EVOKED RESPONSES TO TRANSCRANIAL AND ELECTRICAL STIMULATION DURING ISOMETRIC AND LENGTHENING CONTRACTIONS OF THE SOLEUS MUSCLE

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ABSTRACT

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The aim of this study was to assess differences in motor control between isometric and lengthening contractions of the soleus muscle. Evoked responses to TMS (MEPs) and electrical stimulation of the peripheral nerve (H-reflexes) where recorded at rest and during isometric and lengthening contractions of the soleus muscle at 20%, 40%, 60% and 80% MVC. Torque and background EMG were averaged over a time window of 100ms prior to stimulation. MEPs were found to be significantly lower ($P < 0.05$) during lengthening contractions compared to isometric contractions at 40%, 60% and 80% MVC (10.95 ± 0.05 mV *vs* 9.93 ±0.06 mV ; 1.47 ± 0.07 mV *vs* 9.7 ± 0.08 mV; 11.48 ± 0.08 mV ± *vs* 10.14 ± 0.07 mV). The H-reflex-to-Mmax ratio was significantly lower ($P < 0.05$) during passive lengthening compared to the passive isometric condition (2.5 ± 1.11 mV *vs* 1.4 ±0.88 mV). In an active muscle, the H-reflex-to-Mmax ratio was similar between isometric and lengthening modes of contraction. Torque production during passive lengthening (1.8 ± 2.8 Nm *vs* 4.6 ± 3.2 Nm) and at 20% MVC lengthening (215.25 ± 68.3 Nm *vs* 300.8 ± 155 Nm) was higher ($P < 0.05$) compared to the corresponding torque produced in isometric conditions.

MEPs represent the excitability of both supraspinal and spinal neurons; H-reflex, on the other hand, reflects only the excitability of the spinal MN pool. Therefore, it was concluded that the motor cortex generates a descending command of lower amplitude in the case of lengthening contractions. This, however, could not be considered as neural inhibition, since recorded torque during muscle lengthening was similar or higher compared to the torque recorded during isometric muscle actions.

Keywords: isometric contractions, lengthening contractions, MEP, H-reflex, soleus, motor control

ABBREVIATIONS

CMS	Cervicomedullary stimulation
EMG	Electromyography/ Electromyographic
H-reflex	Hoffman reflex
ISO	Isometric
LEN	Lengthening
MEP	Motor-evoked potential
Mmax	Maximal M-wave
MN	Motor Neuron
MU	Motor unit
MVC	Maximal voluntary contraction
SHORT	Shortening
SOL	Soleus
TMS	Transcranial Magnetic stimulation
TA	Tibialis anterior

CONTENTS

1 BACKGROUND

1.1 Introduction

Many attempts have been made to understand the central nervous system in relation with the skeletal muscles – or, to put in other words, motor control. Gandevia (2001) has designated the muscle as a motor driven by the central nervous system. For obvious reasons, this representation is not sufficient in order to understand the complex neural pathways mediating voluntary movements. The endless neural connections, called synapses, either between two neurons or between neurons and muscle fibers, give rise to a complex system from a structural, as well as from a functional aspect. In addition to this, reflexes, i.e. automatic responses to a variety of stimuli may arise from other structures of the nervous system, other than the brain. Understanding how muscles are activated in order to produce a sequence of voluntary or automatic movements, implies the understanding of the central nervous system function and organization.

The central nervous system can be divided into two centers of control: a supraspinal and a spinal one. In the latter reside the nuclei of groups of specialised neurons, i.e. α-motorneuron pools, which innervate muscle fibers belonging to the same muscle. However, MNs within the same pool bear dissimilar characteristics, a fact which goes in accordance with the variety of muscle fiber types. In humans, three different types of motor units have been identified, based on the characteristics of the innervating motorneuron and the innervated muscle fibers. The fundemental distinction between fast and slow motor units has been established according to muscle twitch characteristics.

Motor units respond to a descending command from superior control centers, and are activated in a different manner according to the requirements of a given motor task. In other words, in order for a motor task to be performed succesfully, the correct type of motor unit has to be recruited; once recruited, the activity of the motor unit is graded in order to produce the desired movement. The latter is achieved through modulation of the motor unit firing frequency (i.e. rate coding), resulting in a precise control of force

production. In multijoint movements, the parameters of the tasks are multiple and motor units are activated according to complex patterns of activity. When it comes to one-joint movements, deductions concerning motor unit activation can be made more easily, through measurement of the electromyographic (EMG) activity of the muscles crossing the joint and of global torque production.

In contrast to isometric (ISO) contractions, lengthening (LEN) contractions – i.e. stretching of an active muscle – have up to present remained an unsolved mystery for researchers. In fact, numerous assumptions have been made concerning the motor control strategies employed during muscle LEN. A great amount of clarifications concerning the exact neural mechanisms underlying the resulting motor unit activation, and/or the motor unit activation pattern themselves during LEN, has, however, been achieved via stimulation of neural pathways and measurement of the evoked responses. The excitability of different neural structures underlying torque production is therefore determined, and the respective role of each structure in movement control is outlined.

This paper therefore attempts to shed some light to the neural control of LEN tasks with the help of transcranial magnetic stimulation (TMS) and electrical stimulation. The evoked responses to the aforementioned stimulation techniques provide information about the excitability, and as a corollary, about the degree of involvement of central and peripheral neural networks in the performance of LEN tasks. This is followed by a comparison of LEN to ISO contractions, the latter serving as a reference, given the ample amount of information concerning their initiation and control.

1.2 Motor control

1.2.1 The corticospinal tract

The human brain is comprised of numerous structures, which are responsible for fulfilling all the functional purposes of the musculoskeletal system through their interaction via a

complex neural network. The cerebrum, or telencephalon, is the largest part of the brain. Its most superficial layer is the cerebral cortex, which is known to be divided into numerous areas according to the functional purpose of each (Enoka, 2002). The motor areas occupy the agranular section of the frontal lobe of the cortex (for a review, see Rizzolatti et al., 1998). Perhaps the most renown of these areas is Bowman's area 4, the primary motor cortex, considered by many as the most crucial component of the central nervous system (Enoka, 2002; Rivara et al., 2003). It is comprised of large pyramidal, or Betz cells (Rivara et al., 2003) which generate a neural command travelling across fast-conducting cortical axons (Butler et al., 2007). The monosynaptic component of the synapse between these axons and the α-MN pool at the spinal level has been assessed via the examination of post-stimulus time histograms (PSTH) following cortical magnetic stimulation of the motor cortex (Figure 1). A short-duration short latency sharp peak in the firing probability of a motor unit provides infallible evidence of a monosynaptic projection of the cortical neurons to the spinal MN pool (Palmer & Ashby, 1992; Butler et al., 2007).

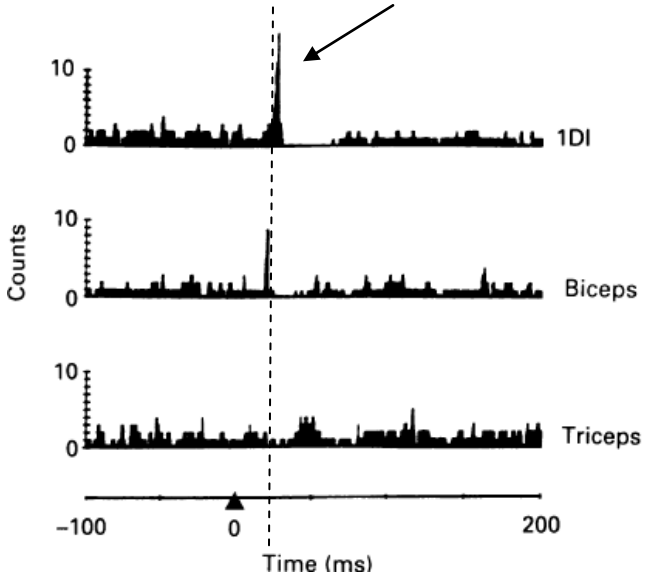

Figure 1. Post-stimulus time histogram. Notice the sharp and short-lasting increase in the firing probability of the motor unit in the upper panel (arrow), at a small latency (28-30ms) following the application of the magnetic stimulus. (Modified from Palmer & Ashby, 1992).

The synapse between the cortical axons and the MN pool has been shown to be free of presynaptic inhibition (Nielsen & Petersen, 1994).

The corticospinal tract has been considered to be constituted not only of the cortical neurons but also of the spinal interneurons and the MNs. (Devanne et al., 1997). The control of a voluntary movement depends, consequently, not only on the output of the cortical cells, but also on the properties of the other elements of the corticospinal tract. Stimulation of the corticospinal tract with increasing intensity and the measurement of the evoked response at each intensity (i.e. *input- output relationship)* has allowed a detailed examination of the neural transmission in the tract (i.e. its functional state). This relationship can be obtained during voluntary activity or in a relaxed state. Devanne et al. (1997) have identified three fundamental properties of the – sigmoidal in shape – input-output relationship whose interpretation allows for conclusions to be made concerning motor control (Figure 2): threshold, maximum slope and plateau level.

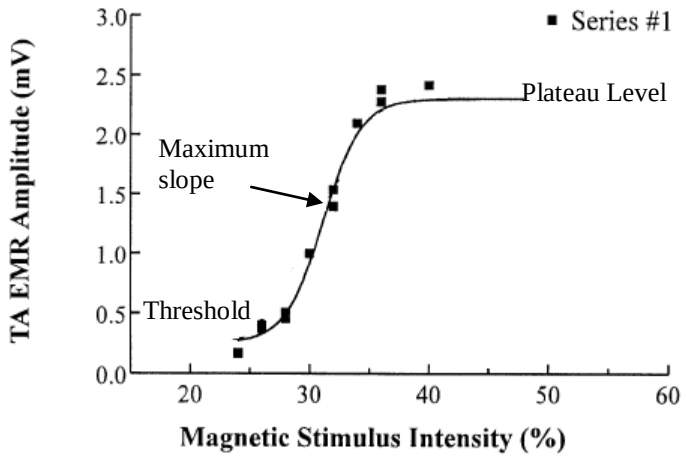

Figure 2. Example of the input-output relationship obtained during an isometric contraction (10% MVC) of the tibialis anterior (TA) muscle. (Modified from Devanne et al., 1997).Note that the threshold represents the lower intensity at which an evoked response (EMR) is obtained (about 23% of maximum stimulator output in this case).

Threshold represents the bias level in the corticospinal pathway (i.e. the susceptibility of the earliest- recruited MNs to respond to the stimulus). The maximum slope reflects the

size of the subliminal fringe of all the elements of the corticospinal tract. Finally, the plateau value reflects the number of the MNs that are ultimately recruited and therefore it answers the question of how inhibitory or excitatory is the descending evoked volley (Sekiguchi et al., 2003).

The methodological tool of transcranial magnetic stimulation used to obtain this relationship is described in the following section. The implications of this relationship concerning motor control are explained in section 3.2.

1.2.2 Assessment of neural cell excitability

Transcranial magnetic stimulation. Transcranial magnetic stimulation (TMS) consists in stimulating the motor cortex *via* a round, conical or size-of-8 coil which creates a magnetic field on the surface of the skull (Figure 3a). Stimulation with a round coil excites quite a large region of cells located deep into the white matter; on the other hand, a figure-of-eight coil attains only the most superficial cortical neurons but is also more focal (Rossi et al., 2009). TMS has hence been shown to excite the cortical cells both deep into the white matter, slightly distal to the cortical cell body, but also trans-synaptically, i.e. through interneurons which converge to the cortical cells (for a review, see Di Lazzaro et al., 1998). The descending volleys following TMS are comprised therefore of a direct (D) wave, of short latency, and multiple indirect (I) waves, corresponding to the trans-synaptic activation (Burke et al., 1990, 1993; Nakamura et al., 1996). These descending volleys cause multiple firing of MNs, forming the motor-evoked potential (MEP) which is recordable with conventional surface EMG electrodes (Figure 3b; Day et al., 1989; Edgley et al., 1997). Bawa and Lemon (1993) have showed that despite the polyphasic and complex nature of the descending volley the recruitment and rate coding of MNs follows the orderly pattern observed with increased descending voluntary command. It is known that the motor cortex follows a topographic organization, so the position of the stimulating coil needs to be adjusted in order to evoke responses in different target muscles. Also, as a consequence of the richer corticospinal projections in upper limb than in lower limb muscles, the threshold for TMS is lower for the former (see the review by Rothwell et al., 1991).

Apart from its use to describe in detail the functional state of the corticospinal tract at *fixed* levels of voluntary activity, TMS has been used extensively as a tool to investigate and compare motor control during different kinds of muscle action (for a review, see Petersen et al., 2003).

a. **b.**

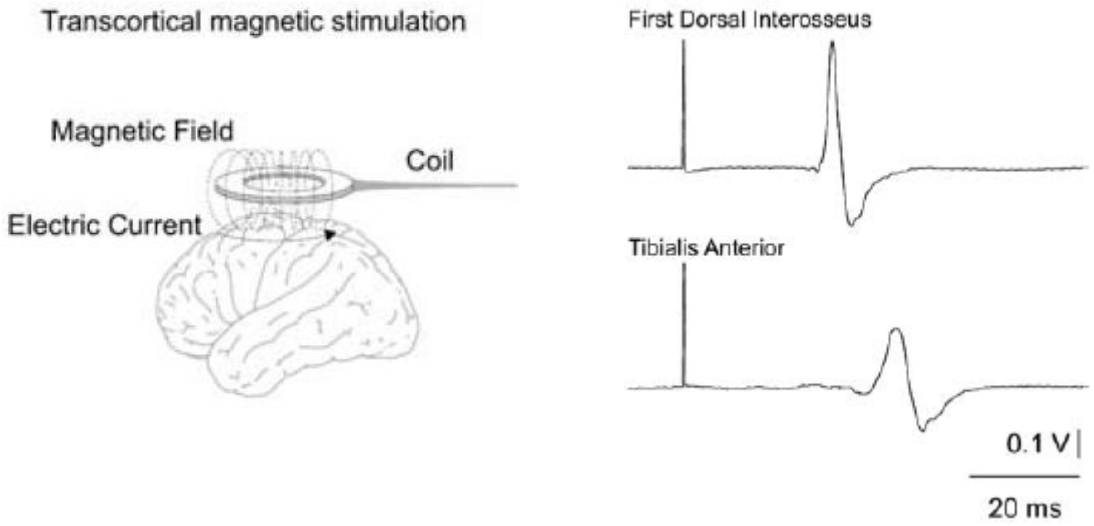

Figure 3. In **a**, the motor cortex cells are activated by means of a round stimulating coil. In **b**, MEPs in the first dorsal interosseous muscle and the TA muscle (a and b adapted from Petersen et al., 2003)

In fact, the MEP is undergoing modulation following initiation and grading of voluntary activity (see section 2.3 below) and during the execution of different motor tasks (Datta et al., 1989; Flament et al., 1993; Edgley & Lemon, 1999). The differences in MEP amplitude observed during the execution of two distinct tasks is attributed to differences in locations and extent of cortical activity, which consequently affects the amplitude and number of the descending volleys (Flament et al., 1993). TMS has also been used as a mean to investigate the transcortical nature of longer-latency components of the stretch reflex (Palmer & Ashby 1992; Petersen et al., 1998; Christensen et al., 2001; Taube et al., 2006). In this case, MEP depression or facilitation, depending on the protocol, was indicative of the involvement of supraspinal centers in the appearance of longer-latency reflex responses associated with muscle stretching. Assessment of fatigue and, specifically, its effect on combined

supraspinal and spinal excitability has also been carried out by investigating the changes in the MEP amplitude (Gandevia et al., 2002; Di Lazzaro et al., 2003)

However, MEP amplitude depends not only on the excitability of cortical output cells, but also on the excitability of the MN pool (e.g. Datta et al., 1989; Nielsen et al., 1995). Consequently, differentiation of excitability changes in a spinal and supraspinal level cannot be made without the use of additional tools. Two of the most commonly used ones are described in the following paragraphs.

The Hoffmann-reflex. When a peripheral nerve is electrically stimulated at a relatively low current (several mA) a response named the Hoffmann-reflex (H-reflex) after Hoffman who first discovered its existence in 1918 – is obtained. The H-reflex owes its appearance to the generation of an action potential in the Ia afferent nerve, which depolarizes the MN pool via the monosynaptic component of the Ia fibers-MN pool connection (Latash, 1998). Hence, H-reflex has traditionally been considered to reflect the excitability of the MN pool (Capaday, 1997). However, it should not be forgotten that the synapse between the Ia afferent and the MN pool is controlled by the so-called Ia afferent presynaptic inhibition (Figure 4a; Romano & Schiepatti, 1987; Capaday, 1997). Presynaptic inhibition itself seems to have its source in supraspinal centers, and functions as a variable gain modulator of the Ia reflex arc. Its contribution to motor output in case of voluntary contractions is different for the upper and lower limb (Meunier & Deseilligny, 1998). Therefore, the H-reflex could be described more accurately as a measure of the efficacy of the synaptic transmission in the Ia reflex arc (Capaday, 1997). As it can be seen from Figure 4b, the H-reflex undergoes modulation following changes in background voluntary activity (Nordlund et al., 2002; Romano & Schieppatti, 1987), changes in mode of muscle action (Pinniger et al., 2000; Nordlund et al., 2002) and between motor tasks (Leukel et al., 2008a; Simonsen & Dyhre-Poulsen, 1998). The activation history of the reflex, i.e. the frequency of peripheral nerve stimulation is also a modulating factor which occurs in conjunction with motor task modulation (Trimble et al., 2000). In addition to this, H-reflex undergoes facilitation following a Jendrassic maneuver, i.e. a contraction of a muscle distal to the muscle from which the H-reflex is measured (Tazoe et al., 1995). Butler et al. (1993) have

also pointed out that H-reflex is sensitive to the direction of the ongoing contraction, i.e. the recorded H-reflex is larger when the contraction force is slightly increasing than when it is kept completely stable. Therefore, a set of modulating parameters have to be kept in mind when interpreting the relationship between H-reflex size and contraction strength.

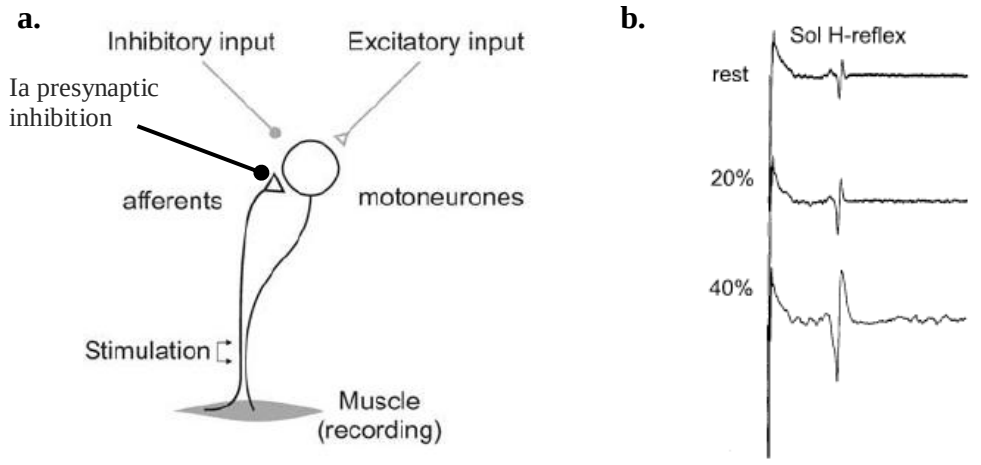

Figure 4. In **a,** the Ia afferent reflex arc which gives rise to the H-reflex. In **b**, the SOL H-reflex measured at different levels of voluntary background EMG (a, modified from Petersen et al., 2003; b, adapted from Petersen et al., 2003)

H/M recruitment curve. When the peripheral nerve is stimulated with progressively increasing stimulation intensity, an input-output curve is obtained, which is called H/M recruitment curve (Figure 5). As stimulation intensity grows, the efferent α-MN axons are also depolarized which gives rise to another evoked response, the M-wave. The M-wave has a shorter latency than the H-reflex since it is generated via direct stimulation of the motor nerve and hence there is no synaptic delay involved (Latash, 1998). Further increase of the stimulation intensity results in peaking and progressive disappearance of the H-reflex, which is due to antidromic collision of action potentials travelling on opposite directions in the Ia-afferent arc (Maffiuletti et al., 2000). On the other hand, the M-wave grows as stimulation intensity increases until it reaches a plateau. This plateau value is called maximal M-wave (Mmax) and it represents the activity of the whole MN pool (Crone et al., 1999).

Practical implications. It should be kept in mind that the susceptibility to facilitation or inhibition of the conditioned reflex changes as a function of the unconditioned reflex size (Crone et al., 1990).

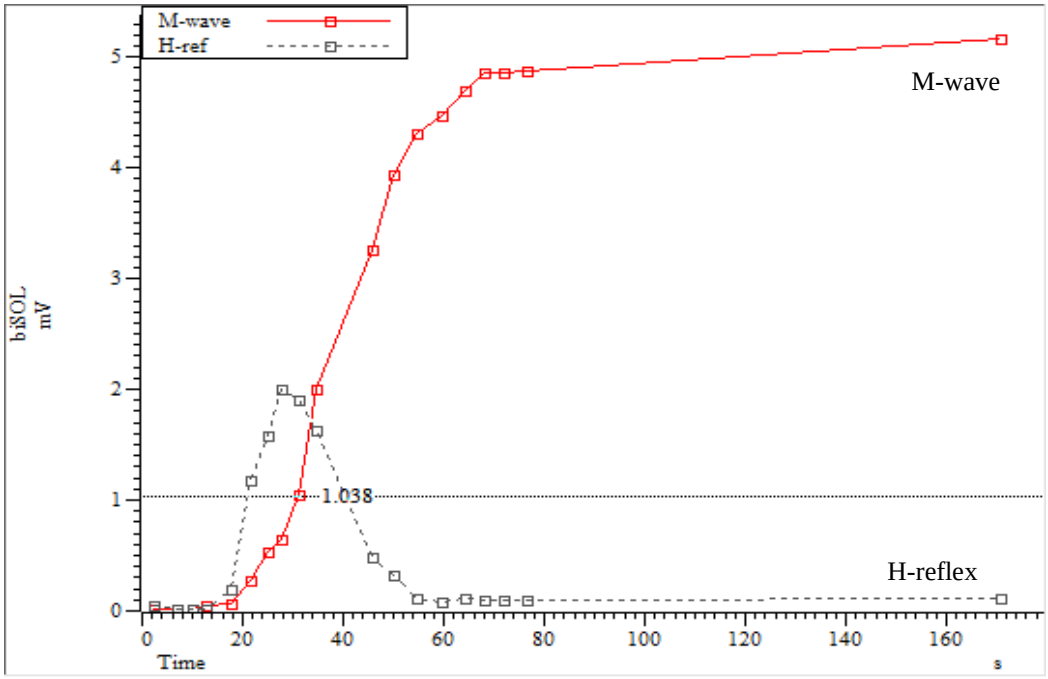

Figure 5. H/M recruitment curve. M-wave is represented with continuous line and H-reflex with a dashed line. Notice the remarkable M-wave growth after the H-reflex has already plateaued (Data from the current experiment).

The methodological implication of the above finding is that it is necessary to measure H-reflex at matched levels of background electromyographic activity, in order to render it comparable between motor tasks (Capaday, 1997). In addition, it is recommendable to choose a stimulation intensity which elicits an H-reflex of 20-25% of the maximal M-wave, on the ascending part of the H/M recruitment curve (Crone et al., 1990). As a consequence, the size of the maximal M-wave itself has to be controlled regularly (and therefore the stimulation intensity changes accordingly – see e.g. Pinniger et al., 2000 or Leukel et al., 2008b) especially in the case of dynamic contractions where the stimulating electrode unavoidably moves with respect to the nerve (Capaday, 1997).

Cervicomedullary Stimulation. A technique to stimulate non-invasively the corticospinal tract which does not have the inherent disadvantages of the H-reflex, consists in the application of electrical or magnetic stimuli at the posterior side of the head, at the level of the medulla – more specifically, at the pyramidal decussation (for a review, see Taylor & Gandevia, 2004). This technique has been named cervicomedullary stimulation (CES or CMS, for electric and magnetic stimuli, respectively) and evokes a single descending volley in the corticospinal neurons, which are known to form monosynaptic connections with the spinal MNs (see section 2.1). This monosynaptic connection is free of presynaptic inhibition, as demonstrated by Nielsen et al. (1994) and further verified by the Petersen et al. (2002). Taylor et al. (2002) have demonstrated that CES/CMS activates the same axons as TMS as occlusion of the responses to TMS was observed when the cervicomedullary stimulation was followed by a magnetic stimulus at appropriate intervals (as a result antidromic collision of volleys travelling on the same axons).

As a corollary, CES or CMS can be reliably used to measure spinal MN excitability, through the cervicomedullary motor evoked potential (CMEP). Comparison of the MEP to the CMEP (MEP-to-CMEP ratio) gives accurate information about the location of excitability changes in the human motor pathway (Martin et al., 2009) given that the test responses are of similar size (Gruber et al., 2009). However, this method can be under circumstances painful, and, in addition, when the stimulation intensity is high enough, the stimulation site may shift from the corticospinal axons to the dorsal roots of the spinal cord (Taylor & Gandevia, 2004).

1.2.3 Effect of voluntary activity on neural cell excitability

When a muscle reverts from rest to an active state (i.e. contraction), the size and number of epidural volleys evoked by TMS increases and their latency decreases. The threshold stimulation intensity at which they appear is also lowered. These observations were not reproduced following stimulation by TES (Di Lazzaro et.al, 1998). Since TMS activates corticospinal neurons trans-synaptically, this implies that voluntary activation substantially increases the excitability of these neurons, with minor changes in the excitability of the

interneuronal axons or the axons of the corticospinal cells themselves (Mazzocchio et al., 1994). However, the EMG responses (i.e. MEPs) show a large increase, which is disproportional compared with the increase in volley size (Di Lazzaro et al., 1998). This suggests a more important contribution of the spinal excitability in the overall EMG response (Ugawa et al., 1995; Taylor et al., 1996). Consistent with this finding, it has been demonstrated that in an already active muscle, the MEP grows as consequence of an increase in ISO contraction strength, for a variety of muscle groups (Figure 6).

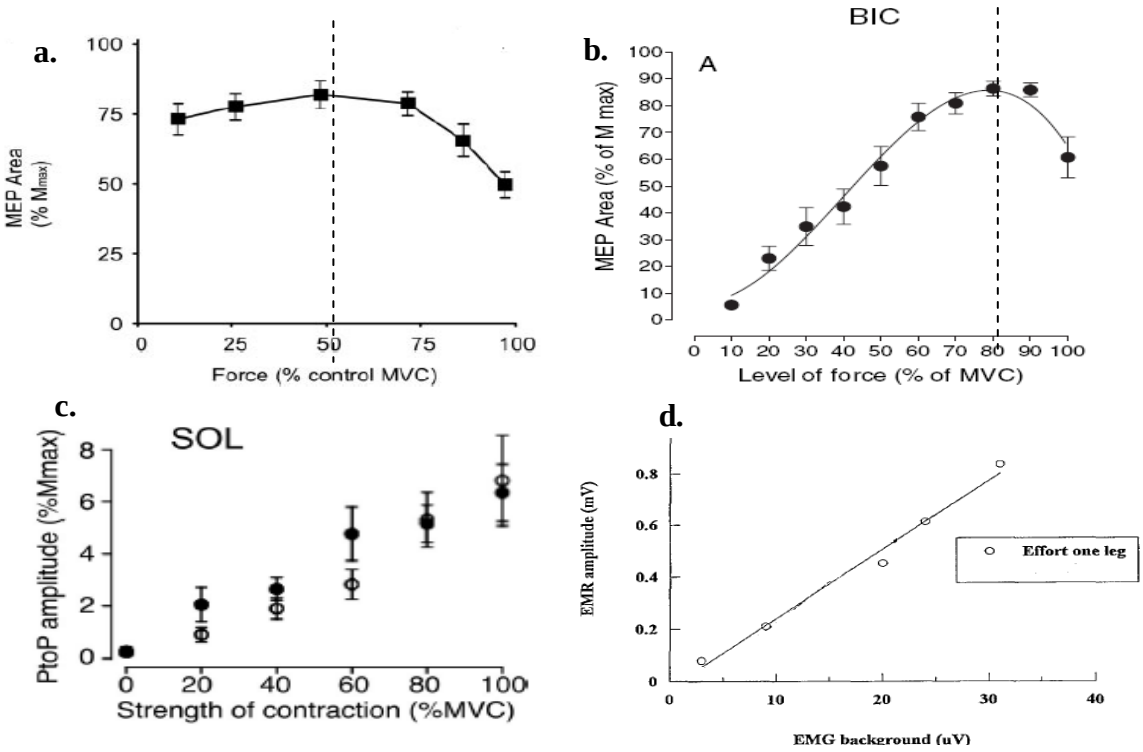

Figure 6. MEP amplitudes plotted against contraction strength for various muscles. In a and b (first dorsal interosseous and biceps brachii, respectively), the intersection of the dotted line with the abscissa reflects the contraction strength at which the MEP peaks. In c and d (both for soleus), the MEP amplitude grows linearly with increased contraction strength. Notice the inter-experiment reproducibility of the relationship. (a and c, modified from Martins et al., 2006; b, modified from Gelli et al., 2008; d, modified from Lavoie et al., 1995).

Surprisingly, this increase does not persist up until maximal voluntary effort (i.e. it is not paralleling the increasing excitability level of the corticospinal tract), but peaks at a contraction strength which has been shown to coincide with the upper recruitment limit of

the muscle (Lavoie et al., 1995, Taylor et al., 1996; Martin et al., 2006; Gelli et al., 2008; Oya et al., 2008).

Therefore, the observation of an increase in MEP amplitude over a broad range of contraction strengths is made in muscles with a wide recruitment range. For instance, the increase in soleus MEP up to MVC (Lavoie et al., 1995; Oya et al., 2008) is in line with the recent finding that recruitment in soleus during ramp ISO contractions persists up to 96.6% of MVC (Oya et al., 2009). The reason for such a behavior of the MEP resides in MN properties and specifically in their incapacity to respond to an additional input when their background firing rate is already high; this is a result of a prolonged refractory period (Matthews, 1996; 1999). In other words, when the recruitment is complete and additional increments in force are attained exclusively by increased firing; the MN pool becomes less and less responsive, resulting in decreased MEP responses. Differences related to recruitment are visible in muscles fulfilling different functions, for instance muscles of the forearm versus muscles of the hand (Gelli et al., 2008) and antagonist muscles of the same joint, for instance elbow flexors versus elbow extensors (Todd et al., 2006).

To conclude, the probability that a MN pool responds to an external stimulus is conditioned by its current state of activation. Therefore, in this kind of experiments, MEP amplitude is indicative of the neural mechanisms operating mainly at a spinal level and which are responsible for force production during ISO contractions. Taking this into account, a subsequent step would be to employ a similar methodology for the investigation of the excitability of the corticospinal tract during dynamic contractions.

1.3 Lengthening contractions

1.3.1 Torque, EMG and voluntary activation

LEN contractions occur when an already active muscle is being forcibly lengthened. In real-life tasks, this is the case of resisting a load or controlling the release of a load, such as in the performance of isokinetic movements and in the braking of rapid movements (Duchateau & Enoka, 2008). Levin & Wyman (1927) were the first to report that when an isolated active muscle was stretched, the subsequently developed maximum tension was much greater than in other forms of muscle work. Later, Hill (1938; 1970) and Katz (1939) also reported that *in vitro*, the force-velocity relationship of a muscle is characterized by a sharp increase in force as the velocities progress towards negative values, i.e. when the muscle is lengthened. This increase in force can be accounted for by short-range stiffness (Rack & Westbury, 1974). Short-range stiffness is due to the elastic elements present the muscle and is the reason why a previously stretched muscle is capable of producing more work during subsequent muscle shortening (Cavagna et al., 1968; Cavagna & Citterio, 1974). Greater loading of the elastic component of the muscle during LEN contractions has been proven not only to enhance torque during one-time measurements, but also to more rapid torque decrements during repeated, fatiguing contractions (Komi & Rusko, 1974). Asmussen (1954) as well as Komi and Buskirk (1973) have identified stretching of the elastic elements of the muscle as the reason for muscle soreness following LEN contractions. Due to the prominent involvement of other elements than pure neural drive, LEN contractions constitute, therefore, a particular case of muscle action which has been investigated with respect to torque production, EMG levels and neural activation.

Komi (1973a, 1973b) has used a custom-built dynamometer to measure torque produced by the elbow flexors during ISO, SHO, and LEN contractions of various velocities. According to the obtained force-velocity curve the maximal torque produced at negative movement velocities, is much greater than for ISO and SHO contractions. These results are in line with those obtained in intact muscles with constant activation (i.e. *in vitro)*. It must be

emphasized that during the measurements *in vivo*, the obtained torque value depends on how the activation is *produced* and *maintained.* If the methodology during *in vivo* measurements follows the same principles as during measurements on an intact muscle (i.e. constant preactivation during the isometric phase), the results obtained are similar for both conditions. Under these circumstances it is would therefore be feasible to obtain a force-velocity curve *in vivo* which is identical to the one obtained *in vitro*. Thus, it could be suggested that the methodological procedure used during the measurements is the key factor affecting the findings of Seger and Thorstensson (2000). In fact, these authors have failed to detect any differences in maximal torque production of the *knee extensors* between ISO and LEN modes of contraction over a large range of LEN velocities (Figure 7a). Methodological issues might as well be the reason for the results of Komi et al. (2000), who have also reported similar elbow flexor torque during LEN compared to ISO at angular velocities of 3 rad/s and 4 rad/s (Figure 7b).

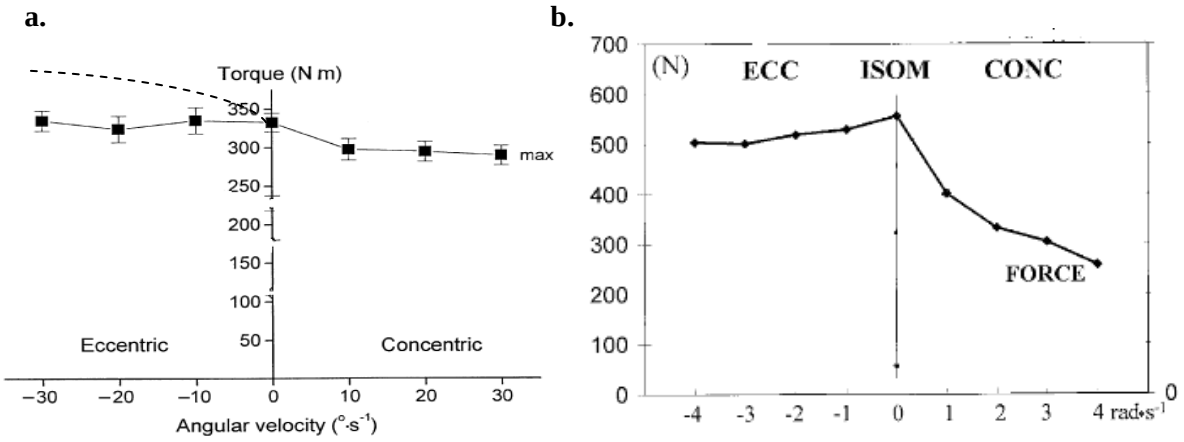

Figure 7. a, the torque-velocity relationship of the knee extensor muscle group obtained *in vivo,* during maximal voluntary effort (continuous line) deviates markedly from that that obtained *in vitro* or by maximal electrical stimulation (dashed line) for negative velocities (i.e. during LEN contractions). **b,** the torque-velocity relationship of the elbow flexor muscle group (note: of *untrained* subjects) is characterized by a relatively stable torque value over a range of LEN speeds (a, modified from Seger and Thorstensson, 2000; b, modified from Komi et al., 2000).

In reference to *how* torque was measured, it has to be noted that in addition to muscle preactivation, the portion of the movement at which measurements are taken also affects the recorded torque. In fact, Figures 7a and 7b refer to torque measured at the middle of the range of motion. Interestingly, in case the torque is measured *immediately* after the onset of muscle LEN, its maximal value is much greater than the maximal torque measured during ISO conditions. Therefore, on the bottom line it seems that LEN torque *does indeed* exceed the values recorded for ISO, but not during the whole range of motion (Aagaard et al. 2000; Komi et al.2000; Linnamo et al. 2006; Pinniger et al. 2000; Seger & Thorstensson 2000; Westing et al. 1991). Additionally, the muscle group from which torque recordings where made might also be a factor causing discrepancies in the torque-velocity curves reported in the literature. Finally, the training status of the muscle may be responsible for impaired torque production, as reported by Komi et al. (2000).

When it comes to findings referring to EMG activity, Bigland and Lippold (1954) have demonstrated that for a given velocity of muscle shortening/lengthening, the slope of the iEMG-tension relationship is lower for LEN compared to SHORT contraction. Simlarly, Asmussen (1956) has reported that iEMG levels are lower during a SHO, compared to a LEN contraction with the same load. These initial findings have later been confirmed by more reports of lower EMG activity during LEN in comparison with ISO (Komi et al., 2000; Linnamo et al., 2006) and SHORT contractions (Komi, 1973a and 1973b; Westing et al., 1991; Komi et al., 2000). However, it should be kept in mind that the maximum developed tension during LEN is, in fact, higher than the maximum tension developed during SHORT. Therefore, if iEMG is plotted against relative tension (in %), there is no difference in the slope of the relationship for both LEN and SHORT modes of action (Komi, 1973), nor in the absolute iEMG values during maximal LEN and SHORT contractions (Komi & Rusko, 1974). Furthermore, according to the findings of Bishop et al., (2000), the integrated EMG amplitude was less in maximal LEN compared to maximal SHORT contractions. On the other hand, when EMG activity is reported as the *peak* EMG amplitude, then its value was greater in LEN compared to SHORT (Bishop et al., 2000). This is consistent with the finding of higher spindle activity during LEN (Burke, 1977). In fact, LEN contractions are accompanied by greater spindle discharge than ISO and SHORT

contractions. Active LEN causes greater spindle discharge than passive LEN, something which might reflect an increased α-γ coactivation (Burke, 1977).

Neural activation (and subsequently, any neural activation deficit) seems to vary as a function of the muscle group under investigation. Komi and Burskirk (1972) have demonstrated the effects of training on torque produced during LEN contractions of the *elbow flexors*. However, these authors have not reported any increase in the iEMG per unit of tension, suggesting increased desynchronisation of motor units as a possible candidate for this improvement in torque. Consequently, they excluded inadequate neural activation as the reason for the pre-training lower torque values. On the other hands, some authors (Westing et al., 1990; Babault et al., 2001) have shown by using twitch interpolation that the voluntary activation level during a maximal LEN contraction of the *knee extensors* is substantially below maximal. This finding could further explain the reported results of Seger and Thorstensson (2000; see above). Similarly, Aagaard et al. (2000) have found lower EMG, lower force, and greater increments in total force following electric stimulation during LEN than during SHO contractions of the quadriceps muscle. The rate of increase of the activation level as a function of contraction strength is smaller for LEN compared to ISO over a range of contraction strengths, which suggests that activation deficit does not occur exclusively during maximal effort but is observed independently of the effort level (Babault et al., 2001). However, Aagaard et al. (2000) have shown that after 14 weeks of resistance training neural activation levels increase up to 100%. Additionally, Amiridis et al. (1996) reported that during LEN contractions of the knee extensors throughout a wide range of angular velocities the co-activation levels of the antagonist muscles were significantly higher than during SHORT contractions. Co-activation was suggested to be the cause of the reduced torque production of the agonist muscle group during LEN. Interestingly, in highly trained athletes co-activation was absent, and the torque-velocity relationship obtained during voluntary effort was identical to the one obtained by electrical stimulation (Amiridis et al., 1996). The effects of training status are also discussed by Westing et al. (1991).

From all the above findings it can be deducted that LEN contractions are characterized, under certain circumstances, by an inhibitory neural component. Consequently, it has been hypothesized that a set of mechanisms acting at a spinal level are responsible for this

possibly reduced muscle activation and, subsequently, the reduced torque output (Aagaard et al., 2000; Linnamo et al., 2006). The exact nature of these mechanisms has not been identified beyond doubt but traditional assumptions include the inhibitory actions of Golgi tendon organs and of afferent fibers conveying nociceptive sensory signals (e.g. Westing et al., 1990; 1991; Seger & Thorstensson, 2000). Trimble et al. (2000) also describe spinal pre- and post-synpaptic mechanisms as being modulating factors which limit motor output during LEN. In contrast to the assumption of spinal disfacilitation, Linnamo et al., (2006) provide an alternative explanation for the lower EMG amplitudes by mentioning phase cancellation (Keenan et al., 2006) as a candidate mechanism.

1.3.2 Motor control strategies of lengthening contractions

The traditional views of exclusively spinally modulated motor output during LEN have recently been put into question. In fact, it has been suggested that motor output during LEN is not merely a reflection of mechanisms operating at a spinal level, but depends largely on descending corticospinal input to the MN pool. In other words, it is believed that the central nervous system generates a *unique* neural command in the case of LEN contractions. This notion has been established on the basis of a number of findings (for a review, see Enoka, 1996). This possibility does seem likely according to the findings of Tax et al. (1989), which reported that MN behavior (recruitment threshold and firing frequency) is different during LEN contractions of the elbow flexors than during ISO contractions. It seems that the reason for this discrepancy is the different distribution of descending activity in the MN pool between these two modes of muscle action (Tax et al., 1989). In addition to this, it has been shown (Fang et al., 2001) that cortical potentials related to movement planning and initiation occur earlier, and are of greater magnitude in the case of LEN compared to SHO contractions. In order to explain their observations, these authors have advanced the hypotheses of increased degree of difficulty of LEN contractions, the necessity to prevent muscle damage and the possibility of a differential control strategy compared to other muscle action modes. Grimby and Hannerz (1977) provided an early support for the latter hypothesis, as they were among the first to suggest that during certain circumstances,

recruitment in the active muscle deviates from the classical "size principle" scheme. However, they did not cite LEN contractions as a special case. More specifically, Nardone et al. (1988, 1989) have investigated the hypothesis that during LEN contractions of the plantarflexors, muscle activation is redistributed within the synergist muscle group, resulting in a greater relative activation of the fast-twitch gastrii in comparison to the slow-twitch soleus. These authors concluded that high-threshold motor units are preferentially activated during tasks involving muscle LEN. In contrast, the mean frequency of the EMG power spectrum (an indicator of MU recruitment) decreases during LEN compared to SHO contractions. This implies a lower global muscle conduction velocity, which subsequently might reflect *derecruitment* of high-threshold motor units during muscle LEN (Komi et al., 2000). The possibility of a disruption in recruitment order is also excluded according to the findings of Tax et al. (1989), Sekiguchi et al. (2001) and Gruber et al. (2009).

The studies focusing on differential recruitment pattern fail, in fact, to adequately explain the decreased torque production during LEN. The use of evoked responses to different kinds of stimulations, on the other hand, provides a valuable insight to the neural mechanisms involved. Sekiguchi et al. (2001; 2003) measured the input-output relationship of the corticospinal tract during rather weak LEN and SHO contractions (of similar background EMG levels) and found evidence of a decrease in the *overall* excitability of the corticospinal tract during LEN compared to SHO (Figure 8).

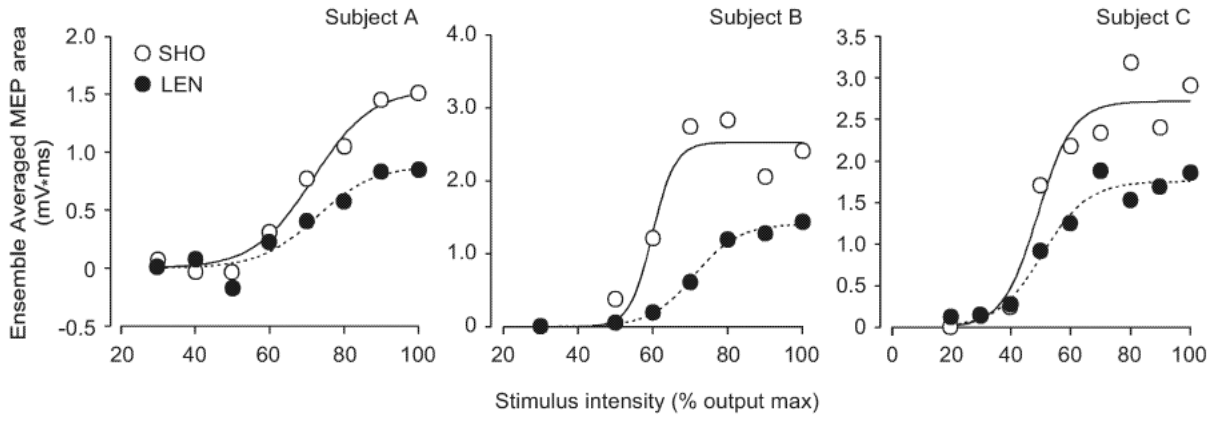

Figure 8. Observation of lower maximal slope and lower plateau values (i.e. maximal MEP area) during the performance of weak LEN contractions compared to SHO, at similar bEMG. The observations are consistent across subjects. (Sekiguchi et al., 2003)

Although there were no changes in the threshold between LEN and SHO, the maximal slope and plateau value of the relationship were significantly lower during LEN.

The above findings are in agreement with the decreased MEP amplitude observed during the performace of LEN contractions compared to SHO and ISO contractions (Abbruzzese et al., 1994). Interestingly, Gruber et al. (2009) reported *increased* MEP-to-CMEP ratio, in conjunction with lowered CMEPs, during the performance of submaximal and maximal LEN contractions with respect to the ISO action mode. Finally, H-reflex studies (Romano & Schieppati, 1987; Pinniger et al., 2000; Nordlund et al., 2002) have showed that the H-reflex of the SOL muscle modulated towards lower values in both active and passive LEN, in comparison to SHO and ISO contractions of similar background activity levels.

An array of studies provides insights to the above observations. According to Sekiguchi et al. (2001; 2003), a decreased maximal slope signifies lower gain in the corticospinal pathway, and the lower plateau value implies that the inhibitory component of the descending command to the MNs is stronger during LEN. This could explain the decreased responsiveness of the MN pool to any kind of additional input, illustrated by a decreased CMEP (Gruber et al., 2009). In addition to this, the increased MEP-to-CMEP ratio indicated that cortical excitability during LEN is slightly higher (Gruber et al., 2009), which supports the findings of Fang et al. (2001) of enhanced brain potentials in the case of LEN. Concerning the H-reflex down-regulation, it could be hypothesised that both the postsynaptic input to MNs and the presynaptic inhibition to the Ia afferent terminals contributes to the observed values (Sekiguchi et al., 2003). However, both Pinniger et al. (2000) and Nordlund et al. (2002) have pointed out that the down-regulation occurs in *passive* conditions, where there is no descending postsynaptic input to MNs. Therefore, presynaptic inhibition – probably controlled by supraspinal centers (Meunier & Pierrot-Desseilligny, 1998) – most likely comes into play and decreases the transmission efficacy in the Ia afferent pathway. It appears that this is done in order to limit the spindle output during LEN, in favor of the performance of fine motor actions (Romano & Schieppati, 1987).

It therefore appears that in the existing literature there is quite substantial evidence arguing towards a neural command which is specific to LEN contractions. Authors have quite often

advanced the explanation of a need to limit MN output during LEN, either by increasing the inhibitory component of the descending command, and/or by limiting the excitatory effect of the Ia afferent volleys. In both cases, the corticospinal pathway as a whole (i.e. corticospinal neurons, MNs and spinal interneurons; Devanne et al., 1997) is considered as less responsive in the case of LEN. This specific neural control scheme is said to exist for the sake of functionality, i.e. in order to protect the muscle from excessive torque production and to fine-tune motor performance, matching it therefore to task requirements (Gruber et al., 2009).

1.4 Anatomy and function of the soleus muscle

The soleus muscle (SOL) belongs to the triceps surae muscle group, which is principally responsible for ankle plantarflexion. Anatomically, soleus muscle fibers are organized in a pennate fashion, i.e. at an angle with respect to the parallel plane (Reeves et al., 2005a). Due to its anatomy, SOL is a powerful force capacitor. From a neural control point of view, it is comprised primarily of slow-twitch motor units, in contrast to its "fast" synergist muscles grastrocnemius medialis and lateralis (Nardone et al., 1998; 1989). The SOL MN pool is characterized by neural input homogeneity, i.e. synaptic input (mainly Ia and corticospinal in origin) is equally distributed to the whole population of SOL MNs (Morita et al., 2000). Due its importance in locomotion, the modulation of the soleus H-reflex during walking and running has been repetitively examined in the past (Capaday & Stein, 1986; Edamura et al., 1991; Petersen et al., 1998; Simonsen & Dyhre-Poulsen, 1999). Soleus as a postural muscle is involved in compensatory responses following perturbations (Cronin et al., 2009a; 2009b). The possibility of cortical control of these responses cannot be excluded, as there is evidence of their mediation via a long-latency transcortical reflex pathway (Taube et al., 2006). Lavoie et al. (1995) agree with the above finding, as they concluded that the motor cortex participates in the control of the soleus muscle not only in the case of volitional tasks, but also in the case of postural regulations. In addition to this, there is a strong spinal component to shorter-latency responses which adapts soleus muscle

activity during locomotion according to the characteristics of the task (Klint et al., 2010). Therefore, both peripheral feedback and descending commands from supraspinal centers contribute to neural control of the muscle (Nielsen et al., 1995).

2 PURPOSE OF THE STUDY

The purpose of the present study was to carry out a comparison between ISO and LEN plantarflexions of five different strengths (REST, 20%, 40%, 60%, 80% MVC), this comparison aiming to put into perspective any differences in neural control and recruitment strategies between the two contraction modes (Figure 1). The tools used in outlining neural control were TMS and electrical stimulation. The evoked responses to these two types of stimulation (i.e. MEP and H-reflex, respectively) were considered as indicators of the degree of involvement of supraspinal and spinal structures in the control of the SOL muscle during ISO and LEN contractions of increasing strength. Thereafter, MEP and H-reflex amplitudes were plotted against contraction strength for both contraction modes.

It was hypothesized that MEP and/or H-reflex amplitudes would be lower in the case of LEN throughout the range of contraction strengths, thus indicating the possible involvement of inhibitory mechanisms limiting total motor output. It was also hypothesized that the pattern of change of the MEP *versus* contraction strength relationship for LEN would not be linear, thus suggesting that recruitment of SOL MUs during LEN is done, in contrast to the ISO condition, in a *non-orderly* manner.

A secondary purpose of the study was to compare the obtained results for ISO with the quite numerous already-existing set of data on evoked responses measured during ISO contractions of the SOL muscle.

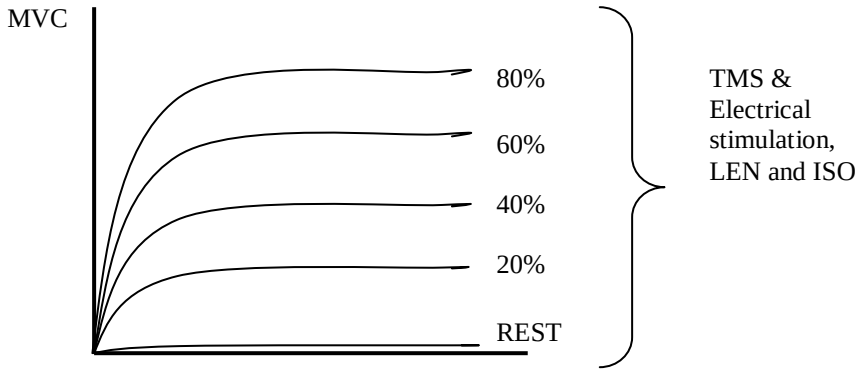

Figure 1. Protocol overview.

3 MANUSCRIPT FOR PUBLICATION

Evoked responses to transcranial and electrical stimulation during isometric and lengthening contractions of the soleus

[Authors, name of University and contact information]

Abstract

3.1 Introduction

Lengthening (LEN) contractions occur when an already active muscle is being forcibly lengthened. In real-life tasks, this is the case of resisting a load or controlling the release of a load, such as in the performance of isokinetic movements and in the braking of rapid movements (Duchateau & Enoka, 2008). Levin & Wyman (1927) as well as Hill (1938; 1970) and Katz (1939) have reported that *in vitro*, the force-velocity relationship of a muscle is characterized by a sharp increase in force as the velocity progresses towards negative values, i.e. when the muscle is lengthened. Lengthening force has been reported to equal 1.5 times the maximal ISO force (Rack & Westbury, 1974), this fact being attributed to the short-range stiffness of the muscle's in-series elements (Cavagna et al., 1968; Cavagna & Citterio, 1974; Rack & Westbury, 1974). However, *in vivo*, enhanced torque production during LEN is not observed in all circumstances. LEN contractions are, indeed, characterized by greater torque (Komi, 1973a; 1973b) but not throughout the whole range of motion (Westing et al. 1991; Aagaard et al., 2000; Komi et al., 2000; Pinniger et al. 2000; Seger and Thorstensson 2000; Linnamo et al. 2006;). On the other hand, EMG levels during LEN have been reported to be lower than for ISO (Komi et al., 2000; Linnamo et al., 2006) and for SHORT contractions (Westing et al., 1991; Komi et al., 2000). When it comes to voluntary activation levels, findings support the presence of neural deficit during LEN (Westing et al., 1991; 1993; Aagard 2000).

Numerous spinal mechanisms have been put forward as candidate factors explaining the above-mentioned observations in the untrained muscle. Trimble et al. (2000) describe spinal pre- and post-synaptic mechanisms as modulators which limit motor output during LEN. This set of mechanisms presumably includes the inhibitory actions of Golgi tendon organs and of afferent fibers conveying nociceptive sensory signals (e.g. Westing et al., 1990; 1991; Seger & Thorstensson, 2000). These mechanisms seem to be dependent on training status of the muscle, since LEN-specific muscle conditioning leads to their eventual disappearance (Komi & Burskirk, 1972; Amridis et al., 1996; Aagard et al., 2000). As a corollary, it has recently been suggested that a *unique* neural control scheme is elaborated by the central nervous system in the case of LEN contractions (for a review, see Enoka, 1996). Investigation of a possibly specific neural input to an active muscle

undergoing LEN requires the use of transcranial magnetic stimulation (TMS). TMS enables to outline the parameters of the descending motor input due to the fact that it activates cortical cells trans-synaptically (Edgley et al., 1997). The subsequently obtained response, i.e. the motor evoked potential (MEP) is representative of the excitability of the corticospinal tract as a whole, i.e. cortical neurons, motorneurons (MNs) and spinal inter-neurons (Devanne et al., 1997). Corticospinal tract excitability is, according to an extensive set of evidence, lower during LEN as lower MEPs have been recorded for LEN compared to ISO and SHORT contractions during the performance of various tasks, at various background activity levels and for a wide range of muscle groups (Abbruzzese et al. 1994; Sekiguchi et al. 2001; 2003; Gruber et al., 2009).

In addition to this, the H-reflex obtained by electrical stimulation of the peripheral nerve is a measure of the efficiency of the conduction in the Ia afferent arc (Capaday, 1997). The fact that it is controlled by cortically mediated pre-synaptic inhibition (Meunier & Pierrot-Deseilligny, 1998) compromises its reliability when it comes to assessing the excitability of the spinal MN pool (Capaday et al., 1997). However, H-reflex has been extensively used when it comes to clarifying neural control of LEN contractions (Romano & Schieppatti, 1987; Pinniger et al., 2000; Nordlund et al., 2002).

The specificity of LEN contractions has also been addressed in reference to altered recruitment schemes, and namely the lowering of recruitment thresholds during LEN contractions (Tax et al., 1989; Theeuwen et al., 1994). Furthermore, others (Nardone & Schiepatti, 1988; Nardone et al., 1989) have suggested that during LEN there is reversal of the traditional size-principle recruitment scheme (Grimby & Hannerz, 1977).

Previous studies (e.g. Lavoie et al., 1995; Lavoie et al., 1995; Martin et al., 2006; Oya et al., 2008) have demonstrated that the pattern of change of the MEP when ISO force increases up to its maximal value is indicative of the recruitment patterns underlying this increasing force production. In such a context, the soleus muscle (SOL), considered as a key muscle in postural regulation (Taube et al., 2006; Cronin et al., 2009a; 2009b) and during gait (e.g. Faist et al., 1996), has often been a subject of investigation. During ISO contractions of the SOL, H-reflexes (Loscher et al., 1996) as well as MEPs (Lavoie et al., 1995; Oya et al., 2008) have been shown to increase linearly up to MVC. However, there is

a lack of information in the literature concerning the neural control of the SOL during LEN contractions.

The present study has been designed to compare ISO and LEN plantarflexions of five different strengths (REST, 20%, 40%, 60%, 80% MVC), in the aim to put into perspective any differences in neural control and recruitment strategies between these two contraction modes. The tools used in outlining the neural input to the SOL MN pool were the relatively new technique of TMS and the more commonly used electrical stimulation. The amplitudes of the evoked responses (i.e. MEP and H-reflex, for TMS and electrical stimulation, respectively) were thereby plotted against contraction strength for both contraction modes.

It was hypothesized that MEP and/or H-reflex amplitudes in the SOL would be lower for LEN contractions throughout the range of contraction strengths, thus indicating the possible involvement of inhibitory mechanisms limiting total motor output. It was also hypothesized that the pattern of change of the MEP *versus* contraction strength for LEN would not be linear, suggesting that recruitment of SOL MUs during LEN is done, in contrast to the ISO condition, in a *non-orderly* manner.

3.2 Methods

Subjects

Ten healthy, physically active subjects (9 females, 1 male) participated in the study. They had no history of neuromuscular problems related to the calf muscles or the ankle joint. They were not engaged in any form of training which involved specifically lengthening muscle actions. Information was given about possible risks and discomfort; subsequently, all subjects gave their written informed consent to participate. The subject was free to withdraw his or her participation at any stage of the experimental session without having to give any particular reason or explanation. The study was conducted according to the declaration of Helsinki and was approved by the ethics committee of the University of Jyväskylä.

Experimental procedures

Once the subject gave informed consent concerning the measurement procedures the EMG and stimulating electrodes were placed. Thereafter, the subjects were asked to sit on the dynamometer chair which was adjusted in order to ensure maximum comfort and optimal measurement procedure. The trunk was afterwards fixed to the chair with safety belts and the hip, knee and angle joint were secured with straps in order to minimize movements and facilitate the exclusive contribution of the ankle joint to the produced torque. A computer screen where the EMG and torque traces were visualized was set in front of the participants, slightly above eye level. The subjects were then familiarized with the experiment setting by performing some contractions (in both static and dynamic conditions) while observing simultaneously the screen for deviations in the EMG and torque traces.

Afterwards, subjects were asked to perform three MVCs in 90° ankle angle, followed by three MVCs at 115° ankle joint angle. Verbal encouragement from the experimenters was given to ensure maximal effort. The maximal torque value produced during each MVC was recorded, and an average of three trials was calculated for each ankle angle. Afterwards, 20%, 40%, 60% and 80% of each average MVC value was calculated.

After a short pause H/M recruitment curve was recorded by increasing the stimulation intensity in steps of 2 mA. The starting stimulation intensity was for all subjects 2 mA. The stimulation intensity after which the M-wave reached a plateau was multiplied by a 1.5, and this supramaximal stimulation intensity was used throughout the experiment for measuring the Mmax.

After the preliminary measures, the actual measurement session was carried out either by starting first the H-reflex experiment or the TMS experiment. The order in which the experiments were performed was always random. Within experiments, the order of contraction modes and contraction strengths was also varied from subject to subject in a random manner. The subjects were asked to produce torque in the aim to match the torque level to a cursor which was displayed on the screen. During ISO tasks, the subject was asked to produce a certain amount of pre-activation torque at 90° ankle angle. When the torque was held constant at the desired level for several ms, the stimulus, either magnetic or electric in nature depending on the experiment, was manually triggered. During LEN tasks, the subject was asked to produce a certain amount of pre-activation torque at 115° ankle angle. After 2 seconds of maintaining a stable amount of torque, the dynamometer was automatically triggered, whereby the dynamometer plate moved from 115° to 85° ankle angle, i.e. the SOL fascicles were lengthened. During muscle lengthening, the subject was instructed to *"keep the same amount of effort"* throughout the lengthening disregarding the torque trace displayed on the screen. The stimulus was delivered automatically at 90° ankle joint angle.

Therefore, the stimuli where delivered at the *same* ankle joint position for both ISO and LEN, i.e. at the same muscle length (but with different MU activities).

Prior to the actual set of trials at a given contraction mode, contraction strength and stimulation type, the active Mmax – i.e. with muscle contraction and, in the case of LEN, with ankle joint movement – was always measured. For the TMS measurements, a total of five trials were carried out for all five contraction strengths at each contraction mode. For the H-reflex measurements the number of trials was in the order of ten, with the aim to obtain three valid trials. The validity of the trial was judged according to the size of the M-wave accompanying H-reflex. In order for the H-reflex value to be considered as valid the

amplitude of the accompanying M-wave should lie between 15 and 25% of the Mmax. Each set of trials was conducted in a rather rapid manner, the resting periods between each trial being in the order of several seconds, with the exception of the strongest contraction level (i.e. 80% MVC) where the inter-trial period was longer. Between contraction strengths one-minute rest periods were kept. In total, an approximate total number of 160 contractions were performed during the course of a single measurement session.

Before experiments and tasks, the subjects were given time to familiarize themselves with the functioning of the dynamometer and the nature of the stimuli. Throughout the measurement session verbal guidance and encouragement was provided to the subject in order to about the upcoming stimulation type and contraction mode and also in order to achieve and maintain the desired torque level. In addition, the participant was given time to relax and stretch between experiments. The bands securing the hip, knee and ankle joints were released if needed.

Due to the quite long duration of the experiment and the considerable total number of contractions performed it was necessary to verify whether fatigue was present when the protocol was completed. Therefore, at the end of the experiment the subjects were asked to perform an MVC at 90° of ankle angle, which was compared to the average MVC measured before the start of the experimental procedure.

Measurement of muscle torque

Participants sat in a chair, which was adjusted as to ensure maximal possible comfort throughout the duration of the experiment. The plant of their right foot was brought in contact with the dynamometer's strain pedal gauge transducer. Their torso and knee and ankle joints were stabilized in order to ensure the exclusive participation of the ankle joint muscles to the total torque production. Measurements were performed isometrically always at 90°, and dynamically from 115° to 85° of plantarflexion with a constant angular velocity of 30°/s. The range of motion of only 30 ° was chosen to avoid potential fatiguing effects due to the low angular velocity used (in accordance with Seger & Thorstensson, 2000).

Electromyography

EMG activity was recorded from the right SOL of all subjects using self-adhesive Ag/AgCl electrodes (Blue Sensor N-00-S, Medicotest) placed in a bipolar configuration. The process of electrode placement was carried out in accordance to the SENIAM recommendations. Electrodes were placed on the SOL belly in accordance with the underlying muscle fiber direction (interelectrode distance = 20 mm; interelectrode resistance = 2 kΩ). Prior to electrode placement, the skin was prepared by shaving percutaneous hair and removing dead skin by gentle rubbing with sandpaper. Additionally, EMG activity was recorded from the synergist medial gastrocnemius and the antagonist TA in order to verify the predominance of SOL muscle activity during the tasks performed. The criterion for the proper alignment of the electrodes was a smooth bipolar M-wave shape during quiet stance. The signals were filtered (10 Hz to 1 kHz), amplified (500 times, amplifier NL824-153, Digitimer, Welwyn, Garden City, UK) and sampled at 5 kHz through an AD-Interface (CED 2701 with Signal software, Cambridge Electronic Devices, Cambridge, UK).

Stimulation methods

Two types of stimulation were used: magnetic stimulation of the motor cortex by TMS and electric stimulation of the posterior tibial nerve. Stimuli were always delivered at 90° ankle joint angle.

Transcranial magnetic stimulation. MEPs were elicited in the right SOL muscle by means of a magnetic stimulator (Magstim 200, SA34 0HR; Magstim, Whitland, UK). The figure-of-eight coil was placed on the left hemisphere of the cortex and moved with respect to the vertex until the optimal site (”hot spot”) was determined, which corresponded to the point on the skull where a visible response could be elicited with minimum stimulus intensity. Generally this point was located latero-posteriorly (1cm back and 1cm to the left) with respect to the vertex. The coil was then secured with the aid of a holder and an elastic band positioned below the chin. Coil position was checked throughout the experiment with reference to markings made on the skull. In all the subjects, the stimulation intensity was

set to be equal to resting motor threshold which was defined as the stimulus intensity eliciting a MEP of at least 50 μV with 50% probability in a fully relaxed muscle (Rossini et al., 1994; Rossi et al., 2009). This intensity was on average 51.36% of maximal stimulator output and was kept constant throughout the experiment. The safety and ethical considerations presented in the paper by Rossi et al. (2009) were taken into account when carrying out stimulation by TMS.

Electrical stimulation. H-reflexes and M-waves were evoked in the right SOL muscle by percutaneous stimulation of the tibial nerve with a single rectangular pulse (pulse duration = 1ms) delivered from a constant current stimulator (DS7A, Digitimer, Hertfordshire, UK). The optimal stimulation point was determined during quiet stance, when the cathode (77 mm2 in pick-up area, Unilect short-term ECG Electrodes, Ag/AgCl, Unomedical Ltd., UK) was placed stepwise in the popliteal fossa. The optimal stimulation point was hence defined as the spot where the largest SOL H-reflex and the smallest TA H-reflex were recorded. The cathode was then placed carefully over the optimal stimulation point and secured with an adhesive tape in order to maintain the pressure on the nerve. The anode, an oval dispersal pad (5,08 cm x 10,16 cm in dimensions, V-trodes neurostimulation electrodes, Mattler Electronics corp., USA), was fixed on the anterior aspect of the knee above the patella. The sensitivity of the H-reflex to facilitation and inhibition varies with respect to the size of the control H-reflex (Crone et al. 1990). Therefore, to select suitable test stimulus intensities, passive isometric recruitment curves were obtained for each subject at the start of each experiment, when the subject was seated on the chair, keeping the SOL relaxed.

Data processing

Average values of plantarflexor torque and SOL EMG were measured over a 100ms window prior to stimulation (i.e. one second after the beginning of muscle lengthening, for LEN tasks, corresponding to an ankle joint angle range of 88-90°). Subsequently, a mean of three trials for each subject was computed. Peak-to-peak amplitudes of Mmax, MEPs and H-reflexes were calculated from the initial deflection from the background

electromyographic activity to the second crossing of the horizontal axis. For the MEPs, the mean of five trials was calculated. This mean value was then normalised to the Mmax value recorded during contractions of the same intensity and mode. In the case of H-reflex, three valid trials were accepted for each contraction level and mode (see experimental procedures above). Each valid H-reflex was thereby divided by the accompanying M-wave and the average value of three H-reflexes over M-wave was calculated.

Statistical analysis

Group data are presented as means ± SD unless otherwise stated. Initially, variables were tested for normality using the Kolmogorov-Smirnov test. Since all variables were normally distributed, parametric tests were performed in order to reveal differences between pooled averages of all measured variables. For a given contraction mode, comparisons between values of evoked responses (MEPs, H-reflexes, Mmax) recorded at all contraction strengths (within-subject factor) were carried out using repeated measures analysis of variance (ANOVA). In case of significant F-values ($F < 0.05$), specific comparisons were carried out using Student's paired t-test. For a given contraction strength, Student's paired t-test was used in order to reveal differences between ISO and LEN contraction modes with respect to torque, EMG, MEP, H-reflex and Mmax values. The level of significance was always set at to $P \leq 0.05$ (2-tailed).

3.3 Results

EMG and Torque

EMG activity 100ms prior to stimulation showed a general tendency to be lower in LEN compared to ISO (Figure1a). However, this difference reached significance only in the case of 60% MVC (0 .098 ± 0.035 mV for ISO *vs* 0.078 ± 0.027 mV for LEN, $P < 0.01$). The effect of muscle LEN on torque was quite prominent (Figure 1b and 1c).

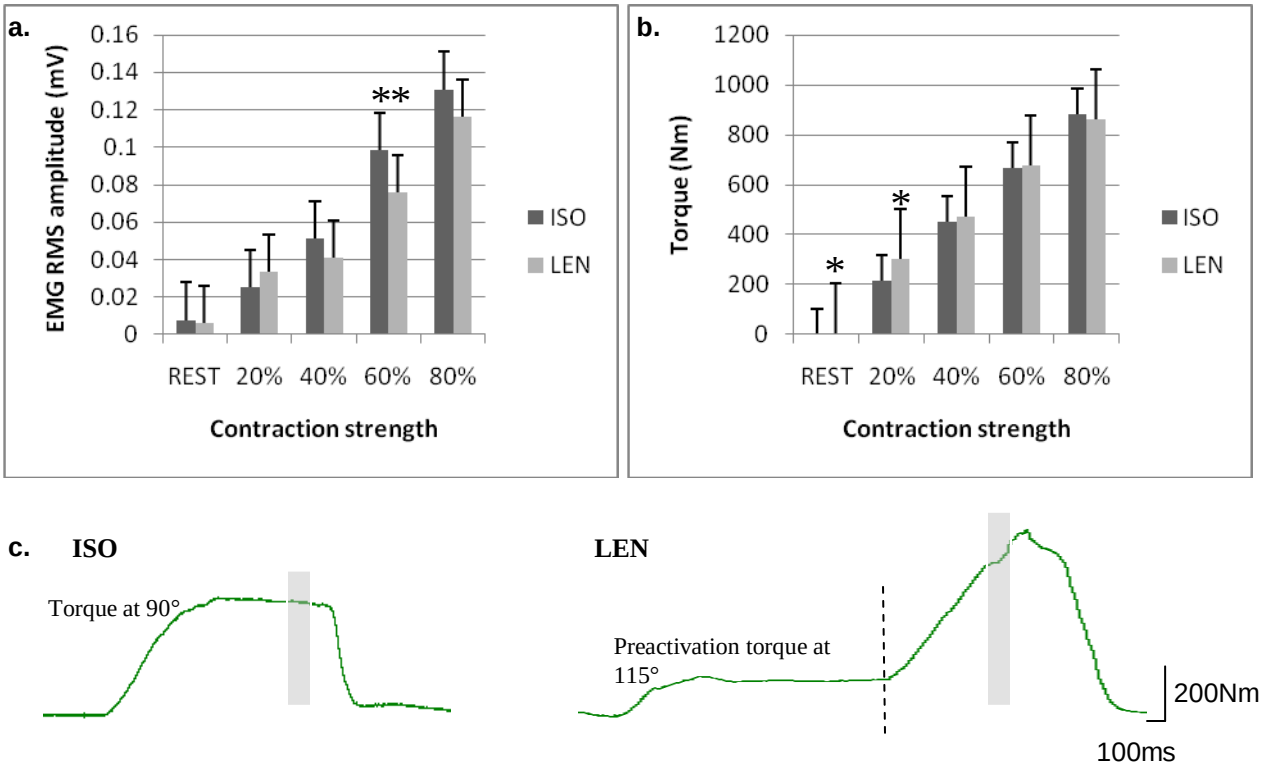

Figure 1. a., EMG RMS amplitudes for ISO and LEN over the entire range of contraction strengths. **b.,** Same as in **a.** for torque. Significant differences are depicted by asterisks. **c.,** Torque at 20% MVC for one representative subject, during an ISO (left) and LEN (right) contraction. Please notice the prominent increase in torque due to muscle lengthening (dashed line). The comparison was carried out on the basis of the average torque developed 100ms prior to stimulation (shaded area). * $P < 0.05$, ** $P < 0.01$.

Interestingly, significant differences were observed at REST (1.8 ± 2.8 Nm for ISO *vs* 4.6 ± 3.28 Nm for LEN) and at 20% MVC (215.25 ± 68.3 Nm for ISO *vs* 300.8± 155.05 Nm for LEN) where the LEN torque was significantly higher than ISO torque ($P < 0.05$).

Evoked responses

Table 1 summarises the absolute values and the relative to Mmax or M-wave values of MEP and H-reflex, respectively, for ISO and LEN contraction modes.

Table 1. Values of Mmax, MEP (normalized and absolute) and H-reflex (absolute and divided by the corresponding M-wave, i.e. H/M-ratio)

	REST	*20%*	*40%*	*60%*	*80%*
Mmax (mV)*					
ISO	9.6 ± 0.02	10.6± 0.02	10.95± 0.05	11.47± 0.07	11.48± 0.08
LEN	8.5 ± 0.003	9.5 ± 0.06	9.63 ± 0.06	9.7 ± 0.08	10.14± 0.07
MEP (mV)					
ISO	0.07 ± 0.05	0.59 ± 0.5	1.10 ± 0.7	1.47 ± 0.8	1.33 ± 0.4
LEN	0.05 ± 0.04	0.46 ± 0.4	0.61 ± 0.4	0.82 ± 0.3	0.93 ± 0.3
MEP/Mmax					
ISO	0.01± 0.004	0,06± 0.04	0.11 ± 0.06	0.13 ± 0.05	0.11 ± 0.02
LEN	0.01± 0.007	0.05± 0.04	0.07 ± 0.05	0.07 ± 0.05	0.07 ± 0.02
H-reflex (mV)					
ISO	3.37 ± 1.6	3.76 ± 1.8	4.32 ± 1.7	4.7 ± 1.7	5.19 ± 1.8
LEN	2.86 ± 1.4	4.07 ± 1.7	4.13 ± 1.7	4.56 ± 1.8	4.73 ± 1.7
H/M ratio					
ISO	2.5 ± 1.1	2.38 ± 0.9	2.75 ± 0.7	2.87 ± 0.8	2.87 ± 0.95
LEN	1.40 ± 0.9	2.44 ± 0.8	2.39 ± 0.6	2.84 ± 1.1	2.47 ± 0.9

*Mmax values are given only for the TMS experiment

Figure 2 depicts real signals obtained from one representative subject (H-reflex plus the accompanying M-wave, as well as MEP) at REST and at 80% MVC during both ISO and LEN modes of contraction.

Size of Mmax

The results reported here are valid for both TMS and H-reflex experiments.

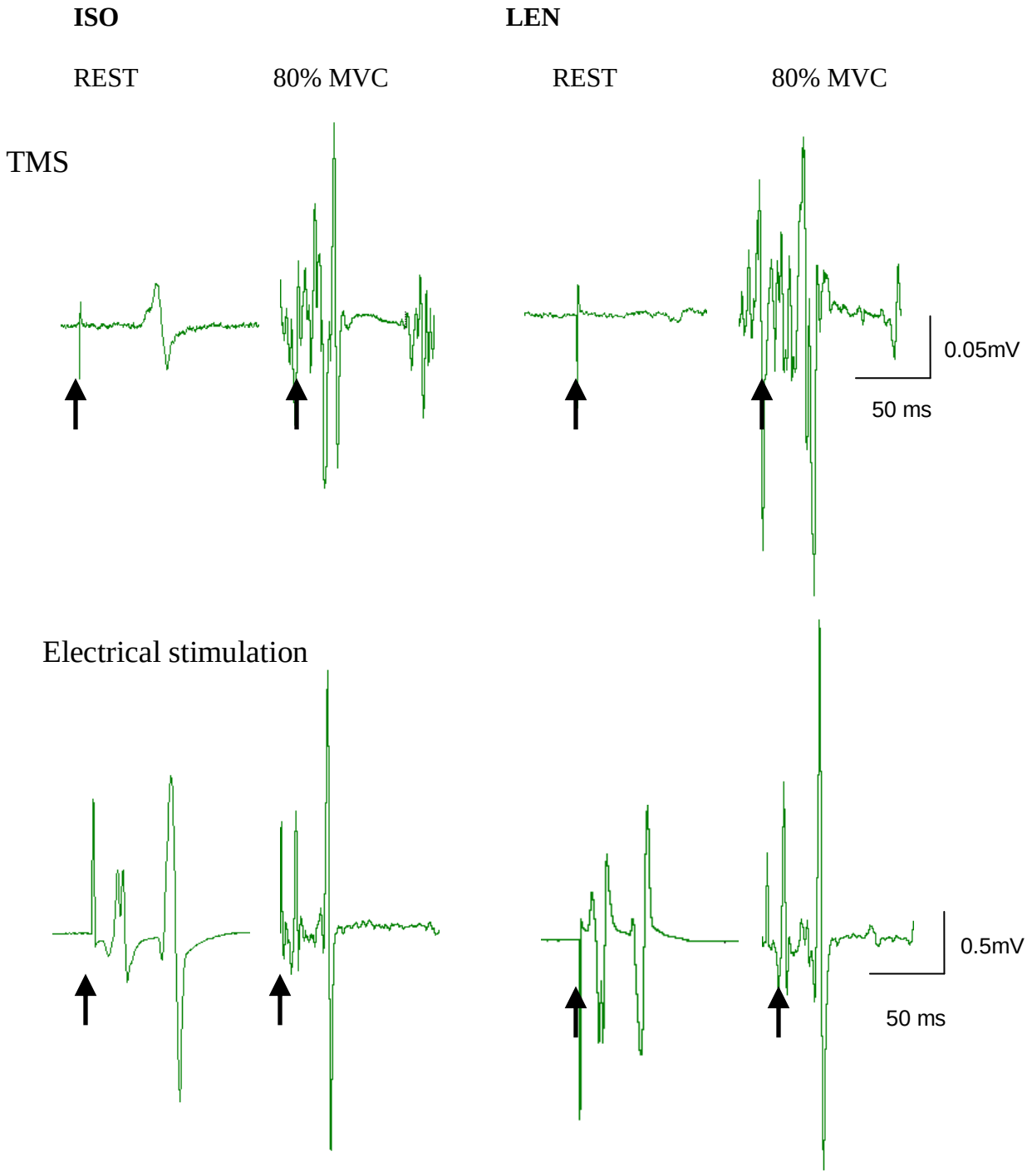

Figure 2. Superimposed responses of a single subject to TMS (MEPs) and electrical stimulation (H-reflex plus M-wave) at REST and at 80% MVC. *Left:* ISO contractions. *Right:* LEN contractions. The stimulation instant is indicated in each case by a bold arrow. Please notice the different voltage scale between the two types of stimulation, as well as the differences in amplitude of the evoked responses between contraction strengths and contraction modes.

As expected, the Mmax grew in accordance with the increase in contraction strength. Between contraction modes, no significant differences in Mmax size were observed when it comes to responses obtained at matched contraction strengths (P always > 0.05).

Size of MEPs

Within contraction modes, the absolute MEP response increased in amplitude in parallel with the increasing contraction strength up to 40% MVC (Figure 3).

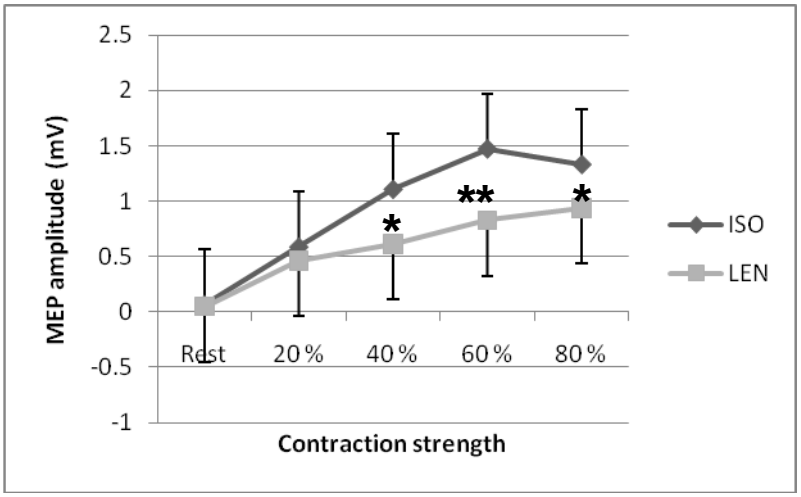

Figure 3. Absolute MEP amplitude plotted against contraction strength for ISO and LEN contraction modes. Significant differences between ISO and LEN MEPs are depicted with asterisks. * P < 0.05, ** P < 0.01.

Specifically, for ISO contraction mode MEP amplitude differed significantly between REST and all other contraction strengths (P < 0.001). Also, significant differences were obtained between the MEP measured at 20% MVC and the corresponding MEPs measured at 40%, 60% and 80% MVC (P < 0.001). When it comes to LEN, significant differences were observed between MEP amplitudes at REST and all other contraction strengths (P < 0.01). Significant differences were also observed between the MEP measured at 20% MVC and the corresponding MEPs measured at 40%, 60% and 80% MVC (P < 0.05). In addition, and in contrast to the ISO condition, the MEP amplitude significantly differed between 40% and 60% MVC (P < 0.05). Therefore the growth of the MEP was non-significant after 40% MVC for ISO and 60 % MVC for LEN contractions. Plateauing of the MEP is thus observed for both contraction modes.

Between contraction-mode comparisons revealed that absolute MEPs were significantly different between ISO and LEN. Significant differences between contraction modes were detected at 40, 60 and 80% MVC ($P < 0.05$). Comparisons involving the normalized to Mmax MEPs (Figure 4) gave exactly identical results as those described above for the absolute MEPs.

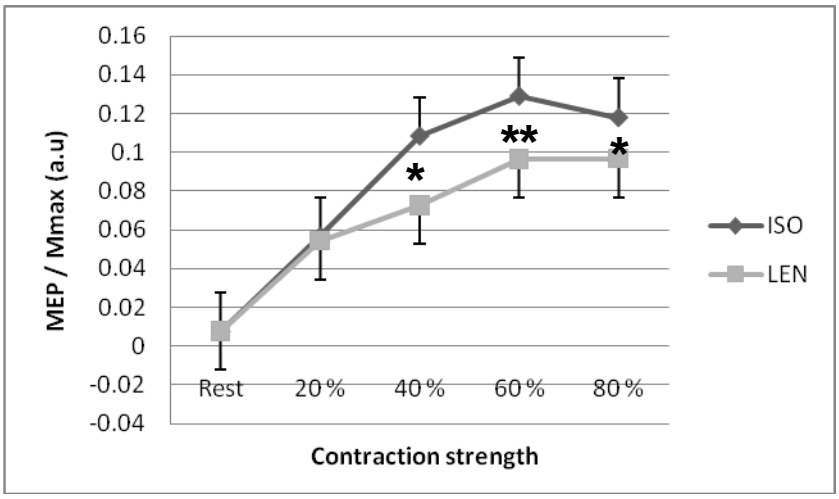

Figure 4. MEP amplitude normalized to Mmax plotted against contraction strength for ISO and LEN contraction modes. Significant differences between ISO and LEN MEPs are depicted with asterisks. * $P < 0.05$, ** $P < 0.01$.

Size of H-reflexes

The absolute H-reflexes demonstrated for both ISO and LEN a linear increase from REST to 80% MVC (Figure 5).

Specifically, for ISO, significant differences were located between REST and 40, 60 and 80% MVC ($P< 0.05$), between 20 and 60% and 80% MVC ($P < 0.05$) and between 40 and 80% MVC ($P < 0.01$). For LEN, significant differences were revealed between REST and 20, 40, 60 and 80% of MVC ($P < 0.01$), 20 and 80% MVC, ($P < 0.05$) and 40 and 80% MVC ($P < 0.05$). Between contraction modes, the *raw* H-reflexes obtained at matched contraction strengths were identical in amplitude between ISO and LEN contraction modes ($P > 0.05$ in all cases).

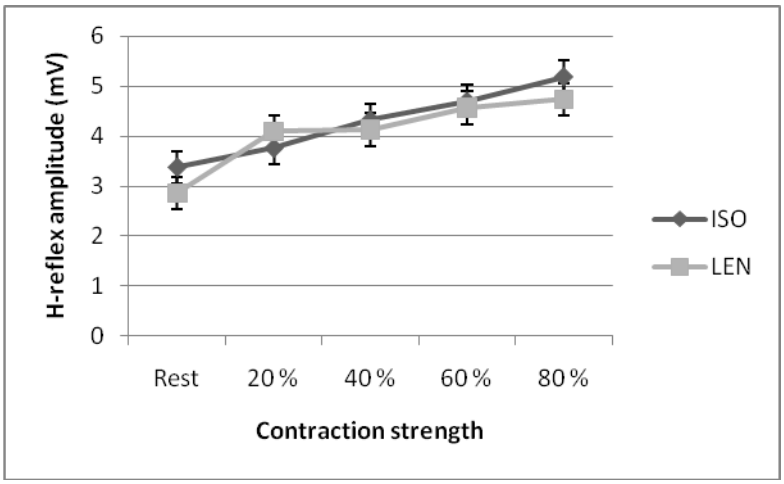

Figure 5. Absolute H-reflex amplitude plotted against contraction strength for ISO and LEN contraction modes. No inter-contraction mode differences were observed in this case. * $P < 0.05$, ** $P < 0.01$.

The H/M-ratio (Figure 6) was, for ISO, stable in amplitude over the range of contractions strengths. On the other hand, H/M-ratio obtained during LEN demonstrated significant differences between REST and 20%, 40%, 60% and 80% MVC ($P < 0.05$ in all cases). Interestingly, H/M-ratio amplitude differed significantly between ISO and LEN exclusively at REST ($P < 0.05$).

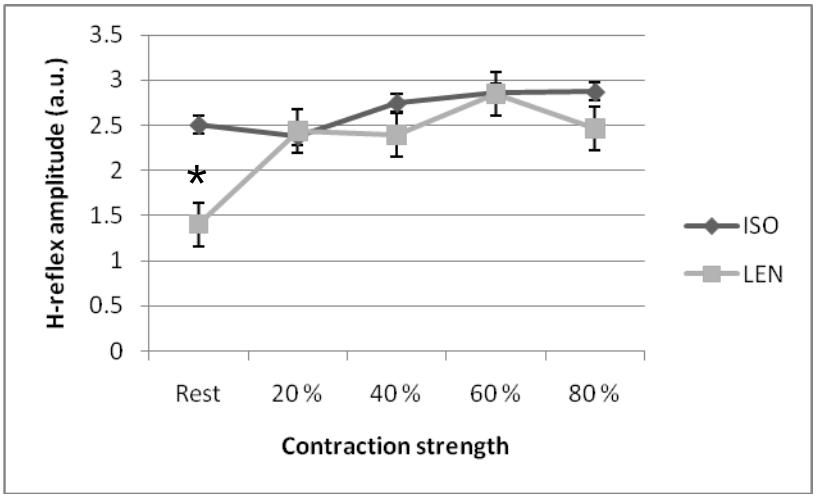

Figure 6. H/M-ratio plotted against contraction strength for ISO and LEN contraction modes. Significant differences between ISO and LEN H-reflexes are depicted with asterisks. * $P < 0.05$, ** $P < 0.01$.

Overall, the comparison between the ISO and LEN contraction modes revealed no differences between H-reflex values, neither for absolute H-reflex nor for H/M-ratio, except at REST only for the latter.

Relative differences of evoked responses

For a given evoked response, the average relative difference between the amplitude of the response at ISO and at LEN was computed for each contraction strength (Figure 7). Thereafter, comparisons were carried out at each contraction level between the respective relative differences calculated for H-reflexes and MEPs. Significant differences were revealed only at REST where the average relative difference is significantly larger for the H-reflex than for the MEP response (43% for H-reflex *vs* 10% for LEN; $P < 0.05$) (Figure 7).

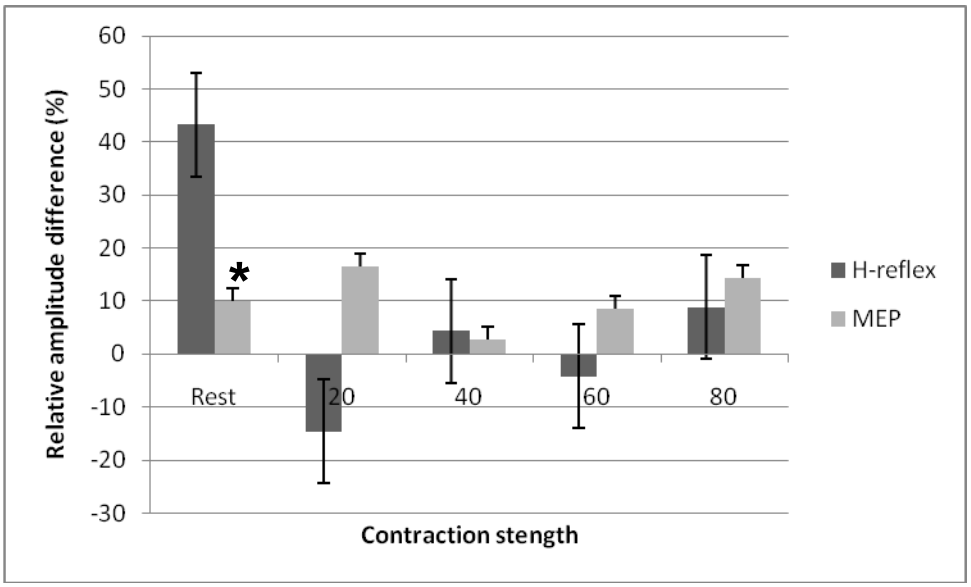

Figure 7. Mean relative difference between the responses obtained in ISO and LEN contraction modes is shown both for H/M-ratios and MEPs. Significant differences are depicted by asterisks. * $P < 0.05$.

3.4 Discussion

The main outcome of this study was significantly smaller MEPs during LEN contractions at 40%, 60% and 80% MVC compared to isometric contractions of similar strength ($P < 0.05$). On the other hand, the H/M-ratio measured was significantly smaller for LEN compared to ISO ($P < 0.05$) only at REST. EMG activity showed a general trend to be lower in LEN, but a significant difference was observed only at 60% MVC ($P < 0.01$). On the contrary, torque during LEN was shown to be either greater than during ISO (REST and 20% MVC, $P < 0.05$) or equal to its ISO counterpart.

EMG and torque

The tendency for decreased EMG during LEN is, in this case, quite puzzling. In fact, large increments in EMG were observed at short latencies after lengthening, which were presumably due to the increased firing of the muscle spindle accompanying increments in muscle length (Burke, 1977; Berardelli et al., 1982). This persisted throughout the whole range of motion and up to 100ms before stimulation. However, despite the additional input from the Ia afferent arc, EMG at LEN failed to match the EMG levels recorded during ISO contractions. An explanation for this could be that to start with, the pre-activation torque was lower for LEN, due to the unfavorable ankle angle of 115° degrees for torque production for SOL (Maganaris, 2001). This could account for the initially lower pre-activation EMG levels, the increase in EMG due to muscle spindle firing being unable to compensate for this deficiency. In any case, this finding comes in agreement with previous reports of decreased EMG during LEN contractions compared to ISO contractions (Komi et al., 2000; Linnamo et al., 2006). LEN seemed to exert a prominent effect on plantarflexor torque as well. This effect was most obvious for contractions performed at 20% MVC. The pre-activation torque was naturally always lower for LEN since it was produced at a greater ankle joint angle which is inherently less favorable for torque production (Maganaris, 2001). However, LEN of the pre-activated muscle resulted in a remarkable rise in torque. In fact, during LEN the torque production was equal to that recorded for the ISO contractions, which were performed at the theoretically optimal ankle joint angle of 90 degrees (Maganaris, 2001). The additional torque is most likely to be due to the elastic

elements present in the muscle in accordance with earlier theories concerning muscle mechanical properties and stiffness (Hill 1938; Huxley, 1958; Rack & Westbury, 1978) and more recent simulation studies (Bohm et al., 2006; Grover et al., 2007). More concretely, the in-series elastic components seem to be functioning as energy-absorbing mechanical buffers, also interestingly referred to muscle power amplifiers (Roberts & Azizi, 2010) which consequently prevent muscle damage under extreme conditions involving impacts (Reeves et al. 2003). In this regard, the greater torque production observed in the present case does not come as a surprise.

Mmax

As a measure of the activation of the total MN pool (Crone et al., 1999), the M-max was taken as the normalizing factor of absolute MEP values. As such, the observed increase in its value with increasing contraction strength is in accordance with the fact that Mmax presumably undergoes potentiation due to enhanced synaptic transmission at the neuromuscular junction as a consequence of voluntary activation (Hicks et al., 1989). An alternative explanation for the Mmax growth was provided by Fitch and McComas (1985) who suggested increased synchronisation of action potentials travelling along the muscle fiber as the mechanism for the observed Mmax potentiation. The fact that no significant differences were found between Mmax measured at matched contraction strengths between ISO and LEN is of practical importance (Duclay & Martin, 2005). The normalization of the MEP response to Mmax could not have been carried out were the Mmax different for ISO and LEN. Another Furthermore, contrary to the findings of Crone et al. (1999), in the present study the Mmax did not decrease in value during the experiment. The Mmax was in fact measured twice for a given contraction mode and contraction strength during a single measurement session: once during the TMS experiment and once during the H-reflex experiment. A considerable amount of time elapsed between the two experiments; however, in contrast with the findings of Crone et al. (1999) the amplitude of the Mmax was not shown to decrease.

Consequently, these findings provide solid justification for the use of the Mmax and M-wave as a normalizing factor in the present study and enable reliable comparison of the remaining set of variables.

MEPs

It can be observed that MEPs demonstrate a similar pattern of change between the two contraction modes. In both ISO and LEN, MEP increases with increasing contraction strength up to a maximum point (plateau). The shape of the relationship is therefore, in both contraction modes, not entirely linear. According to previous findings, MEP increases linearly with increased contraction strength (Morita et al., 2000; Oya et al., 2008) and this relationship plateaus at 60% MVC (Oya et al., 2008). In contrast to Oya et al. (2008) the present results point out towards a plateau for ISO at 40% MVC. However, in the present study the statistical difference between MEPs at 40% and 60% MVC was in fact very close to significance ($P = 0.086$); in addition, the visual appearance of the curve was that of an increase of the MEP from 40% to 60% MVC. An explanation about why the MEP-contraction strength relationship plateaus has been given by Martin et al., (2006) who suggested that the contraction strength at which the MEP amplitude plateaus is indicative of the upper limit of the recruitment range. The subsequent increases in force are therefore achieved exclusively by means of rate coding. According to Matthews (1996; 1999), a MN firing at a relatively high rate is incapable of responding to additional external inputs (in this case the descending drive triggered by the TMS stimulus) due to prolonged refractory period. Given the incapacity of the neuron to produce a response to the incoming extra drive, the incapacity of the MEP to increase any further can be explained. It was shown that for the SOL muscle, recruitment persists up to 95% MVC for ramp ISO contractions (Oya et al., 2009). However, the present and older findings (Oya et al., 2008) suggest that the MEP-contraction strength curve does not strictly follow the above finding, given that plateauing of the relationship for ISO occurs much before the attainment of maximal force.

Furthermore, the present findings also give rise to the question of the mechanism responsible for the later-occurring plateauing of the relationship for LEN compared to the ISO contractions. Bigland and Lippold (1954) have provided evidence that during LEN contractions the absolute force exerted by individual motor units is higher than during ISO contractions. Also, some authors (Tax et al., 1989; Theeuwen et al., 1994) have suggested lowered recruitment thresholds of MUs for LEN contractions associated with lower initial firing rates. In this light, the later-occurring plateauing for the MEP-contraction strength

curve for LEN contractions can be explained. It can thus be speculated that if the recruitment is done in a manner that, in overall, relies less on rate coding, and that, once recruited, the MN is intrinsically capable of producing more force, then the MN pool might possess a larger "excitability margin" which makes the MEP increase possible throughout a larger range of contraction strengths. Interestingly, despite the larger margin of MEP growth, MEP amplitude during LEN was always lower than ISO – and significantly so for 40%, 60% and 80% MVC. This significant inter-contraction mode difference agrees with previous findings (Sekiguchi et al. 2001; 2003) according to which the MEPs in the SOL are lower for LEN contractions of moderate background EMG levels compared to SHORT contractions. In addition to this, Gruber et al. (2009) and Abbruzzese et al. (1994) have reported lower MEPs of the elbow flexor muscle group during LEN compared to ISO and SHORT contractions, respectively. The observation of lower amplitude MEPs during LEN contractions is referred to in more detail in conjunction with other measured variables in the *Comparison of MEPs and H-reflexes* paragraph (page 48).

Taken as a whole, the present findings provide support to the argument of rate coding being the limiting factor for MEP growth (Martin et al., 2006).

Nardone et al. (1988; 1989) have provided so far the only piece of evidence of preferential recruitment of high-threshold MUs during muscle lengthening at low contraction strengths. However, in the present study, it seems that MU recruitment during lengthening actions has indeed followed the size principle, given the striking similarity between the MEP-contraction strength relationship obtained for ISO and LEN. Furthermore, this relationship is characterized for both contraction modes by a progressive pattern of change, suggestive of orderly recruitment. In addition to this, given that the high-threshold MUs demonstrate a higher initial firing frequency at their recruitment threshold than low-threshold MUs, the overall MEP should be significantly lower for LEN from the beginning of the contraction range, since high-firing MUs are less responsive to additional inputs (see the previous paragraph).

However, MEP amplitude is known to be a global indicator of the responsiveness of the corticospinal tract as a whole (e.g. Devanne et al., 1997). It is not therefore possible to distinguish between the supraspinal and spinal level when attempting to define the exact location of reduced excitability responsible for the lower MEPs at LEN. Comparison of the

MEP and H-reflex responses is therefore necessary in order to draw any conclusions regarding the excitability of isolated elements of the corticospinal tract (Schieppatti et al., 1996; Morita et al., 2000).

H/M-ratio

H/M-ratio remained fairly constant despite increasing contraction strength for both ISO and LEN and not surprisingly so. From a mathematical point of view, a constant value was something to be expected, since the increasing raw H-reflex amplitude was counterbalanced by the increasing M-wave amplitude. This result comes in accordance with the results of Ruegg et al. (1990) who superimposed H-reflex on steady ISO contractions of the plantarflexors. They found that the normalized H-reflex amplitude was independent of the amount of force produced, i.e. H-reflex amplitude undergoes no modulation related to the pre-existing level of motor discharge. The same pattern of H-reflex behavior is observed during LEN contractions, i.e. the Hmax/Mmax ratio remained unchanged for LEN contractions of the SOL muscle at 10, 20 and 30% MVC (Nordlund et al., 2002).

Comparison between contraction strengths did not reveal any significant differences between H-reflex amplitudes except for REST, i.e. for resting muscle at constant length (ISO) and for passively lengthening muscle (LEN). Firstly, the observation of a significant difference at REST comes in agreement with the previous reports (Nordlund et al. 2002; Duclay & Martin, 2005) of a significantly lower Hmax/Mmax ratio for passive LEN versus passive SHORT actions of the plantarflexors. Furthermore, Pinniger et al. (2001) have reported a severe depression of the inactive SOL H-reflex for LEN compared to ISO. Comparison of responses obtained from an active muscle during LEN and SHORT contractions revealed a significantly lower Hmax/Mmax ratio for LEN than for SHORT (Nordlund et al., 2002; Sekiguchi et al., 2003). In addition to the numerous reports about differences between LEN and SHORT contractions, there has also been some evidence (Duclay & Martin, 2005) of lower Hmax/Mmax ratio during passive LEN compared to passive ISO actions.

The proposed explanation for the aforementioned results could be strongly related to muscle history (Wood et al., 1996). If H-reflex measurement in a passive muscle is

preceded by muscle contraction– as it was the case in the current study – the subsequent H-reflex response is depressed (Wood et al., 1996). This might be due to the fact that during the performance of passive movements the Ia afferent presynaptic inhibition is accentuated (Duclay & Martin, 2005); on the other hand, voluntary activation is a modulating factor which induces dishinhibition (Faist et al., 1996; Wood et al., 1996; Nordlund et al., 2002). Ia presynaptic inhibition is thought to be controlled by supraspinal centers (Meunier & Pierrot-Desseilligny, 1998), but might also include a peripheral component (Ruegg et al., 1990). Additionally, in the case of an active muscle, muscle LEN is accompanied by high muscle spindle discharge (Burke et al., 1977) which remains high also for a certain time period *after* the contraction (Wood et al., 1996). Thus, the combination of high spindle discharge which might cause the Ia afferent to be irresponsive to additional inputs (Trimble et al., 2000) and the increased presynaptic inhibition (Duclay & Martin, 2005) could be the cause for H-reflex depression during passive LEN of the SOL. During passive movements the agonist muscle usually remains silent. However, parallel activation of the antagonistic muscle (in this case, the TA) in order to stabilize the limb can also lead to heteronymous Ia afferent inhibition (Ruegg et al., 1990).

It should be mentioned that modulation of the H-reflex in functional tasks i.e. walking is done in a different way than for the isolated movements of the ankle joint which were examined in this study. During whole-body coordinated movements, Ia afferent presynaptic inhibition displays a behavior congruent with the goal of the motor task and various environmental constraints (Faist et al., 1996). Furthermore, afferent inputs other than the Ia-pathway play a role in the fine-tuning of total motor output (Klint et al., 2010).

Comparison of MEPs and H-reflexes

Given the absence of any differences between contraction modes for the H/M-ratio except at REST, and the significant differences between the MEPs at 40, 60 and 80% MVC, it can be deducted that differences in control, if any, between ISO and LEN contractions are localized in the supraspinal centers. This comes in contrast to the findings of Gruber et al. (2009) who have reported lower cervicomedullary motor potentials (CMEPs) during LEN contractions which, in combination with slightly higher MEP/CMEP ratios, clearly point out towards impaired *spinal* excitability during LEN. Gruber et al. (2009) have therefore

concluded that the cortical excitability is slightly higher for LEN. This latter finding is supported by evidence of a greater movement-related cortical potential associated with the performance of muscle movements involving LEN (Fang et al. 2001). However, these authors investigated upper limb muscles (i.e. elbow flexors), which have been reported in the past to be subject to more elaborate control strategies than lower limb muscles (Palmer & Ashby, 1992), and as a corollary, the SOL muscle (Nielsen et al., 1995; Taube et al., 2006). It is therefore quite likely that in the case of LEN contractions of the SOL muscle the motor cortex generates a differential command in comparison to ISO contractions. Support for this comes from the fact that despite lower MEPs LEN torque was identical (and greater, even, in the case of 20% MVC) to the torque measured in ISO conditions. Since the H/M-ratio was similar between the two contraction modes, it would seem that lower MEPs account for lower corticospinal excitability (Schieppatti et al., 1996; Morita et al., 2000). This could simply mean that the neural contribution to motor output is of less significance in the case of LEN contractions given the predominance of mechanically-mediated motor output (see *EMG and torque* above). Further evidence from this fact comes from the overall lower EMG levels measured during LEN.

However, during passive movements, synaptic transmission in the neural pathways involved appears to be different than in an active muscle. MEP amplitude was not significantly different at REST between LEN and ISO; however, in accordance to previous findings (Pinniger et al., 2000; Nordlund et al., 2002) H-reflex amplitude was significantly lower during passive ISO and LEN actions. Significantly larger relative differences between ISO and LEN H-reflexes at REST than the corresponding difference for MEPs also underline this fact. Similar MEPs but severely depressed H-reflexes are most likely indicative of MN output modulation by peripheral factors (Schieppatti et al., 1996; Morita et al., 2000). In fact, it seems that a spinally mediated mechanism aiming to keep MN pool output within reasonable limits comes into play during passive LEN (Pinniger et al., 2000). However, this spinal mechanism disappears at tasks involving an active muscle, something which leads to the reasonable assumption that this mechanism consists mainly in Ia presynaptic inhibition (Meunier & Pierrot-Desseilligny, 1998; Pinniger et al., 2000).

Methodological considerations

The interpretation of the present results should also be done taking into account any possible methodological limitations of this study.

One potential factor which might have influenced (although only slightly) the obtained results could be the appearance of neuromuscular fatigue during the course of the experiment. In order to limit the effect of any possible fatigue-related influence of the obtained results, trials were randomized. On the other hand, Mmax amplitude – whose decrease in value is an indicator of fatigue – did not change during the course of the experiment. Furthermore, no significant differences were observed between the MVC measured before and after the end of the experiment. It is widely thought that the primary indicator of fatigue is a reduction of measured maximal voluntary force. However, it should also be kept in mind that fatigue is also manifested through an increased effort to produce a certain amount force, although the capacity to produce this force remains intact. Effort-related indicators of neuromuscular fatigue can be, for instance increased MN firing rates (see Gandevia, 2001 for a review). However, this latter component of the definition of fatigue applies only for submaximal contractions as in the case of an MVC the effort of the subject is, naturally, maximal. Taking all these into account, it can be concluded that neuromuscular fatigue does not seem to have been a modulating parameter in the current experiment.

It has to be mentioned that more reliable as well as more reproducible information concerning corticospinal tract excitability could be obtained by using the cervicomedullary (CMS) stimulation technique (Martin et al., 2009). The limitations of the H-reflex, namely the influence of pre-synaptic inhibition to the obtained response could be avoided, since it has been proved that the connection between descending fibers of the corticospinal tract and the spinal MNs is free of pre-synaptic inhibition (Nielsen & Petersen, 1994). Consequently, the use of CMS would have an exclusive measure of spinal MN excitability, without the conjuncture of any other factors. For the purpose of the current study the CMS technique could not be used due to the posture of the subjects during the measurement sessions (sitting) which was dictated mainly by the TMS stimulation and the configuration of the dynamometer.

Another issue could be the stimulation in dynamic conditions, which raises the question of any potential movement of the stimulating electrode in relation to the nerve during the H-reflex experiment (Pinniger et al., 2001). However, given the method used to measure the H-reflex i.e. referenced to an M-wave corresponding to 15-25% of the Mmax, it is most probable that alterations in the nerve-stimulating electrode spatial configurations were mostly compensated for (Simonsen & Dyhre-Poulsen, 1999). However it should be noted that at the relatively high contraction strengths used during this experiment (i.e. 60% and 80%) control of the M-wave was done with great difficulty. However, this was not thought to compromise the reliability of the measured H-reflex values.

Final conclusions and functional implications

The present study offers a novel insight into the motor control of ISO and LEN contractions of the SOL muscle, a comparison which has yet to be performed.

The results obtained argue in favor of a differential control scheme for LEN contractions, a long-standing assumption which has nevertheless severely lacked – and still lacks – undeniable, concrete evidence. The lower MEP responses obtained for LEN compared to ISO contractions associated with unchanged H-reflexes are indicators of lower cortical excitability. This could simply imply that for the SOL muscle, whose capacity for storage and retrieval of elastic energy is quite prominent due to the Achilles tendon properties, the need for cortical descending drive is less pronounced during LEN, where the series elastic elements are capable of contributing to a large extent to the produced torque.

In this light, the hypothesis of a unique neural command for LEN contractions of the SOL muscle can be confirmed, but not in the context of a neural inhibition, as it was repetitively hypothesized in the past. The current results are rather supportive of an *adaptive* aspect of the neural command and namely the capacity of the supraspinal centers bear to modify the neural command issued by the motor cortex given the particularities of a given motor task. This adaptive neural strategy is most likely put forward for the sake of functionality: in the case of this study, any additional neural descending command would have been redundant and would result in unnecessary extra torque production, given the ability of the lengthening SOL to produce torque by itself *via* its mechanical components.

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5 APPENDICES

Appendix 1. Consent form

Jyväskylän yliopisto, Liikuntabiologian laitos

Study participants information and consent form

Theme of the research:
EVOKED RESPONSES TO TRANSCRANIAL AND ELECTRICAL STIMULATION DURING ISO AND LEN CONTRACTIONS OF THE SOLEUS

1. Researchers' contact information

2. Background information about the study

The research is conducted in the Biomechanics lab, in the department of Biology of Physical Activity. The study is part of the broader research project of the department, that is to say, the investigation of the mechanisms of motor control. Motor control is an extremely complex area, which involves the brain (more specifically, the motor cortex), and specialized neurons (called motor neurons) in the spinal cord. Measuring the responsiveness of neurons at both these levels is crucial in order to understand how different movements are initiated and modulated. We are using transcranial magnetic stimulation (TMS), which is a relatively new tool, introduced about 20 years ago in the domain of neurophysiology. It allows in a painless and non-invasive way to measure how responsive is the motor cortex during the performance of different tasks. It has therefore become a valuable tool in motor control studies. In addition to this, its potential utility in rehabilitation is now being investigated.

3. Research data saving methods

The research data are being saved by the researcher in charge, who is also responsible for their safe retention. Once saved, the data will be kept in a locked cabinet. The personal information of the subjects will be kept confidential. For the purpose of the study, the data may be transferred from the hard drive to CD:s.

4. Purpose of the study, aim and significance

The purpose of the study is to give some insight in the manner with different kinds of muscle contractions are controlled by the nervous system. Isometric contractions are those during which the muscle length remains constant; on the other hand, during lengthening contractions the muscle length increases. It has been assumed, and has partly being proved empirically, that the motor strategies employed by the nervous system differ between these two tasks. This study aims to clarify this concept, through a protocol which includes the performance of lengthening and isometric contractions of different force levels. The responses to electric and magnetic stimuli, delivered during the performance of these contractions, provide information about the implication of different neuronal structures. This is a basic study with no direct practical implications. However, the knowledge gained through it can be of use in the domain of medicine and rehabilitation.

5. Procedures used during the experiment

In this study, the participants are young adults (20-35 years), both male and female. The tasks required to be performed by the subjects are not physically challenging. In addition to the voluntary contraction of the soleus muscle, transcranial stimulation of the motor cortex, as well as electrical stimulation of the peripheral nerve will be used. The transcranial stimulation is magnetic in nature, and is painless and harmless to healthy subjects (Wassermann, EM 1998. Electroencephalogr Clin Neurophysiol 1998 Jan; 108 (1) :1-16.). If your family or yourself have a history of epilepsy, or seizures, if your skull is made of metal or magnetic objects, if

you have a high intracranial pressure, or if you are using electronic pacemakers that maintain physiological function (eg. cardiac pacemaker) you cannot participate in this study. Electrical stimulation is safe, but you may feel uncomfortable.

6. Benefits and risks of the study's participants

What will the participants gain from the study

Study participants will have the opportunity to get acquainted with the current methods of measurement of Neurophysiology and during the measurements can take advantage of scientific expertise of researchers. The subjects will receive a summary of the study results. In case they wish to obtain more information about the study itself, or about its outcomes, they may contact the researcher at any time.

Research-related risks and potential disadvantages

For the purpose of measuring the muscle electrical activity (EMG), the dead skin is removed by means of rubbing with sand paper. In this case a minor risk of infection arises. The laboratory staff is well acquainted with first-aid procedures, and has access to medical instruments, should an accident of any kind occur. The magnetic and electric stimulations used are harmless. However, the magnetic stimulation might cause a slight headache if the neck muscles are activated concomitantly. Electric stimulation may feel uncomfortable, but it is a very commonly used method in research.

7. How and where the research results will be used

Results will be used for the purpose of the master's thesis of the student Alexandrou. The results will be submitted for publication in a scientific journal in the field of sports, and might also be presented at congresses.

8. Rights of the participants

Participation to the experiment is entirely voluntary. The participants are entitled at any time during the procedures to suspend the testing, without incurring any penalties. The data obtained are used exclusively by the researcher, and the analyzing and reporting of the results is strictly confidential and anonymous. The participant has the right to request additional information about the study at any moment.

9. Insurance

The participants are insured against accidents due to any external causes. On the other hand, there is no insurance coverage in the case of accident due to extreme muscle effort, due to the fact that this does not lie in the category of external causes. The laboratory disposes of first-aid facilities and equipment, which the staff is able to use.

10. Participant's consent

I am familiar about this study's purpose and content, about the potential risks to the participants, as well as about the participants' rights and insurance protection. I agree to participate in the measurements at my own will, according to the information given to me. I do not participate having any kind of medical condition. I have the right, at any stage of the experimental procedure, to interrupt my participation, and oppose to the taking of measurements. The research results may be used for publication in a scientific journal, in such a form that the identification of the participants is not possible. Following my signature, I receive a copy of the present agreement, which also includes the researcher's signature. The original version remains to the researcher.

_____

Date Participant's signature

_____

Date Researcher's signature

Appendix 2. The declaration of Helsinki

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI
Ethical Principles
for
Medical Research Involving Human Subjects
Adopted by the 18th WMA General Assembly
Helsinki, Finland, June 1964
and amended by the
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53rd WMA General Assembly, Washington 2002 (Note of Clarification on paragraph 29 added)
55th WMA General Assembly, Tokyo 2004 (Note of Clarification on Paragraph 30 added)
59th WMA General Assembly, Seoul, October 2008

A. INTRODUCTION

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects**,** including research on identifiable human material and data. The Declaration is intended to be read as a whole and each of its constituent paragraphs should not be applied without consideration of all other relevant paragraphs.
2. Although the Declaration is addressed primarily to physicians, the WMA encourages other participants in medical research involving human subjects to adopt these principles.

3. It is the duty of the physician to promote and safeguard the health of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.
4. The Declaration of Geneva of the WMA binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the patient's best interest when providing medical care."
5. Medical progress is based on research that ultimately must include studies involving human subjects. Populations that are underrepresented in medical research should be provided appropriate access to participation in research.
6. In medical research involving human subjects, the well-being of the individual research subject must take precedence over all other interests.
7. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best current interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.
8. In medical practice and in medical research, most interventions involve risks and burdens.
9. Medical research is subject to ethical standards that promote respect for all human subjects and protect their health and rights. Some research populations are particularly vulnerable and need special protection. These include those who cannot give or refuse consent for themselves and those who may be vulnerable to coercion or undue influence.
10. Physicians should consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

B. PRINCIPLES FOR ALL MEDICAL RESEARCH

11. It is the duty of physicians who participate in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects.

12. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

13. Appropriate caution must be exercised in the conduct of medical research that may harm the environment.

14. The design and performance of each research study involving human subjects must be clearly described in a research protocol. The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest, incentives for subjects and provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study. The protocol should describe arrangements for post-study access by study subjects to interventions identified as beneficial in the study or access to other appropriate care or benefits.

15. The research protocol must be submitted for consideration, comment, guidance and approval to a research ethics committee before the study begins. This committee must be independent of the researcher, the sponsor and any other undue influence. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration. The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No change to the protocol may be made without consideration and approval by the committee.

16. Medical research involving human subjects must be conducted only by individuals with the appropriate scientific training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional. The responsibility for the protection of research subjects must always rest with the physician or other health care professional and never the research subjects, even though they have given consent.

17. Medical research involving a disadvantaged or vulnerable population or community is only justified if the research is responsive to the health needs and priorities of this population or community and if there is a reasonable likelihood that this population or community stands to benefit from the results of the research.

18. Every medical research study involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and communities involved in the research in comparison with foreseeable benefits to them and to other individuals or communities affected by the condition under investigation.

19. Every clinical trial must be registered in a publicly accessible database before recruitment of the first subject.

20. Physicians may not participate in a research study involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians must immediately stop a study when the risks are found to outweigh the potential benefits or when there is conclusive proof of positive and beneficial results.

21. Medical research involving human subjects may only be conducted if the importance of the objective outweighs the inherent risks and burdens to the research subjects.

22. Participation by competent individuals as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no competent individual may be enrolled in a research study unless he or she freely agrees.

23. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information and to minimize the impact of the study on their physical, mental and social integrity.

24. In medical research involving competent human subjects, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information. After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

25. For medical research using identifiable human material or data, physicians must normally seek consent for the collection, analysis, storage and/or reuse. There may be situations where consent would be impossible or impractical to obtain for such research or would pose a threat to the validity of the research. In such situations the research may be done only after consideration and approval of a research ethics committee.

26. When seeking informed consent for participation in a research study the physician should be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent should be sought by an appropriately qualified individual who is completely independent of this relationship.

27. For a potential research subject who is incompetent, the physician must seek informed consent from the legally authorized representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the population represented by the potential subject, the research cannot instead be performed with competent persons, and the research entails only minimal risk and minimal burden.

28. When a potential research subject who is deemed incompetent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorized representative. The potential subject's dissent should be respected.

29. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research population. In such circumstances the physician should seek informed consent from the legally authorized representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research should be obtained as soon as possible from the subject or a legally authorized representative.

30. Authors, editors and publishers all have ethical obligations with regard to the publication of the results of research. Authors have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. They should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results should be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest should be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

31. The physician may combine medical research with medical care only to the extent that the research is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

32. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best current proven intervention, except in the following circumstances:
• The use of placebo, or no treatment, is acceptable in studies where no current proven intervention exists; or
• Where for compelling and scientifically sound methodological reasons the use of placebo is necessary to determine the efficacy or safety of an intervention and the patients who receive placebo or no treatment will not be subject to any risk of serious or irreversible harm. Extreme care must be taken to avoid abuse of this option.

33. At the conclusion of the study, patients entered into the study are entitled to be informed about the outcome of the study and to share any benefits that result from it, for example, access to interventions identified as beneficial in the study or to other appropriate care or benefits.

34. The physician must fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never interfere with the patient-physician relationship.

35. In the treatment of a patient, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available.