. Fatigue questionnaires will not be considered in the present review and related information can be found elsewhere [4]. Similarly, central fatigue is sometimes associated with alteration of cognitive performance (*e.g.* declines in reaction times or deterioration in continuous performance tasks). Neither will this aspect be treated in the present paper as central fatigue is defined here as the reduction of maximal voluntary activation.

2. Muscle (or peripheral) fatigue

After different types of exercise such as repetitive isometric or dynamic contractions on isokinetic ergometers or custom chairs and whole-body exercise (*e.g.* walking, running, cycling), fatigue can be detected. Peripheral changes can be investigated by stimulating the muscle in the relaxed state, usually by ES, before, during and after the fatiguing exercise. The standard method consists of first determining the optimal stimulus intensity by progressively increasing the intensity of the stimulus until increasing the intensity does not increase the mechanical or electrical responses (*i.e.* optimal intensity). Supramaximal intensity, generally 120–150% of optimal intensity, is classically chosen to ascertain full spatial recruitment with small changes in electrode position even if such a high intensity may induce co-activation in some muscle groups, *e.g.* the triceps brachii can be inadvertently stimulated if the stimulus intensity applied to the biceps brachii is excessive. As explained above, the use of MS for peripheral measurements (mainly for quadriceps assessment) has recently gained popularity, particularly with patients (*e.g.* [1]) in order to minimize discomfort. We [5] ascertained the accord between ES (supramaximal intensity) and MS of the femoral nerve. However, some limits to the ability of MS to produce supramaximal stimuli exist, particularly in overweight subjects. We [6] showed, in an overweight but not obese group (Body Mass Index: 26.1 $kg\,m^{-2}$; Body fat: 18.9%), that maximal responses for both parameters could not be elicited when intensity was $\leqslant$90% and $\leqslant$85% of maximal stimulator output for twitch torque and M-wave amplitude respectively, while maximal responses were obtained at 80% of maximal stimulator output in the lean group. It was concluded that the capacity of femoral nerve MS to deliver supramaximal stimulation is altered when fat thickness below the coil increases. Since it is recommended that optimal intensity be increased by 20–50% to take into account movements of the stimulating tool, the MS technique may be limited by stimulator power. Also, a reduction of excitability may be observed with fatigue, *i.e.* the activation threshold of motor nerve axons increases after several minutes of repetitive use. Thus, MS at the peripheral level may be limited by the stimulator output for fatigue studies even with slightly overweight (*i.e.* fat) subjects.

Different types of stimuli can be evoked to non-invasively investigate the (i) neuromuscular propagation of action potentials along the sarcolemma (M-wave, high-frequency fatigue), (ii) excitation–contraction coupling (low-frequency fatigue (LFF)) and (iii) intrinsic force (high-frequency stimulation at supramaximal intensity). A single stimulus allows measurements of mechanical (twitch) and EMG (M-wave) responses. However, the mechanical response of every muscle cannot be measured by nerve stimulation, possibly because the nerve is not superficial enough. Another problem might be that some nerves evoke responses in both agonist and antagonist muscles. For instance, stimulation of the musculocutaneous nerve to evoke a motor response of the elbow flexors induces co-contraction of the elbow extensors invalidating the mechanical response. In this case, nerve stimulation can be used to obtain the M-wave but motor point stimulation is required to measure the mechanical response (*e.g.* [7]). Another methodological point to be considered during repeated contractions such as those used in fatiguing tasks is the contradictory effects of potentiation and fatigue. The change in twitch tension from before to after a sustained contraction depends on potentiation (the primary mechanism being phosphorylation of the myosin light chains that is known to induce increased Ca^{2+} sensitivity), and fatigue-associated effects. This is the reason it is always recommended to measure the baseline twitch in the fully potentiated condition so as to not underestimate fatigue [8]. Systematic use of fully potentiated twitches has not been used in the literature. Other parameters such as musculo-tendinous stiffness may also affect the mechanical response to a single ES or MS pulse.

The force–frequency relationship is another tool used to characterize contractile properties of a muscle, usually from several stimulus trains at different frequencies [9]. During *in vivo* studies conducted in humans, it is possible to use as few as two stimulus trains; one at low- (below the fusion frequency, *e.g.* 10–20 Hz) and one at high-frequencies (above the fusion frequency, *e.g.* 50–100 Hz). From the ratio of the mechanical response at low- and high-frequencies, the type of peripheral fatigue can be determined. LFF is characterized by a higher relative loss of force at low frequencies of stimulation and slow recovery [9]. Because the term LFF is sometimes improperly used to describe fatigue induced by low frequency

stimulation, the term "prolonged low-frequency force depression" has been proposed to avoid confusion [10]. LFF is usually associated with a failure in the excitation–contraction coupling since intracellular measurements have shown that LFF is due to a reduction in Ca^{2+} release [11]. LFF is seen after eccentric exercise [5] and this might be due to a reduced level of junctophilins, the proteins involved in transverse (T)-tubule and sarcoplasmic reticulum membrane apposition [12]. Conversely, high-frequency fatigue is characterized by an excessive loss of force at high stimulus frequencies and is attributed, at least in part, to an accumulation of extra-cellular K^+. In high-frequency fatigue, rapid force recovery occurs when the stimulus frequency is reduced. Changes in M-wave characteristics have also been used to investigate the neuromuscular propagation of action potentials along the sarcolemma [13] but the direct correspondence between M-wave amplitude/duration and neuromuscular propagation of action potentials has been questioned.

Another option for assessing peripheral changes is to induce high-frequency tetanus [14]. The problem with this method when applied to large muscle groups is its brutality. Depending on the muscle group, this type of stimulation may be painful and/or induce cramping or injury. Alternatively, the use of an absolute electrically evoked force when tetanus induced by nerve stimulation at supramaximal intensity (high-frequency stimulation) is superimposed on a maximal voluntary contractions (MVC) (*i.e.* similar to the central activation ratio method) as an index of "intrinsic" force [15] has been suggested. While this is slightly less painful than high-frequency evoked tetanus in a relaxed muscle [14], the level of discomfort remains high. For example, it has been reported that a knee cap was dislocated during such an experiment [16]. A compromise for examining contractile response might be to use high-frequency paired pulses [5,8], although this measure is prone to be affected by potentiation and stiffness changes.

Limits must be acknowledged when measuring muscle fatigue with ES and MS in relaxed muscles. For instance, the absence of modification of the low-to-high frequency ratio could result from the combined effects of LFF, which preferentially depresses low-frequency responses, and hyperpolarization, which preferentially depresses high-frequency responses. Also, tetanic stimuli may induce co-activation that limits the significance of the response as an index of maximal "intrinsic" force. Some magnetic and electrical stimuli are not well tolerated because of discomfort or pain, particularly nerve trunk stimulation of large muscle groups. As a consequence, adaptations of stimulation protocols are mandatory with patients or elderly people. For instance, we have shown that LFF is comparable when evaluated with nerve and muscle stimulation [17]. Similarly, LFF could be evaluated by using low- (10 Hz) and high-frequency (100 Hz) doublets [5]. One conceptual difficulty is the fact that in some subjects a 10 Hz doublet has an amplitude virtually identical to that of a 100 Hz doublet. However, changes in the ratio of peak forces measured at 10 and 100 Hz with tetanic stimuli were significantly correlated with changes measured with doublets. This makes the use of low- and high-frequency doublets relevant [5] even if further confirmation of this result is needed. Important problems in the muscular fatigue evaluation of patients are motivation and cooperation since every evaluation assumes that the patient performs to the best of his or her ability. It has been reported that variation in performance for time-to-exhaustion protocols is much higher in patients than controls and that patients show greater variation in MVC force [18]. By using ES (or MS) in relaxed muscle regularly during a test imposing a given load (force or power), it could be possible to make the results of muscle fatigability independent of patient will and motivation. To the best of our knowledge, such a standardized test does not exist. To completely remove the influence on the central nervous system, one solution is to use repeated ES or MS and evaluate the decrement in the kinetics of force. The assessment of muscle fatigability by repetitive peripheral MS has been suggested to be well-tolerated in a clinical study [19].

Finally, it must be considered that an absolute level of peripheral fatigue is highly dependent on (i) the type of stimulation induced and (ii) the time of recovery after the end of the fatiguing task. In high-intensity protocols, a small degree of muscle recovery can have a large effect on the power output of fatigued muscles [20]. In other words, pronounced recovery may occur in only a few seconds so that recommendations of highly standardized protocol must be given in clinical evaluation (unpublished personal data).

3. Central fatigue

To explore central modifications with fatigue, the standard technique is the twitch interpolation method, consisting of stimulating with single stimuli or high-frequency paired pulses at maximal force during MVC and to compare the superimposed mechanical responses to the potentiated mechanical responses obtained in the relaxed muscle. This allows calculation of the maximal voluntary activation level (%VA). Any reduction of %VA due to exercise is considered central fatigue. This technique can be applied to different nerves such as the femoral nerve (quadriceps) or tibial nerve (plantar flexors).

The superimposition of high-frequency (*e.g.* 100 Hz) paired pulses followed by high-frequency paired pulses in the relaxed muscle has been proposed rather than the classical use of single stimuli. Behm et al. [21] found no significant difference in the sensitivity of the twitch interpolation method using either single twitches, doublets or quintuplets. Nevertheless, superimposing high-frequency potentiated paired-pulses is now recommended [8]. Whatever the type of evoked stimulus, there remains debate as to whether the twitch interpolation method provides a valid measure of %VA. Small reductions in central fatigue may

go undetected, thus the method is sometimes considered semi-quantitative [22].

In the context of diseases, it is of interest to report that muscle rather than nerve stimulation can be used to determine %VA. Rutherford et al. [23] compared the use of twitch superimposition evoked by percutaneous stimulation of the human quadriceps at maximally tolerated intensities with stimulation of the femoral nerve. These authors found that the relationship between the extra force generated by the twitch and the level of voluntary contraction was independent of the proportion of the muscle stimulated, *i.e.* the technique was valid at the muscular level for both healthy controls and patients with musculoskeletal disorders. This submaximal technique seems valid whatever the method used to determine the stimulus intensity; for instance, other authors have used an absolute intensity of 100 mA [24] or an intensity to obtain high-frequency tetanus equal to 50% of subject MVC [17]. As explained previously, this technique is also recommended for some muscle groups because nerve stimulation activates both agonist and antagonist muscles. This is the case for elbow flexors since stimulation of the musculocutaneous nerve activates both biceps brachialis and triceps brachialis. It is essential to note that electrical stimulation of the muscle selectively activates nerve-endings within the muscle, and not the muscle fibers directly.

During an exhausting task, the increment of force (superimposed twitch) evoked by motor nerve stimulation during an isometric MVC can increase, suggesting the development of central fatigue. The same concept applies to a superimposed twitch elicited by transcranial magnetic stimulation (TMS). Initially demonstrated during a 3-min MVC of the elbow flexors [25], the increase in superimposed twitch produced by TMS during a maximal contraction was confirmed in various muscle groups and several exercise paradigms. These studies indicate that some fatigue is related to supraspinal mechanisms even if alteration of the neural drive may be located upstream of the motor cortex [25]. The method of calculating cortical %VA is derived from the twitch interpolation technique although the resting twitch is not directly measured as for nerve stimulation. Instead, it is extrapolated from the linear regression between the superimposed twitch and voluntary force at different force levels >50% MVC. It is not appropriate to normalize the superimposed force elicited during voluntary contraction to one evoked in the relaxed muscle

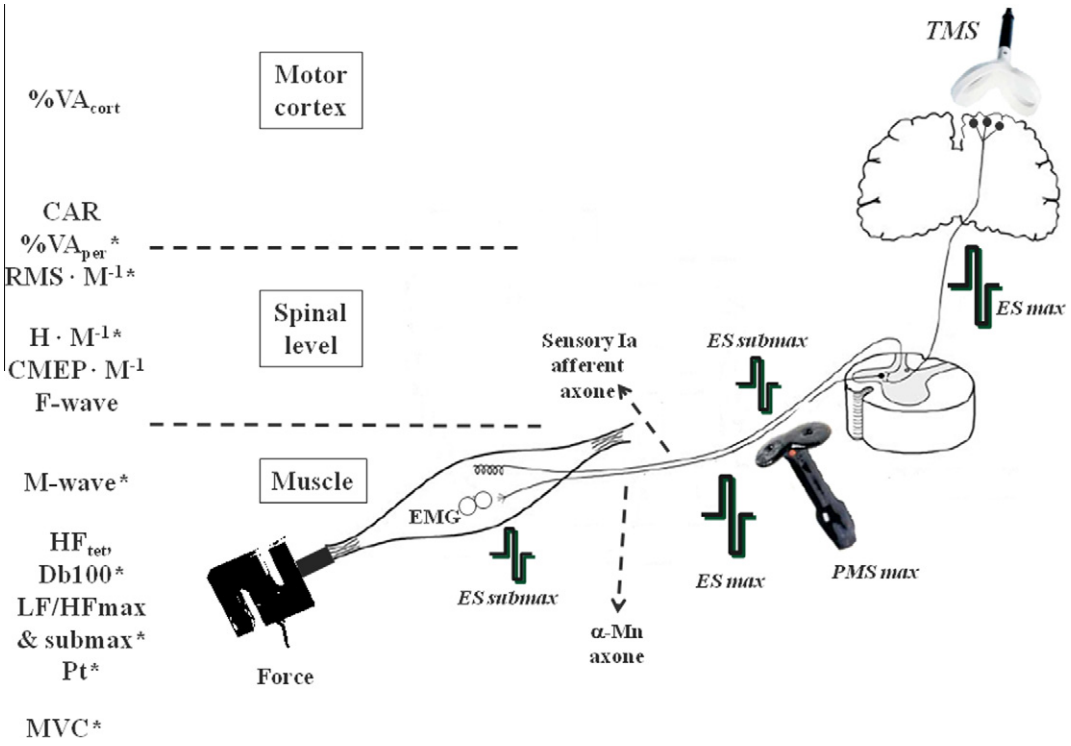

Fig. 1. Schematic view of the main electrical and magnetic stimulation techniques allowing investigation of neuromuscular fatigue. Adapted from Millet et al. [27]. ES: electrical stimulation; TMS: transcranial magnetic stimulation; PMS: peripheral magnetic stimulation; %VAcort: maximal cortical voluntary activation; CAR: central activation ratio; %VA$_{per}$: maximal voluntary activation measured from motor nerve stimulation; RMS $\cdots$ M^{-1}: EMG (root mean square) measured during MVC normalized to M-wave amplitude; H $\cdots$ M^{-1}: H reflex normalized to M-wave amplitude; CMEP $\cdots$ M^{-1}: cervicomedullary motor-evoked potential normalized to M-wave amplitude; M-wave: EMG response to single motor nerve stimulation; HF$_{tet}$: high frequency tetanic stimulation (>50 Hz); Db100: force evoked by paired-pulse at high frequency (usually 100 Hz); LF/HFmax & submax: ratio of force evoked with low-frequency stimulation (usually 10–20 Hz) to force evoked with high-frequency stimulation (>50 Hz), either submaximally in the muscle (submax) or supramaximally by the nerve (max); Pt: peak twitch, force evoked by a singlepulse; MVC: maximal voluntary contraction. $*$ means suitable for clinical populations.

because corticospinal excitability dramatically increases between rest and contractions.

The twitch interpolation technique (either cortical or peripheral) is not the only method used to detect central fatigue. Alternative methods include (i) superimposing a train of stimuli, *i.e.* central activation ratio [15,16,23], (ii) comparing the MVC response to the force evoked by high-frequency tetanus [14] or (iii) examining the change in maximal EMG response (*e.g.* root mean square, RMS) during voluntary contractions, normalized to maximal M-wave, *i.e.* EMG response to a single stimulus. This RMS $\cdots$ M^{-1} index is less reproducible than other methods but allows the examination of modified activation (maximal EMG activity) in the individual muscles of a muscle group, a measure that is not feasible with any other technique based on force measurement. For instance, RMS $\cdots$ M^{-1} of the *vastuslateralis*, *vastusmedialis* and *rectus femoris* can be measured while only %VA of knee extensors may be quantified. In addition, EMG measurements may represent the only way to assess central changes during ballistic contractions. A limit of all these techniques is that they require a MVC which may be problematic with patients or subjects unfamiliar with maximal contractions.

To investigate changes at the spinal level with fatigue, different techniques have been used: Hoffmann reflex (H-reflex), cervicomedullary motor-evoked potentials (CMEP) or F-waves. Because (i) afferents and alpha motoneurones are modulated by presynaptic mechanisms that may change with fatigue (*e.g.* from group III and IV afferent fibers) and (ii) F-waves test only a small portion of the alpha motoneurones pool [26], CMEPs have recently been popularized to detect deteriorated motoneuronal excitability with fatigue since they are not subject to pre-synaptic inhibition. CMEPs must be normalized to M-wave responses to account for any peripheral alteration of the EMG signal, particularly during fatigue studies since M-wave properties are influenced by the type of fatigue and differ between muscles.

It is tempting to consider the fact that EMG levels at the end of a sustained exhausting task remain below maximal EMG as an index of central fatigue. However, the relation between surface EMG amplitude and muscle force varies during fatiguing contractions meaning the neural drive cannot be reliably estimated from EMG amplitude during fatiguing contractions [3].

In conclusion, electrical and magnetic stimulation are extensively used in research to measure alterations in neuromuscular function with fatigue; however, they are still not common in clinical practice. One reason is likely that analysis of the force and EMG signals measured either during the test or pre/post the exhausting exercise exceeds time availability; thus, physicians are more prone to assess patients' perceptions of fatigue. We believe that these techniques can help to non-invasively investigate central and peripheral origins of fatigue (Fig. 1) so that clinicians should be encouraged to use them in order to better assess their patients, particularly their resistance to fatigue in their daily life. Clinical use would be aided by a standardized test to measure patients' fatigability.

4. Conflict of interest

None.

References

[1] Polkey MI, Kyroussis D, Hamnegard CH, et al. Quadriceps strength and fatigue assessed by magnetic stimulation of the femoral nerve in man. Muscle Nerve 1996;19:549–55.
[2] Taylor JL, Butler JE, Allen GM, Gandevia SC. Changes in motor cortical excitability during human muscle fatigue. J Physiol 1996;490(Pt 2):519–28.
[3] Enoka RM. Muscle fatigue – from motor units to clinical symptoms. J Biomech 2012;45:427–33.
[4] Feasson L, Camdessanche JP, El Mandhi L, Calmels P, Millet GY. Fatigue and neuromuscular diseases. Ann Readapt Med Phys 2006;49:289–300, 375–284.
[5] Verges S, Maffiuletti NA, Kerherve H, et al. Comparison of electrical and magnetic stimulations to assess quadriceps muscle function. J Appl Physiol 2009;106:701–10.
[6] Tomazin K, Verges S, Decorte N, et al. Fat tissue alters quadriceps response to femoral nerve magnetic stimulation. Clin Neurophysiol 2011;122:842–7.
[7] Todd G, Taylor JL, Gandevia SC. Measurement of voluntary activation of fresh and fatigued human muscles using transcranial magnetic stimulation. J Physiol 2003;551:661–71.
[8] Place N, Maffiuletti NA, Martin A, Lepers R. Assessment of the reliability of central and peripheral fatigue after sustained maximal voluntary contraction of the quadriceps muscle. Muscle Nerve 2007;35:486–95.
[9] Edwards RH, Hill DK, Jones DA, Merton PA. Fatigue of long duration in human skeletal muscle after exercise. J Physiol 1977;272:769–78.
[10] Bruton JD, Place N, Yamada T, et al. Reactive oxygen species and fatigue-induced prolonged low-frequency force depression in skeletal muscle fibres of rats, mice and SOD2 overexpressing mice. J Physiol 2008;586:175–84.
[11] Hill CA, Thompson MW, Ruell PA, Thom JM, White MJ. Sarcoplasmic reticulum function and muscle contractile character following fatiguing exercise in humans. J Physiol 2001;531:871–8.
[12] Corona BT, Balog EM, Doyle JA, et al. Junctophilin damage contributes to early strength deficits and EC coupling failure after eccentric contractions. Am J Physiol Cell Physiol 2010;298: C365–76.
[13] Bigland-Ritchie B. EMG and fatigue of human voluntary and stimulated contractions. Ciba Found Symp 1981;82:130–56.
[14] Millet GY, Martin V, Lattier G, Ballay Y. Mechanisms contributing to knee extensor strength loss after prolonged running exercise. J Appl Physiol 2003;94:193–8.
[15] Martin V, Kerhervé H, Messonnier LA, et al. Central and peripheral contributions to neuromuscular fatigue induced by a 24-h treadmill run. J Appl Physiol 2010;108:1224–33.
[16] Bigland-Ritchie B, Jones DA, Hosking GP, Edwards RH. Central and peripheral fatigue in sustained maximum voluntary contractions of human quadriceps muscle. Clin Sci Mol Med 1978;54:609–14.
[17] Martin V, Millet GY, Martin A, Deley G, Lattier G. Assessment of low-frequency fatigue with two methods of electrical stimulation. J Appl Physiol 2004;97:1923–9.
[18] Vallier JM, Gruet M, Mely L, Pensini M, Brisswalter J. Neuromuscular fatigue after maximal exercise in patients with cystic fibrosis. J Electromyogr Kinesiol, in press.

[19] Swallow EB, Gosker HR, Ward KA, et al. A novel technique for nonvolitional assessment of quadriceps muscle endurance in humans. J Appl Physiol 2007;103:739–46.
[20] Allen D, Westerblad H. What limits exercise during high-intensity aerobic exercise? Eur J Appl Physiol 2010;110:661–2 [author reply 663–664].
[21] Behm DG, St-Pierre DM, Perez D. Muscle inactivation: assessment of interpolated twitch technique. J Appl Physiol 1996;81: 2267–73.
[22] de Haan A, Gerrits KH, de Ruiter CJ. Counterpoint: the interpolated twitch does not provide a valid measure of the voluntary activation of muscle. J Appl Physiol 2009;107:355–7.
[23] Rutherford OM, Jones DA, Newham DJ. Clinical and experimental application of the percutaneous twitch superimposition technique for the study of human muscle activation. J Neurol Neurosurg Psychiatry 1986;49:1288–91.
[24] Place N, Casartelli N, Glatthorn JF, Maffiuletti NA. Comparison of quadriceps inactivation between nerve and muscle stimulation. Muscle Nerve 2010;42:894–900.
[25] Gandevia SC, Allen GM, Butler JE, Taylor JL. Supraspinal factors in human muscle fatigue: evidence for suboptimal output from the motor cortex. J Physiol 1996;490(Pt 2):529–36.
[26] Taylor JL, Gandevia SC. Transcranial magnetic stimulation and human muscle fatigue. Muscle Nerve 2001;24:18–29.
[27] Millet GY, Martin V, Martin A, Verges S. Electrical stimulation for testing neuromuscular function: from sport to pathology. Eur J Appl Physiol 2011;111:2489–500.

The use of transcranial magnetic stimulation in locomotor function: methodological issues and application to extreme exercise conditions

Abstract: Transcranial magnetic stimulation (TMS) is a widely-used investigative technique in motor cortical evaluation. TMS is now being used in the investigation of fatigue to help partition the effects of central fatigue. Few studies have utilized this technique to evaluate the effects of locomotor exercise and none in conditions of extreme exercise. Therefore, the purpose of this thesis was twofold; first, to answer methodological questions pertaining to the use of TMS in fatigue evaluation, particularly of the quadriceps, and second, to investigate the effects of extreme exercise conditions on the development of central and supraspinal fatigue and corticospinal excitability and inhibition. In Studies 1 and 2, the effect of approaching a target force in different ways before the delivery a TMS pulse and the difference between commonly-employed methods of determining TMS intensity on the selection of optimal TMS intensity were investigated. In Study 3, the effect of one night sleep deprivation on cognitive and exercise performance and central parameters was investigated. The effect of a 110-km ultra-trail on the supraspinal component of central fatigue was evaluated in Study 4. The principal findings from this thesis are that during TMS evaluation during brief voluntary contractions, it is essential to deliver the TMS pulse once the force has stabilized at the target and that a stimulus-response curve at 20% MVC is appropriate for determining optimal TMS intensity in exercise and fatigue studies. Furthermore, while sleep deprivation negatively-impacted cognitive and exercise performance, it did not influence neuromuscular parameters nor result in greater central fatigue. Supraspinal fatigue develops and corticospinal excitability increases during endurance/ultra-endurance running and cycling, while the effects on inhibitory corticospinal mechanisms are equivocal and probably depend on exercise characteristics and TMS intensity.
Keywords: transcranial magnetic stimulation, cortical voluntary activation, corticospinal excitability, intracortical inhibition, neuromuscular fatigue

---

Utilisation de la stimulation magnétique transcrânienne dans l'évaluation de la fonction motrice : aspects méthodologiques et application à l'exercice extrême

Resumé : *La stimulation magnétique transcrânienne (TMS) est une technique d'investigation classiquement utilisée dans l'évaluation du cortex moteur. La TMS est utilisée dans l'étude de la fatigue afin de distinguer sa composante centrale. Peu d'études ont utilisé cette technique pour évaluer les effets de l'exercice locomoteur et aucune dans des conditions extrêmes. Ainsi, l'objectif de cette thèse était double: d'abord, répondre à certaines questions méthodologiques concernant l'utilisation de la TMS dans l'évaluation de la fatigue, en particulier du muscle quadriceps, et deuxièmement, étudier les effets de l'exercice en conditions extrêmes sur le développement de la fatigue centrale et supraspinal ainsi que sur l'excitabilité et l'inhibition corticospinales. Dans les Etudes 1 et 2, l'effet de différentes approches d'une force cible avant l'application d'une impulsion TMS ainsi que les différences entre les principales méthodes utilisées pour déterminer l'intensité optimale de TMS ont été étudiés. Dans l'Etude 3, l'effet d'une nuit de privation de sommeil sur les performances cognitives et physiques et les paramètres centraux a été étudié. L'effet d'un ultra-trail de 110 km sur la composante supraspinale de la fatigue centrale a été évalué dans l'Etude 4. Les conclusions principales de cette thèse sont, sur le plan méthodologique, i) que lors de l'évaluation par TMS pendant de brèves contractions volontaires, il est essentiel d'appliquer l'impulsion de TMS après que la force produite par le sujet se soit stabilisée à la valeur cible et ii) qu'une courbe stimulus-réponse à 20% de la force maximale volontaire est appropriée pour déterminer l'intensité de TMS optimale dans les études portant sur l'exercice et la fatigue. De plus, bien que la privation de sommeil ait des impacts négatifs sur les performances cognitives et à l'exercice, elle n'a pas d'influence sur des paramètres neuromusculaires ni ne provoque une plus grande fatigue centrale. Une fatigue supraspinale se développe et l'excitabilité corticospinale augmente au cours d'exercices d'endurance/ultra-endurance en course à pied et ne vélo, tandis que les effets sur les mécanismes inhibiteurs corticospinaux sont équivoques et probablement dépendent des caractéristiques de l'exercice et de l'intensité de la TMS.*
Mots clés : *stimulation magnétique transcrânienne, activation volontaire corticale, excitabilité corticospinale, inhibition intracorticale, fatigue neuromusculaire*