# A comparison between motor point and transcranial magnetic stimulation for estimating voluntary activation prior to, during and following fatiguing elbow flexor contractions

By

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**Abstract**

The objective of this thesis was to compare changes in voluntary activation (VA) with two different stimulation techniques in non-fatigued and fatigued elbow flexors. Participants completed a single experimental session in which they preformed four sequences of three consecutive fatiguing contractions (fatigue block). Submaximal muscle contractions were performed in sets of four between fatigue blocks. The contractions began with a 100% effort followed by a 25%, 50% and 75% of MVC contraction in random order (VA block). All VA block contractions were sustained for 5 seconds. VA block was completed prior to the first fatigue block, post 5 and 10 minutes. Stimulations were delivered during the final of three successive fatiguing contractions in the fatigue block and during all_VA block contractions. Transcranial magnetic stimulation (TMS), Erb's point stimulation, and motor point stimulation were delivered to induce motor evoked potentials (MEP), maximal muscle compound action potential (Mmax), and potentiated twitch, respectively. VA decreased throughout the fatigue protocol with motor point stimulation and TMS but TMS was significantly underestimated because of lower estimated resting twitch forces than motor point stimulation. There was no change in triceps/biceps brachii electromyography, biceps/triceps motor evoked potential (MEP) amplitudes or bicep MEP amplitudes throughout the fatigue protocol. In conclusion motor point stimulation as opposed to TMS led to a higher estimation of VA in non-fatigued elbow flexors, therefore stimulation type has substantial effect on predictive equation validity when fatigue is incorporated. Additionally, as fatigue increased, potentiated twitch, EMG amplitude and force production progressively reduced.

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**Table of Contents**

**List of Figures**

**List of Symbols, Nomenclature or Abbreviations (in order of appearance)**

| | |
|---|---|
| ITT | Interpolated twitch technique |
| VA | Voluntary activation |
| MVC | Maximum voluntary contraction |
| TMS | Transcranial magnetic stimulation |
| PAP | Post activation potentiation |
| MP | Motor point |
| EMG | Electromyography |
| MEP | Motor-evoked potential |
| Mmax | Maximal compound muscle action potential |
| Mwave | compound muscle action potential |

**List of Appendices**

## Chapter 1: Review of Literature

### 1.1 Introduction

The Interpolated Twitch Technique (ITT), which includes stimulation at the motor cortex, peripheral nerve branch or muscle belly is used to determine the percentage of voluntary activation (VA) during voluntary contractions (Behm et al., 1996). Voluntary activation is defined as a proportional value related to the maximal possible force that can be produced during an isometric or slow dynamic voluntary effort (Gandevia et al., 1996). It is represented as a quantitative discrepancy between voluntary force production and maximum evoked force during the voluntary force production. Most individuals can voluntarily activate their elbow flexors up to and beyond 90% during a maximal voluntary contraction (MVC) (Gandevia et al., 1996). However, as they fatigue, VA substantially decreases (Gandevia et al., 1996). Furthermore, VA differs depending on the type of stimulation that is used and the anticipation of the stimulus (Gandevia et al., 1996;Button and Behm, 2008). Very few studies have compared motor cortex stimulation to muscle stimulation to estimate VA in fresh and fatigued muscle. The overall purpose of this thesis was to assess the estimation of VA of fresh and fatigued elbow flexors using transcranial magnetic stimulation (TMS) and motor point (MP) stimulation and to compare these methods. This review of literature will discuss the physiology of VA, methodology to assess VA and the effect of fatigue on VA.

### 1.2 Physiology

Within the primary motor cortex of the human brain there are neurones that synapse to the spinal cord through descending pathways. These descending motor neurones are sub categorized as upper and lower motor neurones. The upper motor neurones synapse with the

lower motor neurones, which project to skeletal muscle (Hodgson et al., 2005). The neurones that innervate the limbs decussate at the level of the medulla and are referred to as the lateral corticospinal tract. The distribution of axons along their respective pathways is 90% lateral (limb) and 10% anterior (proximal trunk) (Hodgson et al., 2005). These potentials from the primary motor cortex then innervate muscle fibers, creating voluntary muscle activation and human movement (Hodgson et al., 2005).

Muscle fibers, which are excited by the aforementioned descending signals, are a collection of cells that are surrounded by a cell membrane. The cell membrane contains several pumps and ion channels necessary to sustain a negative resting membrane potential (Huxley, 1974). The cells within these muscle fibers contain voltage gated ion channels required for generation of an action potential. The membrane potential of these cells at any time is a function of the net electrochemical gradient of ions, to which the membrane is permeable. When intra-cellular and extra-cellular concentrations are static, it is referred to as equilibrem potential (Huxley, 1974). When electrical stimulation is delivered via motor point or nerve root during a voluntary contraction, the dormant motor units not firing through voluntary activation are activated by the secondary efferent input. During electrical stimulation, motor units operating with a submaximal rate coding can also have an increase in firing frequency leading to a greater activation of the muscle (Z'Graggen W et al., 2011).

There is a change in muscle activation that occurs following a sustained contraction, which is known as post activation potentiation (PAP). PAP is defined as an increase in maximum electrically evoked twitch following brief, non-fatiguing maximal contraction. This can be observed during voluntary force production as well as evoked force production via stimulation (Hodgson et al., 2005). PAP influences the mechanical performance of muscle contractions

based on the contractile history of a muscle (Lorenz, 2011). In other words, the force produced by a muscle is increased because of its previous contraction.

Physiologically, PAP stems from two main sources: 1) phosphorylation of myosin light chains (Grange et al., 1993;Sweeney et al., 1993) and 2) changes in calcium kinetics (Ismailov et al., 2004). The two mechanisms work in conjunction with one another. It is the phosphorylation of myosin regulatory light chains that leaves actin-myosin sensitive to calcium released from the sarcoplasmic reticulum during subsequent muscle contractions. These factors increase force production of a muscle. Vandervoort and colleagues (1983) demonstrated that the intensity of contraction correlated with the onset of post-activation in a linear fashion during an MVC lasting up to 10 seconds. Beyond this point fatigue manifestation lead to a depression in PAP (Behm et al., 2004). They also showed that submaximal contraction of brief duration can induce PAP. Regarding the length of the effect of PAP on the muscle, it has been reported to last from as little as 5, up to 35 minutes (Vandervoort et al., 1983;Chiu et al., 2003). PAP is a component of VA research that needs to be taken into consideration by investigators in the development of research methodology. Its effect on the muscle being tested must be monitored during testing and accounted for in data analysis on account of its potential influence on voluntary force production.

### 1.3 Measurement of Voluntary Activation

Voluntary activation is defined as a proportional value related to the maximal possible force that can be produced during an isometric or slow dynamic voluntary effort (Gandevia et al., 1996). A secondary more descriptive definition is the complete muscle activation an individual can achieve relative to complete activation elicited via superimposed stimulation. This measure is quantified by the ITT. The ITT can be defined as the delivery of stimulation during voluntary contraction in attempt to assess a participant's ability to create high levels of muscle activation;

the discrepancy between voluntary effort and a stimulus evoked twitch force during voluntary effort provides this information (Merton, 1954). It is assessed via a single supramaximal stimulus to the motor nerve during a sustained contraction, followed by a second stimulation delivered to the same muscle during rest directly following the completion of the contraction (Denny-Brown and Liddell, 1928;Merton, 1954). The interpolated twitch technique was investigated, defined and applied after Denny Brown by Merton in his assessment of muscle activation (Denny-Brown and Liddell, 1928;Merton, 1954). Merton's research affirmed that as muscle contraction intensity increases, the superimposed stimulus-evoked twitch force decreases. Thus the twitch force response diminishes until ultimately disappearing in a fully activated muscle (Merton, 1954;Belanger and McComas, 1981;Herbert and Gandevia, 1999). If the superimposed stimulus evokes an increase in force, the stimulated axons were not all voluntarily recruited or their firing rate was submaximal (Belanger and McComas, 1981;Herbert and Gandevia, 1999). To determine the level of VA, the stimulus responses are expressed as a ratio of the superimposed stimulus-evoked twitch force and the resting potentiated twitch force following the voluntary contraction. This is referred to as the ITT. This ratio can be applied to the formula: VA (%) = [1 – (superimposed twitch/potentiated twitch)] x 100(Shield and Zhou, 2004)This equation allows for VA to be expressed as a percent of maximum force producing potential.

Motor point (MP) stimulation in the context of biceps brachii refers to direct stimulus of the muscle through two electrodes adhered to the skin. One over the distal musculotendinous junction of the biceps, and the other adhered to the medial side of the upper arm half way between the proximal and distal insertion of the short head of biceps brachii. MP stimulation represents properties of the muscle at rest in absence of an effect from changes at the supraspinal, spinal, or proximal nerve level (Behm et al., 2002) or changes at the supraspinal,

spinal, or proximal nerve level during a voluntary contraction. Thus, MP stimulation cannot determine the central nervous system level at which fatigue is occurring, whether it be supraspinal or spinal or a combination thereof. However, other stimulation techniques can be used in conjunction with MP to assess central nervous system fatigue and changes along the corticospinal tract such as transcranial magnetic stimulation, which will be discussed later.

### 1.4 Relationship between force and VA

Since the ITT ratio was used by Merton (Merton, 1954), multiple muscles have been the focus of research incorporating his technique using MP stimulation. Just as the technique has been tested across various muscles, the relationship between voluntary and stimulus evoked contraction has also been researched. Particularly how changes occur with increasing contraction intensity. The simplest way to make inter-study comparisons regarding the relationship between voluntary and stimulus evoked contraction is through a graph of the two measures. The following section describes a graph of the curvilinear relationship that develops between these two measures as contraction intensity increases (Behm and Sale, 1993). The high contraction intensities make up the portion of the graph with the highest tangential slope, the moderate contraction intensities make up the central/average portion, and the lower contraction intensities make up the portion of the graph with the lowest tangential slope. These trends have an effect on the use of MP stimulation to estimate and assess VA.

It has been shown that at high contraction intensities, the graphical representation of force and VA becomes more vertical. This was the case in the work of Belanger and McComas (Belanger and McComas, 1981) in their research assessing ankle dorsiflexors, plantar-flexors and tibialis anterior. Their research consisted of 28 individuals seated in an apparatus designed to assess torque in two fixed ankle positions, 10 degrees of dorsiflexion and 20 degrees of plantar

flexion. Stimulations were delivered at 200 to 500 microsecond durations. The researchers demonstrated the difference in complete muscle activation capability of the dorsiflexors and plantar flexors . Specifically the relative ease with which complete activation was achieved in tibialis anterior compared to the plantar flexors (Belanger and McComas, 1981) The research highlights how the relationship is varied between muscles and contraction intensities, specifically the plantarflexor and dorsiflexor groups.

Similar conclusions regarding the curvilinear graphical relationship were established by Behm, St-Pierre and Perez (Behm et al., 1996). Their research focussed on the accuracy of predicted MVC and its dependency on the intensity of the actual voluntary contraction used as a predictor. When 40% MVC was used, a margin of error of 33.3% was present in the prediction of MVC, and when voluntary contraction intensities of less than 80% of MVC were used there was a 13-16% discrepancy of the same measure. This shows us the importance of achieving high intensity submaximal contractions in the prediction of estimated MVC.

Behm and colleagues (Behm et al., 1996) also investigated the validity and reliability of the ITT when measured in the plantar flexor and leg extensor muscles. Inactivation of the muscle was investigated by comparing evoked contractions forces with submaximal and maximal voluntary contraction forces. The researchers compared EMG to induced torque forces to assess which stimulus (singlet, doublet, quintuplet) was the most effective to determine discrepancy between voluntary activation and stimulus induced maximal contraction. Behm and colleagues(Behm et al., 1996) state that even at the appropriate stimulus and contraction intensity, an interruption in background EMG does affect the validity of assessing increase in activation with superimposed stimulation (Behm et al., 1996). Their results show that there was no significant difference in the ITT sensitivity when singlets, doublets, or quintuplets were used.

This specific finding was considered in the selection of what stimulus was most appropriate for our research.

The curvilinear nature of the relationship has been affirmed at higher contraction intensities. For example, Bulow and colleagues (Bulow et al., 1993), stated that when assessing muscle force, it is necessary to use a 75% of maximum contraction intensity to achieve a sufficiently accurate prediction. Their protocol consisted of 7 different experiments all of which had different initiatives. The experiments included effects on twitch size of potentiation, time lag after potentiation, magnitude of voluntary force, stimulus amplitude, stimulus duration, angle of the knee, and angle of the hip. Their results also show that a curvilinear relationship was present between the size of the superimposed twitch and voluntary force produced by the subject in the absence of stimulation. However, this relationship between twitch size and the force's linearity only developed when the contraction intensity exceeded 25% of maximum (Bulow et al., 1993).

MP stimulation has also been used to extrapolate the estimated maximum force producing potential of a muscle. This process involves using MP stimulation during submaximal contractions to draw a regression line to the Y intercept of a graph plotting stimulus evoked muscle contraction and submaximal voluntary contraction, thus creating estimated maximal force production. The limitations of this method are closely related to the above-mentioned conditions under which the relationship can be curvilinear at various contraction intensities. An example of how this technique limits estimation is Dowling and colleagues (Dowling et al., 1994). Their methodology consisted of three brief initial MVC's followed by target contraction intensities, assigned in 10% increments from 0 to 100% of MVC. Participants were instructed to match the displayed intensity in a random sequence with several minutes of rest between contractions to mitigate the effects of fatigue. During each 5-second contraction five MP doublet

stimulations were delivered at even time increments. Five contractions were completed between the contraction intensities 30%-60% of MVC, and ten contractions were completed between the contraction intensities 70%-100%. Dowling's results yielded an estimated curvilinear graph that did not cross the Y-axis. They state that this issue can lead to an overestimation of true maximal force producing potential.

### 1.4 Role of synergists and antagonists in VA

A potential limitation of MP stimulation as an assessment of VA is the inability of the stimulus to create activation of synergists that will contribute to force production at high voluntary contraction intensities. This added voluntary force can contribute to the non-linear relationship (Allen et al., 1998). An example of this was in the work of Allen and colleagues (Allen et al., 1998) who assessed factors affecting maximal voluntary torque and the assessment of the level of voluntary drive in the elbow flexor muscles by completing three different studies. All their experiments began with a brief MVC, superimposed twitch and potentiated twitch to assess VA and peripheral fatigue. Their first study focussed on VA under varied stimulus conditions (single, paired or train of four stimuli) at high voluntary contraction forces. Thirty brief maximal contractions were performed followed by a stimulus to the resting muscle followed by a contraction 10 seconds later at 25%, 50%, 75%, 90% or 100% of the previously tested MVC. The effect of the different stimulus sequencing was established by delivering either the single, paired, or train of stimuli in random order after 3 seconds of sustained contraction had elapsed (Allen et al., 1998). Their second study focussed on the activation of brachioradialis during elbow flexion at high voluntary forces using the ITT. This was assessed by having subjects attempt 42 maximal contractions separated by 2 minutes with stimuli delivered to the biceps or brachioradialis in random sequence. The 2 minute rest was in effort to mitigate fatigue.

This information was used to investigate the contribution of synergist muscles in elbow flexion (Allen et al., 1998). Their third protocol focussed on the effect of changes in muscle length during elbow flexion. This was indirectly assessed by measuring vertical shoulder displacement during isometric elbow flexion. This measure was assessed using a magnetometer during 15-20 brief MVC's. The culminating results of the three projects show that voluntary force increases were in absence of voluntary activation, the increase in force was not due to an increase in voluntary activation. They showed that small increases in muscle length and contribution of synergists at high intensity contractions were secondary contributors to the increase in force production(Allen et al., 1998), it was not attributed to an increase in VA of the muscle. Another related finding of this research was that across a variety of MP stimulation protocols at high forces, a curvilinear relationship developed when both a single stimulus and a paired supramaxamal stimulus were used.

During assessment of VA of the elbow flexors, the effect of the antagonist triceps brachi muscle group also needs to be considered. When comparing voluntary to evoked contraction we must not mistake submaximal force production of the elbow flexors for activation of the antagonist triceps brachii, especially at high contraction intensities when we are most likely to see co-activation of the agonist and antagonist (Allen et al., 1995). In addition to the assessment of force production, assessment of EMG of antagonist musculature is essential when investigating VA of the elbow flexors. This measure acts as a control factor when assessing neural drive to the muscle. If EMG of the antagonist is controlled for, it allows the researcher to attribute changes in VA to changes in neural drive to the agonist elbow flexor with greater confidence (Bigland-Ritchie et al., 2000).

### 1.5 Intermuscular differences

The ability of a single muscle or muscle group to achieve complete voluntary activation has been attempted with varying outcomes. Near Complete muscle activation of the quadriceps has been previously shown (Rutherford et al., 1986;Phillips et al., 1992;Rice et al., 1992), as has an inability to achieve complete activation of the quadriceps (Belanger and McComas, 1981;Strojnik, 1995;Kalmar and Cafarelli, 1999;Urbach and Awiszus, 2000). This outcome variability was also present in the investigation of the plantar flexors where some research groups were able to demonstrate complete activation (Behm and St-Pierre, 1997a;b) and while others were not (Behm and St-Pierre, 1997c). In addition to this across study conflict, inter-subject variability of the plantarflexors and dorsiflexors has been assessed (McComas et al., 1983).

These conflicting results lead Behm and colleagues (Behm et al., 2002) to conduct a comprehensive experiment on the most commonly assessed muscles in VA research, the knee extensors, elbow flexors, plantar flexors and dorsiflexors. VA was assessed using the ITT with two evoked doublets delivered at 1.5 second intervals during submaximal and maximal contractions that lasted 4 seconds. The submaximal contraction intensities were 25, 50, and 75% of MVC. No more than 3 trials were completed per muscle group per contraction intensity. Following the completion of each contraction, a potentiated doublet was recorded 1.5 s after the completion of each voluntary contraction. Their results demonstrated that quadriceps were significantly more inactive compared to plantar flexors, dorsiflexors, and elbow flexors. Plantar flexors and elbow flexors were comparable in levels of VA, and dorsiflexors reached the greatest level of VA. The recorded activation levels were 84.5%, 95%, 95% and 98.7% for the quadriceps, plantar flexors, elbow flexors and dorsiflexors respectively. The researchers did not

attribute the significant discrepancy to a greater number of motor units in the quadriceps remaining inactive. It is stated that the estimated number of quadriceps motor units is not significantly variable in comparison to those innervating the other muscles of study (Rich et al., 1998;Lemmer et al., 2001).

There is evidence that subjects can reach complete muscle activation of the elbow flexors (Lloyd et al., 1991;McKenzie and Gandevia, 1991;De Serres and Enoka, 1998). However, this statement is based on research that focussed on individuals outside of the general population, making it challenging to draw comparisons. De Serres and colleagues studied older individuals (De Serres and Enoka, 1998), Loyd and colleagues studied individuals with Chronic Fatigue Syndrome (Lloyd et al., 1991). There are examples of research documenting complete voluntary muscle activation of the elbow flexors in healthy individuals. One example is Mckenzie, Gandevia and colleagues comparison of fatigue in the limb musculature versus the diaphragm musculature (McKenzie and Gandevia, 1991). Mackenzie and colleagues demonstrated the presence of central fatigue and a previously unreported fatigue resistance of the diaphragm (McKenzie and Gandevia, 1991). Another is Gandevia's work on short muscle lengths during MVC that yielded complete muscle activation (Gandevia and McKenzie, 1988). A third example of complete muscle activation was during trials by all participants in the work of Allen and Gandevia (Allen et al., 1995) in their assessment of the reliability of the ITT. They concluded that although all participants achieved complete muscle activation, it was only achieved in 25% of contractions and stated that this inability was not due to antagonist co-contraction (Allen et al., 1995).

Motor point stimulation acts as an assessment tool in the investigation of the relationship between force and VA as it pertains to various levels of contraction duration and intensity.

However, it is limited in its ability to assess the location of change along the corticospinal tract. Technological advancement has allowed researches to partially circumvent this issue through the use of transcanial magnetic stimulation. Stimulation at the cortex through TMS, and stimulation at the muscle belly through MP stimulation allows researchers to decipher not only that change has occurred, but in part, the site of said change.

### 1.6 Transcranial Magnetic Stimulation (TMS)

TMS stimulates regions of the motor cortex without being physically invasive to the participant while still delivering substantial transsynaptic activation of corticospinal neurones (Brakemeier et al., 2008;Carroll et al., 2009). During the stimulation, a coil producing a magnetic field is placed in contact with, or near the skull of an individual. The coil is connected to a pulse generator, or stimulator, that delivers electrical current to the coil (Lisanby and Belmaker, 2000). The stimulator produces small magnetic currents, which are intended to stimulate the specific region of the brain being researched, through electromagnetic induction. The goal of the stimulus delivery is to elicit a motor evoked potential (MEP) in a given muscle. A MEP is a distinguishable action potential in EMG in a given muscle evoked via TMS. It can be characterized by multiple factors including the appropriate latency (time from stimulus artifact to the start of the action potential), and its wave like configuration, as well as the duration of the silent period that follows the MEP response. The silent period following the wave like response to TMS is recognizable as a relative absence of EMG activity in the muscle following stimulation and is physiologically due to the creation of intercortical inhibition by the stimulus (Wilson et al., 1993). Quiescence of the trace is noticeable for approximately 100ms.

Motor cortex stimulation has been used to help specify where changes in corticospinal excitability along the corticospinal spinal track of fresh and fatigued muscle occurs (Todd et al.,

2003) and is compared to the maximal compound muscle action potential via Erb's point stimulation to account for potential changes in the peripheral nervous system. Erb's point stimulation is elicited by placing a cathode electrode in the subclavicular fossa of the participant. The anode electrode is placed approximately 1cm lateral to the AC joint on the same side of the body. This electrode placement aims to stimulate the brachial plexus. The response elicited in the muscle is called a compound muscle action potential (Mwave). The Mwave represents a peripheral response in the muscle to stimulation of the brachial plexus. Mwave is the response to stimulation represented through EMG, Mmax is the optimized response where stimulation intensity elicits the largest Mwave amplitude.

Stimulating the brachial plexus is used to establish non-fatigue or fatigue-induced changes in the nervous system at the peripheral level. It is commonly used as a comparator to stimulation at the corticospinal level to assess changes in corticospinal excitability (Palmer and Ashby, 1992). This is achieved by normalizing the MEP to Mwave, allowing for the influence of peripheral excitability on corticospinal excitability to be accounted for.

Because it cannot be assured that all motor units are activated via cortical stimulation, a MEP amplitude of 80% of Mmax is considered to represent a large number of firing motoneurones (Hess et al., 1987). However, this large value does not represent optimal activation level of the agonist and antagonist. It has been reported that the optimized intensity for cortical stimulation is an intensity that elicits large MEP in the agonist (>50%Mmax) and a small MEP in the antagonist (<20%Mmax) while maximal voluntary contraction is maintained for a brief period (Matthews, 1999). These percent measurements are a comparison of Mmax and MEP amplitudes. This comparison assures that we can attribute the EMG representation to

corticospinal input and not peripheral input from the nerve or neuromuscular junction. This was described by Matthews and colleagues through a motoneurone model (Matthews, 1999).

In addition to measuring corticospinal excitability, TMS can also be delivered during a muscle contraction to stimulate the motor cortex as a measure of motor cortex voluntary activation. Delivering TMS to a participant during a MVC attempts to provide an assessment of complete force producing potential via supraspinal output(Gandevia, 2001) which is similar to that of MP stimulation. The manner in which a muscle responds to TMS can be subject to external stimuli, and excitability of the motor cortex (Gandevia, 2001). Different levels of excitability can create variability in the motor cortex and subsequent responses in the muscle of study (Carroll et al., 2009). Thus, it is essential to achieve optimal environmental control during testing. However, it is inappropriate to use the same fractional expression of VA for TMS as is used for MP stimulation. Response to TMS in a resting muscle evokes less cortical output and subsequently very little force from the muscle when compared to the same stimulus delivered during activity. This is due to the decreased number of motor units firing in response to the stimulus when the muscle is at rest (Di Lazzaro et al., 1999). Because of this, when stimulating the motor cortex an alternate method for assessing VA must be used.

When stimulating the motor cortex, linear regression equations provide an alternative estimation of resting twitch force (Di Lazzaro et al., 1999;Todd et al., 2003;2004). This is required because corticospinal and motor cortical neurons are less excitable during rest when compared to muscle contraction. This linear equation is derived from motor cortical stimulation delivered at a variety of contraction intensities between 50% and 100% of maximal voluntary contraction. This specific protocol was outlined by Todd and colleagues(Todd et al., 2004) in their work in the early 2000's on elbow flexor response to cortical stimulation. The

superimposed cortical stimuli are graphed in order of contraction strength from lowest to highest and a regression line is then drawn to intersect the Y-axis. This point of intersection is then deemed the estimated resting potentiated twitch as elicited via TMS. This value is now inserted in to the VA equation (VA% = [1 – (superimposed twitch/potentiated twitch)] x 100) as potentiated twitch to produce a percent VA assessment via transcranial magnetic stimulation (Todd et al., 2004). The rationale for comparison across these measures is the fact that during brief non-fatiguing contractions, response to superimposed motor cortical stimulation decreases as voluntary contraction intensity increases. This is due to an increased percentage of motor units being activated voluntarily (Hunter et al., 2008).

### 1.7 Comparison between MP Stimulation and TMS for VA

In non-fatigued muscles when the agonist and antagonist MEP amplitude and MEP to Mmax ratio parameters outlined above are met, a tight correlation between TMS and MP stimulation as predictors of VA have been reported (Todd et al., 2003;2004). This linear relationship between these two measures of voluntary activation was specifically shown at contraction intensities above 50% of maximum voluntary effort (Todd et al., 2003;2004). In individuals with successful cortically evoked estimated voluntary activation, the inter-day repeatability of this measure is extremely high. It is comparable to measures of VA derived from motor point stimulation. However, when compared to the within session repeatability of cortical stimulation versus MP stimulation, cortical stimulation provides a less consistent measure (Todd et al., 2004).

Another key component of comparing MP stimulation and TMS is how the two stimuli evoke a physiological response during rest versus during submaximal contraction, as well as how stimuli differ in their ability to assess VA of the upper versus lower extremities. As previously

described this comparison must take into account the necessity of estimation of resting twitch with TMS due to the stimulus' inability to assure activation of all motor units being investigated Todd and colleagues (Todd et al., 2016) highlighted the difference between TMS and MP stimulation in their review paper stating that with three contractions between 50 and 100% of MVC, TMS estimates resting twitch amplitude at 16.6 +/- 3.5% of MVC while MP stimulation evokes a resting twitch at 10.6 +/-4.0% of MVC (Todd et al., 2016). The researchers attribute the increased percent estimated twitch to the nonspecific nature of TMS and its congruent stimulation of brachialis in addition of the biceps brachii.

Another comparison of TMS and MP stimulation is how these stimuli assess the upper versus lower extremity. A comprehensive breakdown of the upper and lower limb using both stimuli is challenging as MEP response in the lower limb is difficult to quantify due to inconsistency in obtaining a small antagonist MEP (Todd et al., 2016). Regardless, estimated resting twitch evoked via TMS in the elbow flexors is 23+/-8% of MVC while estimated twitch evoked via TMS in the knee extensors is 21+/-9% of MVC. These measures are averages drawn from 13 and 10 studies respectively. In contrast the resting twitch evoked by MP stimulation of the femoral nerve in these studies was 25+/-10% MVC and MP stimulation of the elbow flexors was 10.6 +/-4.0% of MVC (Todd et al., 2016). Of all the studies this information was drawn from in their review, only three demonstrated an equivalent MP evoked resting twitch and TMS evoked estimated resting twitch regardless of whether the upper or lower limb was assessed. In all other instances the estimated resting twitch force from TMS was smaller. This indicates a clear underrepresentation of resting twitch via TMS, making MP stimulation more representative of resting twitch when assessing the upper and lower limb (Todd et al., 2016).

The following is an example of research that incorporates the above information into multiple projects with the intent to compare and investigate TMS and MP stimulation. The research is entitled Supraspinal factors in human muscle fatigue: evidence for suboptimal output from the motor cortex by Gandevia, Allen, Butler and Taylor (Gandevia et al., 1996). The muscle of investigation, stimulations used, and participant population are highly comparable to the research this review of literature surrounds.

This work was broken down into two experimental sessions, one session focussed on TMS, the other on MP stimulation. Across both sessions 8 participants were studied. The first session used MP stimulation, subjects preformed 3 brief MVC's for 2-3 seconds separated by 1 min. During and 5 seconds following each contraction biceps stimulation was delivered. After the completion of the short duration MVC's a 3 minute maximal voluntary contraction was sustained, during which a stimulus was delivered every 10 seconds. At rest following the contraction 3 stimulus were delivered within a 15 second window. The investigation using TMS in the second experimental session followed a very similar protocol. However, the brief 2-3 second non-fatiguing contractions were in sets of 5 in comparison to sets of 3 in the MP stimulation session, the 1 minute rest period was maintained as before. The sustained fatiguing contractions were 2 minutes in duration rather than 3 during which TMS was delivered every 10 to 15 seconds. The post contraction brief MVC's were 30 seconds, 1, 2, and 3 minutes post contraction. During each of these brief contractions TMS was delivered (Gandevia et al., 1996).

Their results indicated that the sustained contraction decreased to 25.9 +/- 8.6% of the initial peak voluntary force. The stimulation delivered immediately post contraction to the muscle at rest generated an evoked force of 29.5 +/- 5.1%. This depicts a decline in force attributed to peripheral fatigue. The component of the study using transcranial magnetic

stimulation saw participants decrease their voluntary force producing potential to 39.2 +/- 9.1% of initial peak production from control MVC's. TMS evoked force production in the resting muscle post contraction reached 9.8 +/- 8.3% of peak force. This research is closely aligned with our project due to the incorporation of fatigue and a comparison of the TMS and MP stimulation.

### 1.8 Limitations of TMS and Motor Point Stimulation for Predicting Voluntary Activation

TMS is unable to evoke firing in all desired motor units, making it inappropriate to compare TMS and MP stimulation under the same conditions (Hess et al., 1987;Thomas et al., 1989;Ugawa et al., 1995;Di Lazzaro et al., 1999). TMS does not exclusively activate the cortical representation of the desired agonist muscle. The general nature of TMS stimulates the representation of multiple muscles in the motor homunculus, including the antagonist muscle (Palmer and Ashby, 1992). In addition, how effective TMS is in completely activating the motoneurone pool is uncertain. MP stimulation is also limited in its ability to create complete muscle activation, a large current may be needed to activate deeper fibres. This has the potential to create conduction to the antagonist muscle and inhibit agonist force production.

The relationship between voluntary activation and evoked activation or force production is not only expressed through linear regression. It can also be expressed through polynomial and exponential functions (Bulow et al., 1993;Dowling et al., 1994;Behm et al., 1996). A problem occurs when trying to decide which method to use, as they typically provide different results. There is substantial disagreement as to which method is the most appropriate (Bulow et al., 1993;Dowling et al., 1994;Behm et al., 1996;Allen et al., 1998;De Serres and Enoka, 1998;Herbert and Gandevia, 1999;Scaglioni et al., 2002). Criticism of the ratio ITT as an assessor voluntary activation stems from its inability to address non-linearity of the evoked-

voluntary relationship. However, the validity of the extrapolation is more challenging than the ratio (Allen et al., 1998;Herbert and Gandevia, 1999). Multiple reasons can lead to non-linearity such as muscle synergists or a small increase in muscle length during contractions near-maximal and maximal intensity, which could increase force independently of the voluntary activation. These factors only affect the extrapolation method as the ratio method is constructed by scaling-evoked forces of the superimposed and control responses.

Technique selection is not the only aspect of VA research that needs to be addressed as a limitation. The ITT has inherent limitations as outlined by Shied and Zhou (Shield and Zhou, 2004). Site of stimulation is the first component. Appropriate electrode placement is pertinent to stimulation of the intended site, if electrodes are improperly positioned there is potential for activation of antagonist and synergist muscles that can lead to an under or over representation of force production respectively (Gandevia and McKenzie, 1988). Inappropriately sized or spaced electrode pairs can also exacerbate this issue.

Selection of stimulation intensity is another potential erroneous aspect of the ITT. In an effort to avoid the above-mentioned synergist and antagonist stimulation, researchers may elect to deliver submaximal stimuli. However, this may lead to varying muscle fibers being stimulated at different times or parts of an experimental session as body position/joint angle affect the section of the muscle the electrodes adhered to the skin innervate.

Another limitation of the ITT is the signal to noise ratio and its influence on the number of successive stimuli used in a protocol. Single stimuli have been reported to yield higher variability in force increments across multiple trials (Suter and Herzog, 2001). Because of this they have been frequently replaced by doublet and trains of up to 4 stimulations 10 milliseconds

apart (Suter and Herzog, 2001). The appropriate stimulus sequencing is another important potential limitation to consider. Unfamiliarized participants are often unable to preform consistent maximal isometric contraction, resulting in underestimation of their voluntary activation. Adequate time for a participant to become familiar with the skill of achieving an isometric MVC and overcoming the detrimental anticipation of the stimulus (Button and Behm, 2008) is essential to accurate use of the ITT.

All of the above are markers that can be addressed in methodology construction however it takes conscious effort on behalf of a research team to tease out the potential flaws that limit this particular technique. Regardless of which technique is used, whether it be the ratio or the relationship method, there are limitations and debate on how to correctly quantify muscle activation using the ITT method (Shield and Zhou, 2004).

### 1.9 Neuromuscular Fatigue

Fatigue is an activity-induced reduction in one's maximal capacity for strength or power, regardless of one's ability to continue in the task they are performing (Bigland-Ritchie et al., 1983). Fatigue can be attributed to changes at multiple levels of the central and peripheral nervous systems or the muscle itself. Sites include the supraspinal circuits, the spinal level (e.g., motoneurone), neuromuscular junction, and the muscle fiber (Taylor et al., 2000). The challenge in determining where fatigue has occurred is outlining where along the brain to muscle pathway physiological changes occurred and how these changes contribute to reduced force production (Taylor et al., 2000). In terms of the muscle the physiological changes that occur with fatigue include metabolite accumulation (Fitts, 1994), substrate depletion, altered excitation contraction coupling due to electrolyte accumulation (McLester, 1997) and impairment of Ca++ release and uptake (Sjogaard, 1996) as well as mechanical damage to the ultrastructure (Proske and Morgan,

2001). Afferent changes in the peripheral receptor also contribute to decreased force production, examples include partial/altered input from the tendon organ, muscle spindle(Burke et al., 1978) and group III and IV afferents (Garland et al., 1988). For a comprehensive list of the physiological mechanisms of neuromuscular fatigue see Gandevia review (Gandevia, 2001).

Changes at the corticospinal level can also be responsible for fatigue as described by Sieck and colleagues (Sieck and Prakash, 1995). Changes at the corticospinal level are also responsible for a drop in force production during fatiguing exercise (Bigland-Ritchie et al., 1983), confirmed by the fact that during sustained contraction the increase in fatigue and decrease in force production, are apparent with an increase in force production evoked through cortical stimulation (Gandevia et al., 1996). This increase in force signifies a reduction in optimal supraspinal drive to the muscle due to the onset of fatigue (Todd et al., 2003). Another indicator of central fatigue would be if the force due to superimposed nerve stimulation increases when expressed relative to potentiated nerve stimulation delivered at the same intensity in the resting muscle post contraction (Thomas et al., 1989;Lloyd et al., 1991). When these values are inputted into the VA equation (previously discussed), a decrease in voluntary activation occurs as fatigue increases (Gandevia et al., 1995).

According to Todd and colleaguues (Todd et al., 2003), regardless of fatigue state, both cortical stimulation and nerve or muscle stimulation provide a linear, repeatable measure of voluntary activation (Todd et al., 2003). This statement is not universally accepted in the literature, it has been found on multiple occasions that nonlinearity develops more frequently with fatigue onset in comparison to the non fatigued state when TMS is the stimulus used to assess VA (Hunter et al., 2008) (Kennedy et al., 2013) (Hunter et al., 2006). However, researchers have been able to work around this issue, an example of this is Hunter and colleagues

(Hunter et al., 2006). They excluded estimated resting twitches derived from linear regressions with a correlation coefficient less than 0.9. Necessary exclusion of participants due to non-linearity under the same measure was reported by Thomas and colleagues (Thomas et al., 2015) who excluded two of 13 participants due to an insufficient agonist MEP during knee extension contractions. Another example of the same issue was van Duinen and colleagues (van Duinen et al., 2010) who excluded an eighth of their participants for non linear representation of a resting twitch found through regression of TMS stimulation. The interruption in assessment of resting twitch is not as apparent when MP stimulation is used in comparison to TMS (Hunter et al., 2006;Hunter et al., 2008;Kennedy et al., 2013;Keller-Ross et al., 2014) The limitations of TMS versus MP stimulation are discussed by Gandevia (Gandevia, 2001) who describes the limited ability of TMS to accurately assess incremental changes in force production. The researcher cites the general nature of TMS and its potential to activate synergist as the cause of inaccuracies. This is an issue that does not arise during assessment of fatigue via MP stimulation.

This study was broken into two components/studies. It was organized similarly to the Gandevia work outlined at length previously in this review (Gandevia et al., 1996). One of the studies was comprised of two experimental sessions. The first experiment of the study was an assessment of unfatigued elbow flexors using motor cortex and motor nerve stimulation. Subjects completed control MVCs that were 1-2 seconds in length. These were used to establish submaximal contraction strength norms at 25%,50%,75% and 90% of MVC. Across these different contraction intensities subjects completed 40 contraction pairs with 1-2 minutes rest between pairs. The pairs consisted of a brief MVC, followed 8 seconds later by a contraction at one of the pre-established submaximal intensities, The 40 contractions were 4 groups of 5, one for each of the four submaximal contraction intensities.

The second experiment of the protocol followed similar parameters however, the two stimulations were motor cortex and brachial plexus stimulation with the intention to investigate the effect of stimulus strength on superimposed twitch via TMS. In this condition the MVC's were now held until the subject fatigued to 60% of the force production achieved when fatigue was not present. In this second experimental session three short duration control MVC's were completed, 50 pairs of contractions were then done with as little rest as possible between sets to keep the level of fatigue as high as possible. Eight seconds later, a contraction of 1-2 seconds was held at 25,50,75 or 90% of that produced by the fatigued muscle. During each 1-2 second contraction either a nerve or cortical stimulation was delivered depending on the trial. Five sets of contractions were completed at each stimulation intensity, for both conditions. This leaves 25 pairs of contractions for each stimulation type. After approximately 2 minutes of rest another 30 pairs of contractions were completed so stimulus could be delivered during sustained contraction.

Their results indicate that when the muscle was not fatigued (first study), both superimposed nerve stimulation and superimposed TMS response increased as VA decreased. The relationship between contraction strength and twitch amplitude was curvilinear for nerve stimulation and linear for cortical stimulation. This was only the case for TMS when the contraction strength was between 50 and 100% of MVC. At these intensities the evoked twitch was larger for TMS compared to nerve stimulation.

In the second fatigue inducing protocol, resting nerve stimulation twitches decreased 43% from pre to post, this represented peripheral fatigue. Central fatigue was also apparent in the protocol due to an increase in superimposed twitch during maximal efforts. This was seen in both

nerve stimulation and TMS. In both the fatigued and unfatigued muscle, superimposed twitch size decreased as voluntary contraction increased for both types of stimulation.

When motor cortical and motor nerve stimulation were plotted on a graph of voluntary activation, the motor cortical stimulation was linear and the motor nerve stimulation was curvilinear and biased towards higher levels of voluntary activation. The shape of the curve was consistent across both fatigued and non-fatigued conditions. During maximal efforts in the unfatigued condition, motor cortical and motor nerve stimulation yielded moderate voluntary activation levels (motor cortex: 93.6 ± 5.6 %; motor nerve: 97.4 ± 2.1 %). During fatigue the same measures were decreased, indicating central fatigue (motor cortex: 79.6 ± 13.0 %; motor nerve: 86.5 ± 9.4 %). At higher contraction intensities, it has been reported that MEP amplitudes remain unchanged, but that at 25-50% of MVC MEP size was decreased in a fatigued muscle compared to a non-fatigued muscle (Todd et al., 2003). Corticospinal excitability has also been shown not to decrease. Some potential physiological mechanisms of fatigue above the periphery have been identified such as intrinsic changes in motor neuron properties, and varied levels of afferent input depending on task demands (Hodgson et al., 2005).

## 1.10 Conclusion

There is an extensive body of research covering TMS, MP stimulation, neuromuscular fatigue and VA. The number of studies begins to thin when assessing these measures in conjunction with one another. The project this review accompanies is designed to fill a specific gap in this branch of neurophysiology research. The question we aim to answer is during sustained MVC, do TMS and MP stimulation provide comparable measures of voluntary activation regardless of fatigue level in the elbow flexors? The intent is to investigate this between stimuli and between actual versus estimated measures of resting twitch force. This

review covered a multitude of topics surrounding this goal. Specific aspects of VA research discussed included the physiology concepts related to VA, the methodologies used to assess VA, as well as limitations and the effect of fatigue on VA. Combing the current literature made it clear that very few studies have compared TMS to MP stimulation to estimate VA in a fresh and fatigued muscle group, leading to the research this literature review precedes.

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**Chapter 2: Co-authorship Statement**

My contributions to this thesis are outlined below:

1. I recruited all participants and analyzed all data collected for this thesis, with the help of my fellow masters' student Mr. Brandon Collins
2. With the assistance of Mr. Brandon Collins (masters' student), Mr. Lucas Stefanelli (masters' student), and Mr. Devin Philpot I collected the experimental data for this thesis.
3. I prepared the manuscript and thesis with the help and guidance of my supervisor, Dr. Duane Button.
4. Dr. Duane Button provided constructive feedback on the manuscript and the

**Chapter 3: A comparison between motor point and transcranial magnetic stimulation for estimating voluntary activation prior to, during and following fatiguing elbow flexor contractions**

**The following manuscript is presented as was accepted in the journal "Frontiers in Physiology".**

(a) Title of the article
Maximal voluntary activation of the elbow flexors is under predicted by transcranial magnetic stimulation compared to motor point stimulation prior to and following muscle fatigue.

(b) Authors' full names
Edward W.J. Cadigan, BKin[1] Brandon W. Collins, BKin[1] Devin T.G. Philpott, MSc[1] Garreth Kippenhuck, BKin[1] Mitchell Brenton, BKin[1] and Duane C. Button, PhD[1,2]*

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(d) Acknowledgments
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(h) Running title
Measures of Voluntary Activation

(i) Ethical publication statement
We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

(j) Disclosure of Conflicts of Interest
None of the authors has any conflict of interest to disclose.

**Abstract**

Transcranial magnetic (TMS) and motor point stimulation have been used to determine voluntary activation (VA). However, very few studies have directly compared the two stimulation techniques for assessing VA of the elbow flexors. The purpose of the study was to compare TMS or motor point stimulation for assessing VA in non-fatigued and fatigued elbow flexors. Participants performed a fatigue protocol that included twelve, 15s isometric elbow flexor contractions. Participants completed a set of isometric elbow flexion contractions at 100, 75, 50 and 25% of maximum voluntary contraction (MVC) prior to and following fatigue contractions 3, 6, 9, and 12 and 5 and 10 minutes post-fatigue. Force and EMG of the bicep and triceps brachii were measured for each contraction. Force responses to TMS and motor point stimulation and EMG responses to TMS (motor evoked potentials, MEPs) and Erb's point stimulation (maximal M-waves, $M_{max}$) were also recorded. VA was estimated using the equation: $\text{VA\%} = (1 - \text{SIT force/PT force}) \times 100$. The resting twitch was measured directly for motor point stimulation and estimated for both motor point stimulation and TMS by extrapolation of the linear regression between the superimposed twitch force and voluntary force. MVC force, potentiated twitch force and VA significantly ($p$<0.05) decreased throughout the elbow flexor fatigue protocol and partially recovered 10 minutes post fatigue. VA was significantly ($p$<0.05) underestimated when using TMS compared to motor point stimulation in non-fatigued and fatigued elbow flexors. Motor point stimulation superimposed twitch forces were significantly ($p$<0.05) higher at 50% MVC than TMS but similar at 75% and 100% MVC. The linear relationship between TMS superimposed twitch force and voluntary force significantly ($p$<0.05) decreased with fatigue. There was

no change in triceps/biceps electromyography, biceps/triceps MEP amplitudes or bicep MEP amplitudes throughout the fatigue protocol at 100% MVC. In conclusion, motor point stimulation as opposed to TMS led to a higher estimation of VA in non-fatigued and fatigued elbow flexors. The decreased linear relationship between TMS superimposed twitch force and voluntary force led to an underestimation of the estimated resting twitch force and thus, a reduced VA.

**Key Words:** Interpolated twitch technique, motor evoked potential, biceps brachii, triceps brachii, fatigue, isometric contractions.

## Introduction

Voluntary activation (VA) is the level of neural drive from the central nervous system to produce a given force output from a muscle. Examining how VA is estimated is important for quantifying the presence of central fatigue in clinical populations and for multiple research purposes (Taylor et al., 1996; Newham and Hsiao, 2001; Todd et al., 2003; Prasartwuth et al., 2005; Todd et al., 2005; Hunter et al., 2008; Cahill et al., 2011; Pearcey et al., 2015; Pearcey et al., 2016). The Interpolated Twitch Technique (ITT) was developed as a way to estimate central VA (Merton, 1954). The amplitude of an evoked superimposed twitch (SIT) force via an electrical stimulus to a nerve during a muscle contraction was expressed as a percentage of the amplitude of an evoked twitch force following the contraction when the muscle was at rest and in a potentiated state (Belanger and McComas, 1981). Transcranial magnetic stimulation (TMS) has also been used to estimate VA (Gandevia et al., 1996; Todd et al., 2003; 2004; Sidhu et al., 2009a). Due to recruitment of very few motor units (Hess et al., 1987; Ugawa et al., 1995; Di Lazzaro et

al., 1998), TMS evokes a low amplitude potentiated twitch (PT) at rest following a muscle contraction. Therefore, a method was developed by linearly extrapolating the regression between TMS evoked SIT forces of submaximal voluntary contractions and MVCs to estimate a TMS-induced resting PT (Todd et al., 2003). Central and, in part, cortical VA can then be estimated by expressing a TMS evoked twitch force during a contraction as a percentage of the estimated PT at rest (Todd et al., 2003; 2004; Goodall et al., 2009; Sidhu et al., 2009a; Hunter et al., 2016).

Studies have directly compared the estimation of VA via nerve stimulation to TMS and have yielded comparable results (Todd et al., 2003; Sidhu et al., 2009a; b; Bachasson et al., 2016). However, they also differ somewhat for several reasons. TMS may activate motor units of synergist muscles leading to greater joint torque, whereas nerve stimulation may fail to activate all motor units, thus leading to differences in SIT force. VA and force forms a curvilinear relationship from 0-100% MVC with nerve stimulation (Todd et al., 2003; Shield and Zhou, 2004) as opposed to a linear relationship from 50-100% MVC with TMS (Todd et al., 2003; 2004; Lee et al., 2008; Goodall et al., 2009; Bachasson et al., 2016). There is a non-equivalent TMS estimated twitch force and the nerve stimulation PT force amplitudes for both the elbow flexors (Todd et al., 2003; 2004; Todd et al., 2005; Smith et al., 2007; Kennedy et al., 2013), and knee extensors (Goodall et al., 2009; Sidhu et al., 2009b; Goodall et al., 2012; Klass et al., 2012; Goodall et al., 2014). Fatigue in the central and peripheral nervous systems (Enoka and Stuart, 1992; Gandevia, 2001; Kent et al., 2016) reduces force production, alters SIT forces during submaximal voluntary contractions and MVCs and decreases estimated or resting PT forces (Todd et al., 2003; Goodall et al., 2009; Kennedy et al., 2013; Keller-Ross et

al., 2014; Pearcey et al., 2015; Pearcey et al., 2016). The linear relationship between voluntary force and TMS evoked SIT force also decreases with fatigue resulting in an altered estimated resting twitch force and subsequently an over or underestimation of VA (Hunter et al., 2006; Hunter et al., 2008; Kennedy et al., 2013; Yoon et al., 2013). There are also other technical challenges as described elsewhere (Shield and Zhou, 2004; Todd et al., 2016) with nerve stimulation and TMS for optimizing the estimation of VA.

There are few studies directly comparing nerve stimulation to TMS for estimating VA of the elbow flexors prior to, throughout and following bouts of fatiguing contractions especially in the elbow flexors. This is especially important because TMS and nerve stimulation are the two most commonly used stimulation techniques for indirectly measuring VA in the elbow flexors and whether or not one stimulation type is superior to another, especially during fatigue, warrants further investigation. Therefore, the purposes of this study was to compare nerve stimulation to TMS for estimating elbow flexor VA and how this estimation changes throughout and following a series of fatiguing MVCs. We hypothesized that nerve stimulation and TMS would estimate VA: 1) similarly in non-fatigued elbow flexors and 2) differently during and following fatigue.

## Materials and methods

### Participants

Based on prior similar research (Taylor et al., 2000), a statistical power analysis determined that 6 participants were necessary to achieve an alpha of .05 with a power of 0.8. Ten resistance-trained males (183.1 ± 5.9 cm, 92.5 ± 12.1 kg, 25.5 ± 4.9 years) from the university population were recruited for the study. Participants were considered resistance trained because they had all trained on average ≥3 sessions a week for ~an

hour each session for at least one year. Participants were verbally informed of the procedures to be used during testing, and all gave informed written consent and completed a magnetic stimulation safety checklist to screen for potential contraindications with magnetic stimulation procedures (Rossi et al., 2009). The study was approved by the Memorial University of Newfoundland Interdisciplinary Committee on Ethics in Human Research (#20161806-HK) and was in accordance with the Tri-Council guidelines in Canada with full disclosure of potential risks to participants.

**Elbow Flexor Force**

Participants were seated in a custom-built chair (Technical Services, Memorial University of Newfoundland) in an upright position, with hips and knees flexed at 90˚, and head strapped in place to minimize movement (see Fig. 1A). Both arms were slightly abducted with elbows resting on padded support at an angle of 90˚. The forearms were held horizontal in a position midway between neutral and supination, and placed in a custom-made orthosis that was connected to a load cell (Omegadyne Inc., Sunbury, Ohio, USA). The load cell detected force output, which was amplified (x1000) (CED 1902, Cambridge Electronic Design Ltd., Cambridge, UK) and displayed on a computer screen. Data was sampled at 2000 Hz. Participants were asked to maintain the upright position during contractions. Verbal encouragement and visual feedback were given to all participants during all contractions.

**Electromyography**

Electromyography (EMG) activity was recorded from the biceps brachii and lateral head of the triceps brachii muscles on the dominant arm using surface EMG recording electrodes (MediTrace Ag-AgCl pellet electrodes, disc shaped and 10 mm in

diameter, Graphic Controls Ltd., Buffalo, N.Y., USA). Electrodes were placed length wise over the middle of the muscle belly with an interelectrode (center-to-center) distance of 2 cm and in accordance with SENIAM recommendations (Hermens et al., 2000). A ground electrode was placed over the lateral epicondyle of the dominant knee. Skin preparation for all recording electrodes included shaving to remove excess hair and cleaning with an isopropyl alcohol swab to removal of dry epithelial cells. An interelectrode impedance of <5 kΩ was obtained via a standard multimeter prior to recording to ensure an adequate signal-to-noise ratio. EMG signals were amplified (×1000) (CED 1902) and filtered using a 3-pole Butterworth filter with cutoff frequencies of 10–1000 Hz. All signals were analog-digitally converted at a sampling rate of 5 kHz using a CED 1401 (Cambridge Electronic Design Ltd., Cambridge, UK) interface.

**Stimulation Conditions**

*Brachial plexus (Erb's point) stimulation*

Stimulation of the brachial plexus (i.e. Erb's point) was used to induce a maximal compound muscle action potential ($M_{max}$). Erb's point was electrically stimulated via a cathode on the skin in the supraclavicular fossa and an anode on the acromion process. Current pulses were delivered as a singlet (200 μs duration, 100-250 mA) via a constant current stimulator (DS7AH, Digitimer Ltd., Welwyn Garden City, UK). The electrical current was gradually increased until $M_{max}$ of the biceps brachii was observed. To ensure maximal stimulation throughout the experiment, a supramaximal stimulation current (i.e., 130% greater than that required to elicit $M_{max}$) was used (Todd et al., 2003; Goodall et al., 2012; Aboodarda et al., 2015; Pageaux et al., 2015).

*Motor point stimulation*

Electrical stimulation was delivered via a cathode placed on the skin over the biceps motor point and an anode on the brachii distal tendon (Smith et al., 2007; Khan et al., 2011; Monks et al., 2016; Pearcey et al., 2016). Current pulses were delivered as a doublet (10 ms apart, 100 μs duration, 100-225 mA) via a constant current stimulator (DS7AH, Digitimer Ltd., Welwyn Garden City, UK). The electrical current was gradually increased until there was no longer an increase in the twitch force of the elbow flexors. A supramaximal stimulation current (i.e. 130% greater than that required to elicit a maximum twitch force) was used for the remainder of the experiment (Allen et al., 1998).

*Transcranial magnetic stimulation (TMS)*

TMS (transcranial magnetic stimulator; Magstim 200, maximal output 2.0 Tesla) was delivered through a circular coil (13 cm outside diameter) placed directly over the vertex (Todd et al., 2003; 2004; McNeil et al., 2011; Forman et al., 2014; Pearcey et al., 2014; Philpott et al., 2015). The vertex was located by marking the measured halfway points between the nasion and inion and tragus to tragus. The intersection of these two points was defined as the vertex. Electrical currents flowed in an anticlockwise direction through the circular coil. The coil was placed horizontally over the vertex so that the direction of the current flow in the coil preferentially activated the right or left motor cortex (A side up for right side, B side up for left) for the activation of the dominant elbow flexors. Stimulation intensity (50-90% MSO) was adjusted to elicit a large MEP in the biceps brachii (>50% of $M_{max}$) and a small MEP in the triceps brachii (<22% of the raw biceps brachii MEP amplitude) in the triceps brachii during elbow flexor MVCs

(Todd et al., 2016). This stimulation intensity was used for the remainder of the experiment.

**Experimental Set-up**

Participants completed a familiarization and an experimental session, which was separated by at least 48 hours. During the familiarization session participants received the stimulation conditions (TMS, brachial plexus and motor point stimulation) to ensure they were comfortable with each stimulation. Participants then performed maximal elbow flexor isometric contractions, with 2 minutes in between each contraction, until they were able to reach peak force within 2 seconds. Next, they practiced elbow flexor contractions at the various percentages of the highest MVC (25, 50, 75%). Finally, the participants completed three fatiguing contractions (15s long) similar to those to be performed in the experimental session.

During the experimental session participants were prepped for the stimulation conditions and EMG. Next, maximal twitch force and $M_{max}$ were obtained at rest through motor and Erb's point stimulation, respectively. Participants then completed a series of brief (2-3s) elbow flexor MVCs. During the MVCs the participants received TMS to determine the necessary intensity to elicit a MEP amplitude that was greater than 50% of the $M_{max}$ which was measured from the biceps brachii during the resting twitches. Following each of the brief MVCs, motor point stimulation was administered once again in order to evoke a PT force and to ensure maximal potentiation (Kufel et al., 2002).

The participants then started the experimental protocol. The protocol consisted of two different types of elbow flexor contractions; contractions to determine VA and

contractions to induce fatigue. All of the contractions to determine VA included a MVC followed by randomly performing 25, 50 and 75% of the MVC. Each contraction was ~5s in duration. The submaximal contractions (25, 50 and 75% of MVC) were always made relative to the 100% MVC in each set. All forces were displayed on a computer screen, which enabled the participants to match the target force. During each maximal and submaximal contraction participants received TMS and motor point stimulation at 2 and 4s, respectively. Two and 3s following the completion of the 5s contraction, when the elbow flexors were at rest, participants received another motor point stimulation and an Erb's point stimulation, respectively. The fatigue contractions consisted of 3, 15s sustained elbow flexor MVCs with 5s rest between each sustained MVC. Although participants force declined during each 15s MVC due to fatigue, they were verbally encouraged to maximally contract the elbow flexors throughout the entire 15s contraction

Initially, participants performed a set of VA elbow flexor contractions. Following the VA contractions, they started the fatigue contractions. After the completion of 3 fatigue contractions they immediately completed another VA set. This process was repeated 3 times. Additional sets of VA contractions were performed at 5 and 10 minutes post-fatigue contractions. In total participants completed 4 sets of fatigue contractions (12 sustained MVCs) and 7 sets of the VA contractions at pre-fatigue (VA-pre), following fatigue sets 1,2 3, and 4 (VA 1-4) and at 5 and 10-min post-fatigue (VA-post 5 and 10) (see Figure 1 for experimental set-up).

**Data Analysis**

The Interpolated Twitch Technique (ITT) was utilized as a measure of the central nervous system's ability to fully activate the contracting muscle (Shield and Zhou, 2004). VA was calculated by comparing the amplitude of the SIT force with the actual or predicted PT force with the following equation: $\text{VA\%} = (1 - \text{SIT force/PT force}) \times 100$ and was quantified by measurement of the elbow flexor force responses to single pulse motor cortical stimulation and to double pulse motor point stimulation during 50, 75, and 100% MVC. The predicted resting PT force for each participant was derived from extrapolating the linear regression ($r^2$ value) between the SIT forces upon the voluntary forces over the force ranges: 50, 75 and 100% MVC. These force ranges were chosen because they gave the best $r^2$ values for TMS predicted twitch force (data not shown) for TMS (Todd et al., 2004; Goodall et al., 2009) and motor point stimulation and will be referred to as TMS predicted and motor point predicted hereafter. The $y$-intercept was taken as the estimated amplitude of the resting PT force. Each set of contractions provided a resting estimated PT force. Furthermore, VA was also quantified by measurement of the elbow flexor force responses to motor point stimulation during 100% MVC and divided by the resting PT force following the MVC (i.e. not using a predicted PT force and referred to as motor point actual hereafter) and a linear regression ($r^2$ values) between the SIT forces upon the voluntary forces over the force ranges (25-100%) and the actual potential twitch (i.e. 0% MVC) and 25-100%. The amplitude of motor point actual PT force was also measured to assess muscle fatigue. The maximal force of the elbow flexors was quantified as the average value over a 500 ms interval that was centered about the peak of the MVC. The biceps and triceps brachii EMG activity

was determined as the root mean square (RMS) value over a 500 ms interval about the same interval of the MVC force measurement. Triceps EMG was also expressed as a percentage of biceps EMG during each elbow flexor MVC.

The amplitudes and areas of MEP and $M_{max}$ of the biceps and triceps brachii evoked by TMS and Erb's point stimulation, respectively, were measured between cursors placed at the beginning and end of the evoked potentials for each set of contractions. Triceps MEP amplitude was also expressed as a percentage of bicep MEP amplitude during each MVC. Because amplitude and area showed similar changes, only amplitude data were reported. All data were measured offline using Signal 4.0 software (Cambridge Electronic Design Ltd., Cambridge, UK).

**Statistical Analysis**

Statistical analyses were computed using SPSS software (SPSS 22.0, IBM Corporation, Armonk, New York, USA). Assumptions of sphericity (Mauchley test) and normality (Shapiro-Wilk test) were tested for all dependent variables. If the assumption of sphericity was violated, the corrected value for non-sphericity with Greenhouse-Geisser epsilon was reported. A one-way ANOVA with repeated measures (time; VA-pre, VA 1-4, and VA-post 5 and 10-min) was performed on MVC force, EMG and PT force. A two-way ANOVA (3×7) with repeated measures (stimulation type; motor point actual, motor point predicted and TMS predicted × time) was performed on VA, actual and predicted twitch forces and $r^2$ values. A two-way ANOVA (2×7) with repeated measures (stimulation type × time) was performed on super imposed twitch force. A Bonferonni Post Hoc test was performed to test for significant differences between

interactions. *F*-ratios were considered statistically significant at the $p<0.05$ levels. Cohen's *d* effects sizes (ES) (Cohen, 1988) were also calculated to determine the magnitude of the differences between interventions and time. The following criteria were used: ES<0.2 "trivial"; ES=0.2-0.49 "small"; ES=0.5-0.79 "medium"; and ES>0.8 "large". Percentage changes and absolute values (mean±SD) are reported in text and absolute values only (mean±SE) are reported in figures (3-5).

## Results

### MVC force and EMG

There was a significant ($F_{(6,54)}=22.47$, $p<0.001$; $F_{(6,54)}=2.57$, $p=0.03$; $F_{(6,54)}=18.77$, $p<0.001$) main effect for time on MVC force, EMG and PT force, respectively. MVC force significantly ($p<0.01$, ES=2.7) decreased from VA-pre VA-4 by 40.5±9.1% (Figure 2A). MVC force remained significantly ($p<0.01$, ES=1.7 and $p<0.01$, ES=1.5) depressed by 27.2±9.3% and 24.9±11.3% at VA-post5 and VA-post10, respectively compared to pre-fatigue. However, MVC force significantly ($p<0.01$, ES=0.9 and $p<0.01$, ES=1.0) increased by 22.2±18.4% and 25.9±19.1% at VA-post5 and VA-post10, respectively compared to VA-4. EMG significantly (range: $p<0.01$–$p=0.03$, ES=0.8-1.4) decreased (range: 24.9±20.1-44.6±31.1%) from VA-pre compared to all other time points (Figure 2B).

### Voluntary activation

There was a significant interaction ($F_{(12,108)}=10.54$, $p<0.001$) between stimulation type and time for VA (Figure 3A). VA when using motor point actual or motor point predicted twitch forces were significantly ($p<0.01$ for all time points, ES=1.6-3.1) higher at each time point (range: 20.9±11.9% to 136.1±39.6%) compared to using TMS

predicted twitch force. VA significantly ($p$<0.01, ES=1.6; $p$<0.01, ES=1.8; $p$<0.01, ES=1.8) decreased from VA-pre to VA-4 by 17.7±9.0%, 20.4±8.4%, and -75.2±99.2% when calculated by using the motor point actual, motor point predicted and TMS predicted twitch forces, respectively. VA remained significantly depressed by 16.7±5.7% and 15.7±6.2% ($p$<0.01, ES=1.1 and $p$<0.01, ES=1.3) when using motor point actual, by 19.4±5.7% and 18.4±6.6% ($p$<0.01, ES=1.2 and $p$<0.01, ES=1.4) when using motor point predicted and by 51.3±44.5% and 58.6±28.6% ($p$<0.01, ES=1.7 and $p$<0.01, ES=1.5) when using TMS predicted twitch forces at VA-post5 and VA-post10, respectively compared to VA-pre. However, VA significantly ($p$<0.05, ES=3.3 and $p$<0.05, ES=4.0) increased by 176.9±46.6% and 191.3±44.3% at VA-post5 and VA-post10, respectively compared to VA-pre, when using TMS predicted twitch forces.

There was a significant interaction ($F_{(12,108)}$=9.33, $p$<0.001) between stimulation type and time for PT force (Figure 3B). Motor point actual and motor point predicted resting potentiated twitch forces were significantly ($p$<0.01 for all time points, ES=2.7-5.2) higher at each time point (range: 50.1±23.6% to 79.6±13.1%) compared to TMS predicted resting potentiated twitch force. Motor point actual was significantly ($p$<0.05, ES=1.7) higher by 19% at VA-pre compared to motor point predicted resting potentiated twitch force at VA-pre. Motor point actual and TMS predicted resting potentiated twitch forces significantly ($p$=0.001-$p$=0.003, ES=1.8-2.2 and $p$<0.01-$p$=0.034, ES=0.7-3.6) decreased from VA-pre compared to all other time points, respectively by 19.9±8.5-30.5±13.3% and 27.1±25.1-40.1±34.1%. Motor point predicted resting potentiated twitch forces significantly ($p$<0.01, ES=1.3) increased by 17.7±10.2% from VA-pre to VA-1

and significantly ($p$<0.01, ES=1.1 and $p$<0.01, ES=1.1) decreased by 14.5±14.7% and 15.4±12.1% from VA-1 to VA-post5 and VA-post10, respectively.

There was a significant ($F_{(6,54)}$=8.64, $p$<0.001) main effect for time on SIT force at 100% MVC. SIT force significantly ($p$<0.01 for all time points, ES=0.7-1.3) increased (range: 159.4±120.1-253.9±210.1%) from VA-pre compared to all other time points (Figure 4A). There was a significant interaction ($F_{(6, 36)}$=6.0, $p$<0.001) between stimulation type and time for SIT force at 50% MVC. TMS SIT force was 30.2±20.2-35.7±12.9% lower ($p$<0.05 for all time points, ES=1.0-1.6) than motor point stimulation from VA-1 to VA-post10, respectively (Figure 4A).

There was a significant main effect ($F_{(1,8)}$=5.9, $p$<0.05) for stimulation type on $r^2$ values. Overall, $r^2$ values were significantly ($p$<0.05 and $p$<0.05) lower by 19.9±5.1% and 14.2±5.5% for TMS predicted than motor point actual and predicted, respectively (Figure 4B).

**Biceps and triceps EMG and MEP and $M_{max}$ amplitudes**

There was no significant ($F_{(6,54)}$=0.83, $p$=0.53; $F_{(6,54)}$=2.66, $p$=0.08; $F_{(6,54)}$= 3.82, $p$=0.06; $F_{(6,54)}$=2.55, $p$=0.08) main effect for time on 100% elbow flexor MVC triceps/biceps EMG, biceps MEP and $M_{max}$ amplitudes, and triceps/biceps MEP amplitude, respectively. Triceps EMG ranged from 18.9±12.1-22.2±14.1% of biceps EMG from VA-pre to VA-post10. Biceps MEP and $M_{max}$ amplitudes ranged from 6.7±4.5-7.8±4.1 mV and 13.5±6.2-13.9±5.8 mV, respectively from VA-pre to VA-post10. Triceps MEP ranged from 15.1±8.2-21.4±11.2% of biceps MEP from VA-pre to VA-post10.

## Discussion

Overall elbow flexor fatigue was due to a combination of reduced output from the central and peripheral nervous systems. Interestingly, VA was substantially underestimated when using TMS compared to motor point stimulation in non-fatigued and fatigued elbow flexors. As elbow flexor fatigue developed, this underestimation became dispersed. The dispersed underestimation of VA could not be explained by a fatigue-induced increase of triceps brachii activation, but instead a reduced linearity between the TMS evoked SIT force and voluntary force of the elbow flexors. The decreased linearity subsequently yielded a reduced TMS, but not motor point stimulation, predicted resting twitch force leading to an underestimation of VA. The reduced linearity may be due to TMS evoked SIT force being much smaller than motor point stimulation evoked SIT forces at 50% MVC.

The elbow flexor fatigue protocol in the current study induced fatigue both centrally and peripherally. Participants could no longer voluntarily drive the muscle the same way as pre-fatigue. Following the fatiguing contractions, motor point stimulation evoked larger SIT forces during the MVCs than when the muscles were not fatigued indicating that the axons of the motoneurones were capable of increased output but that there was a reduction in central nervous system output to (i.e. at the corticomotoneuronal synapse) (Gandevia et al., 1999) or within the motoneurone itself (i.e. decreased intrinsic excitability) (Khan et al., 2012). During the same MVCs, the increased SIT force due to TMS indicates that the reduced output to the motoneurone was due, in part, to altered synaptic activity from the motor cortex (Taylor et al., 1996; Ranieri and Di Lazzaro, 2012). Because there was no change in the biceps brachii MEP amplitude, the

corticospinal pathway probably never played a role in the reduced MVC force. Thus, the altered synaptic activity to the motoneurone may be upstream from this pathway. Mechanisms of fatigue-induced changes in central nervous system output have been reviewed elsewhere (Gandevia, 2001; Ranieri and Di Lazzaro, 2012; Kent et al., 2016). The reduction in PT force indicates that the reduction in MVC force was, in part, due to fatigue induced changes in the elbow flexor muscles. The reduction in PT illustrates that there were impairments to: 1) muscle excitation-contraction coupling, such as sarcoplasmic reticulum release, restoration of intracellular calcium and sensitivity of calcium to contractile protein interactions, 2) H+, 3) PCr breakdown, 4) muscle deoxygenation and 5) others, which have all been reviewed in detail (Enoka and Stuart, 1992; Fitts, 1994; Allen et al., 2008; Kent et al., 2016).

The most interesting finding in the current study was the disperse differences in estimated VA via TMS compared to motor point stimulation, especially during the development of elbow flexor fatigue. A potential reason for these differences is antagonist co-activation. Because cortical stimulation is not focal, there may be an activation of corticospinal cells that project to various muscles including the antagonist. Activation of the antagonist during an agonist contraction would reduce the size of the SIT force and subsequently result in an over- or underestimation of VA (Todd et al., 2016). In the current study, at all contraction intensities (data only shown for 100% MVC) and throughout the development of fatigue the triceps/biceps MEP and EMG ratios were approximately 20% or less. Thus, increased antagonist activation could not explain the disperse VA differences between TMS and motor point stimulation or the decrease in VA via TMS from VA-pre to VA-4.

The main reason for the disperse differences in VA as the participants fatigued was poor linear regression. When linear regression between motor point evoked SIT force and voluntary force was made the average r-values at all times points were high for the elbow flexors with and without fatigue. For TMS the linear regression average r-value was high only for the elbow flexors in a non-fatigued state. It has been shown that non-linearity of the regression between TMS evoked SIT force and voluntary force occurs more often with fatigued compared to non-fatigue muscle (Hunter et al., 2006; Hunter et al., 2008; Girard et al., 2013; Kennedy et al., 2013; Keller-Ross et al., 2014). Based on the current findings, the difference in the linear regression between TMS and motor point stimulation was due to the 50% but not 75 or 100% MVCs. The evoked SIT forces were similar for both TMS and motor point stimulation at 75% and 100% MVC but much smaller for TMS at 50% MVC. These differences lead to an underestimation of resting twitch force for TMS. In fact, because of these differences in linearity, the TMS predicted resting twitch force became so underestimated that by VA-4 the SIT was larger than the predicted resting twitch, and thus a negative VA occurred. As the SIT force at 50% MVC started to recover post-fatigue there was an increase in the estimated resting twitch force and VA became positive again.

There were several methodological considerations for this study. Typically, the TMS predicted resting twitch force is larger in the elbow flexors compared to the resting potentiated twitch force evoked by motor point stimulation (Todd et al., 2003; 2004; Kennedy et al., 2013), which is opposite to what happened in the current study. The differences between TMS and motor point predicted VA compared to other studies (Todd et al., 2003; 2004; Kennedy et al., 2013) may have occurred for several reasons. First, in

the aforementioned studies a single motor point stimulus was delivered, whereas a double stimulus was used here. A double rather than a single stimulus was used to evoke twitch forces because it has been shown to produce a higher signal to noise ratio (Behm et al., 1996). The double stimulus at the motor point recruited the elbow flexor muscle fibers differently than TMS at 50% MVC especially during the fatiguing contractions. Second, we recruited chronically strength-trained participants, whereas other studies (Todd et al., 2003; 2004; Kennedy et al., 2013) did not. Although strength-training alters various sites in the central nervous system (Carroll et al., 2011) it does not appear to affect VA of upper limb muscles (Herbert et al., 1998; Lee et al., 2009). Lastly, in the current study, the elbow joint was flexed to 90° and the shoulder at 0° with the forearm parallel to the ground and supinated with the force at the wrist being upwards. In other studies, (Todd et al., 2003; 2004; Kennedy et al., 2013) the elbow and shoulder joints were flexed to 90° with the forearm vertical and supinated with the force at the wrist being backwards. Changes in forearm and shoulder positions alters CSE of the biceps brachii (Forman et al., 2016; Nuzzo et al., 2016) and potentially could affect VA of the elbow flexors. Todd et al. (2003) showed a high linear regression between TMS evoked SIT forces and voluntary force (50-100% MVC) in fatigued elbow flexors with the elbow and shoulder joints were flexed to 90°, which was opposite to the current results. However, to the best of our knowledge no studies to date have determined the combined effects of fatigue, shoulder position, stimulation type and training on elbow flexor VA.

**Conclusion**

The estimation of VA or the level of neural drive from the central nervous system to produce force is important for quantifying the presence of central fatigue in various

physiological conditions. Compared to motor point stimulation, VA of the elbow flexors was underestimated prior to and even more so during fatigue when using TMS. During fatigue the stimulus evoked SIT forces responded differently to TMS at submaximal compared to near maximal or maximal voluntary contractions leading to an underestimation of the resting twitch and subsequently underestimation of VA. TMS during voluntary contraction does have the advantage over motor point stimulation to indicate that a change in VA is, in part, cortex dependent. However, based on the current findings and the conditions in which VA was measured the use of TMS to estimate VA of the elbow flexors may not be an appropriate technique especially following fatigue. Overall motor point stimulation was the more appropriate technique for estimating VA of the elbow flexors.

**Abbreviations**

VA, Voluntary Activation; CSE, corticospinal excitability; TMS, transcranial magnetic stimulation; MEP, motor evoked potential; MVC, maximum voluntary contraction; Mmax, maximal muscle compound action potential; EMG, electromyography; PT, potentiated twitch, ITT, interpolated twitch technique; SIT, superimposed twitch; RMS, root mean square.

**Authors contributions**

EC, BC, DP, GK, MB and DB contributed to the conception or design of the work, the acquisition, analysis, and interpretation of data for the work and final submission of the manuscript. All authors agree to be accountable for the work.

## Conflict of interest

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

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**Figure Legends**

**Figure 1. (A)** Picture of the experimental set-up to measure elbow flexors submaximal and maximum voluntary contractions (MVC), voluntary activation (VA) and electromyography (EMG) and placement of EMG electrodes transcranial magnetic stimulation (TMS), motor point stimulation and Erb's point stimulation. **(B)** Participants' performed an experimental protocol that consisted of a set of pre-fatigue voluntary contractions (100, 75, 50, 25% MVC, VA-pre), 4 sets of fatiguing contractions and 4 sets of voluntary contractions (100, 75, 50, 25% MVC, VA 1-4) and a set of voluntary contractions (100, 75, 50, 25% MVC) at 5 and 10-min post-fatigue (VA-post 5 and 10). A set of VA contractions was always performed following a set of fatiguing contractions. The VA contraction sets were performed in order to derive an estimated potentiated resting twitch (see methods for details) to estimate VA. The black arrows indicate that the participant received several stimuli. The blue boxes indicate VA set contractions and the red bars represent fatiguing set contractions. **(C)** For each contraction (25-100% MVC) in the VA set, participants received TMS and motor point stimulation (at 2 and 4 s, respectively) and motor point stimulation and Erb's point stimulation (at 2 and 3 s, respectively) following the contraction when the elbow flexors were at rest. The blue trace represents one contraction in the VA set.

**Figure 2.** Raw Data recorded from one participant. The red and blue traces represent measures taken from 100% MVC of the elbow flexors in the VA-pre set and VA 4 set, respectively. Top of the figure shows the MVC forces and when the stimulation occurred during the MVCs. It also shows the stimulus evoked superimposed twitch forces during

the MVCs and the stimulus evoked potentiated twitch forces following the MVCs. Notice the reduction in MVC and potentiated twitch forces and the increased superimposed twitch forces in VA 4 compared to VA-pre. The middle and bottom portions of the figure show the biceps and triceps brachii EMG recorded during the MVCs traces from the top of the figure. A MEP and $M_{max}$ occurred in response to TMS and Erb's point stimulation, respectively for both the biceps and triceps brachii and are amplified for clarity. Notice the decrease in EMG during the VA-4 MVC compare to the VA-pre MVC. Also, there was no change in the MEP or $M_{max}$ response.

**Figure 3.** Change in **(A)** MVC force and **(B)** EMG during recorded during the MVC for each VA set from VA-pre to VA-post10. * indicates a significant difference ($p$<0.01) between VA-pre and all other time points and ** indicates a significant difference ($p$<0.05) from VA-4. Each data point represents the group mean ± SE.

**Figure 4. (A)** Change in voluntary activation during the MVC for each VA set from VA-pre to VA-post10. * indicates a significant difference (p<0.01) between TMS predicted compared to motor point actual and motor point predicted, ** indicates a significant difference ($p$<0.05) from VA-4 for TMS predicted only and # represents a significant main effect ($p$<0.05) for time for VA-pre compared to VA-4. **(B)** Change in motor point actual, motor point predicted and TMS predicted resting potentiated twitch forces (black filled circles) and motor point and TMS evoked superimposed twitch forces (white filled circles) at 100% MVC (see also Figure 4B 100% MVC for more details) (at). * indicates a significant difference ($p$<0.01) between VA-pre and all other time points for both motor

point actual and TMS predicted and VA-1 and all other time points for motor point predicted resting potentiated twitch forces. All time points for motor point actual and motor point predicted resting potentiated twitch forces were significantly different ($p$<0.01) than those of TMS predicted resting potentiated twitch forces (not symbols shown to denote difference). Each data point (A and B) represents the group mean ± SE.

**Figure 5. (A)** Change in motor point stimulation and TMS evoked superimposed twitch forces during 50% (left), 75% (middle) and 100% (right) MVCs. * indicates a significant difference ($p$<0.05) between stimulation type and ** indicates a significant difference ($p$<0.05) between VA-pre and all other time points. **(B)** Change in $r^2$ values for predicting resting potentiated twitch force from motor point stimulation and TMS for each set of voluntary contractions from VA-pre to VA-post10. $r^2$ values were calculated as the relationship between motor point and TMS evoked superimposed twitch forces at 50, 75 and 100% MVC and 50, 75 and 100% MVC forces. $R^2$ values were also calculated as the relationship between motor point evoked superimposed twitch forces at 0 (i.e. following the 100% MVC at rest), 25, 50, 75 and 100% MVC and 0, 25, 50, 75 and 100% MVC forces. * indicates a significant main effect ($p$<0.01) for stimulation type. Each data point (A and B) represents the group mean ± SE.

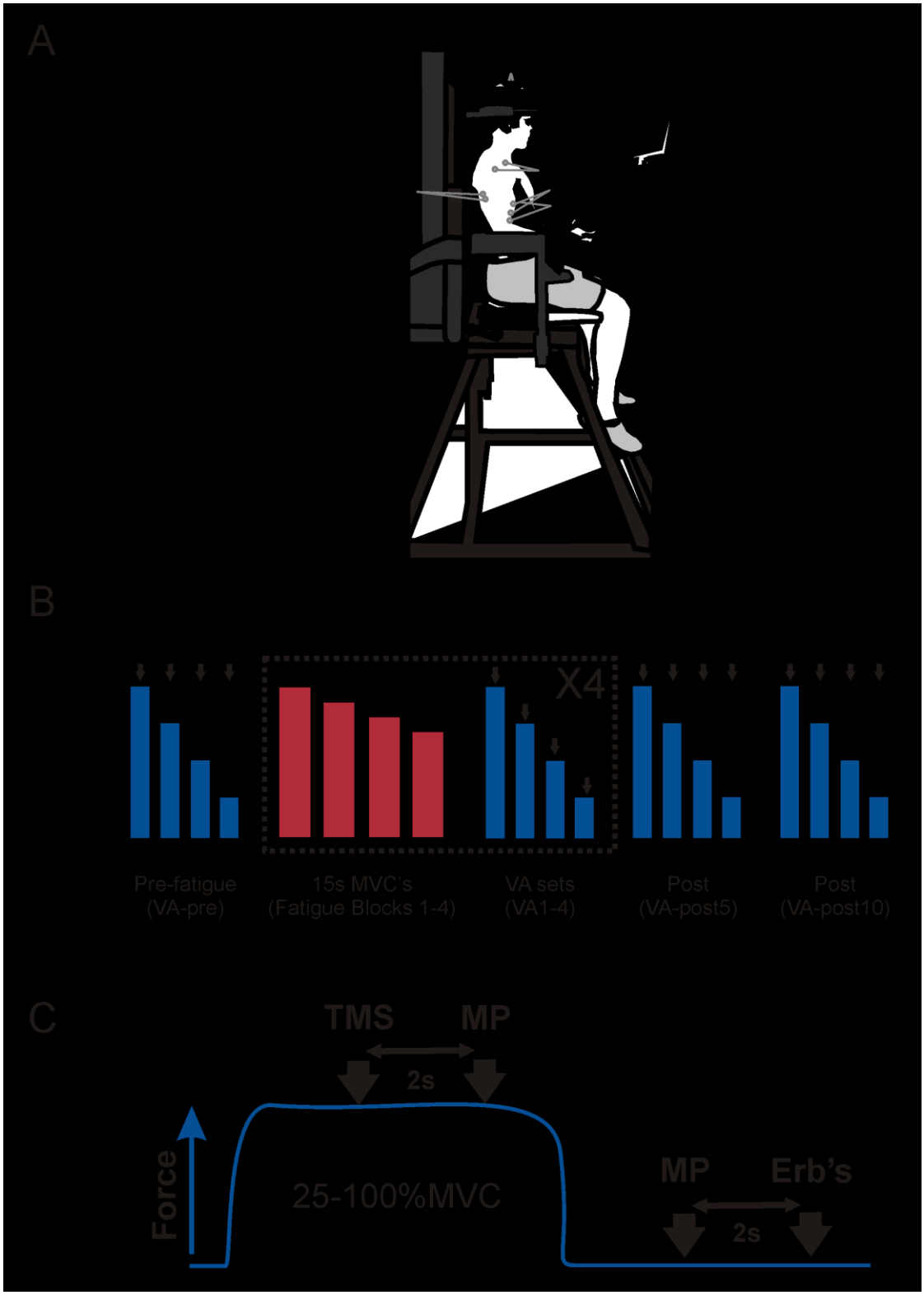
A
B
X4
Pre-fatigue
(VA-pre)
15s MVC's
(Fatigue Blocks 1-4)
VA sets
(VA1-4)
Post
(VA-post5)
Post
(VA-post10)
C
TMS
MP
2s
Force
25-100%MVC
MP
Erb's
2s

Figure 1

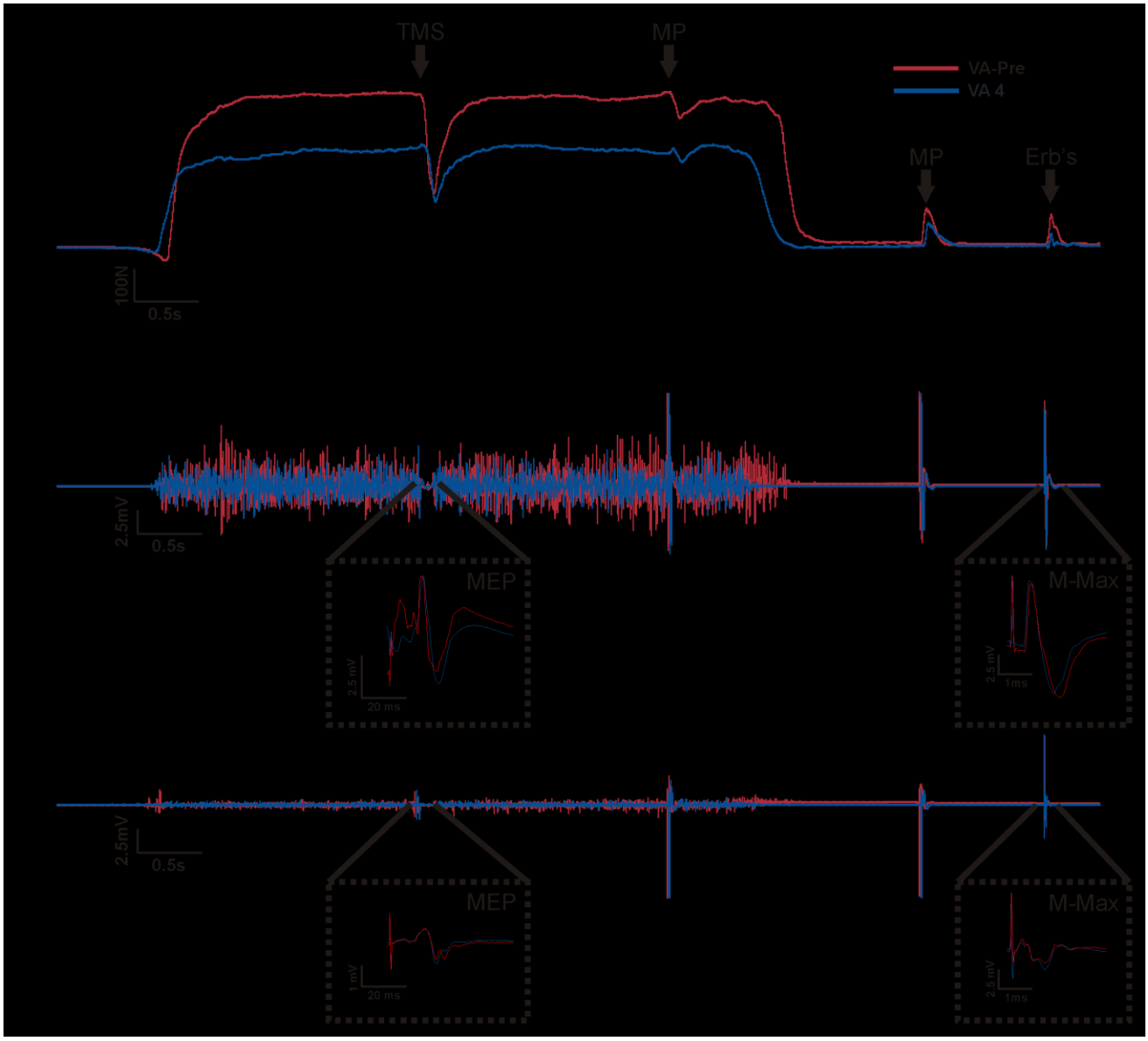
TMS
MP
VA-Pre
VA 4
MP
Erb's
100N
0.5s
2.5mV
0.5s
MEP
M-Max
2.5 mV
20 ms
2.5 mV
1ms
2.5mV
0.5s
MEP
M-Max
1 mV
20 ms
2.5 mV
1ms

Figure 2

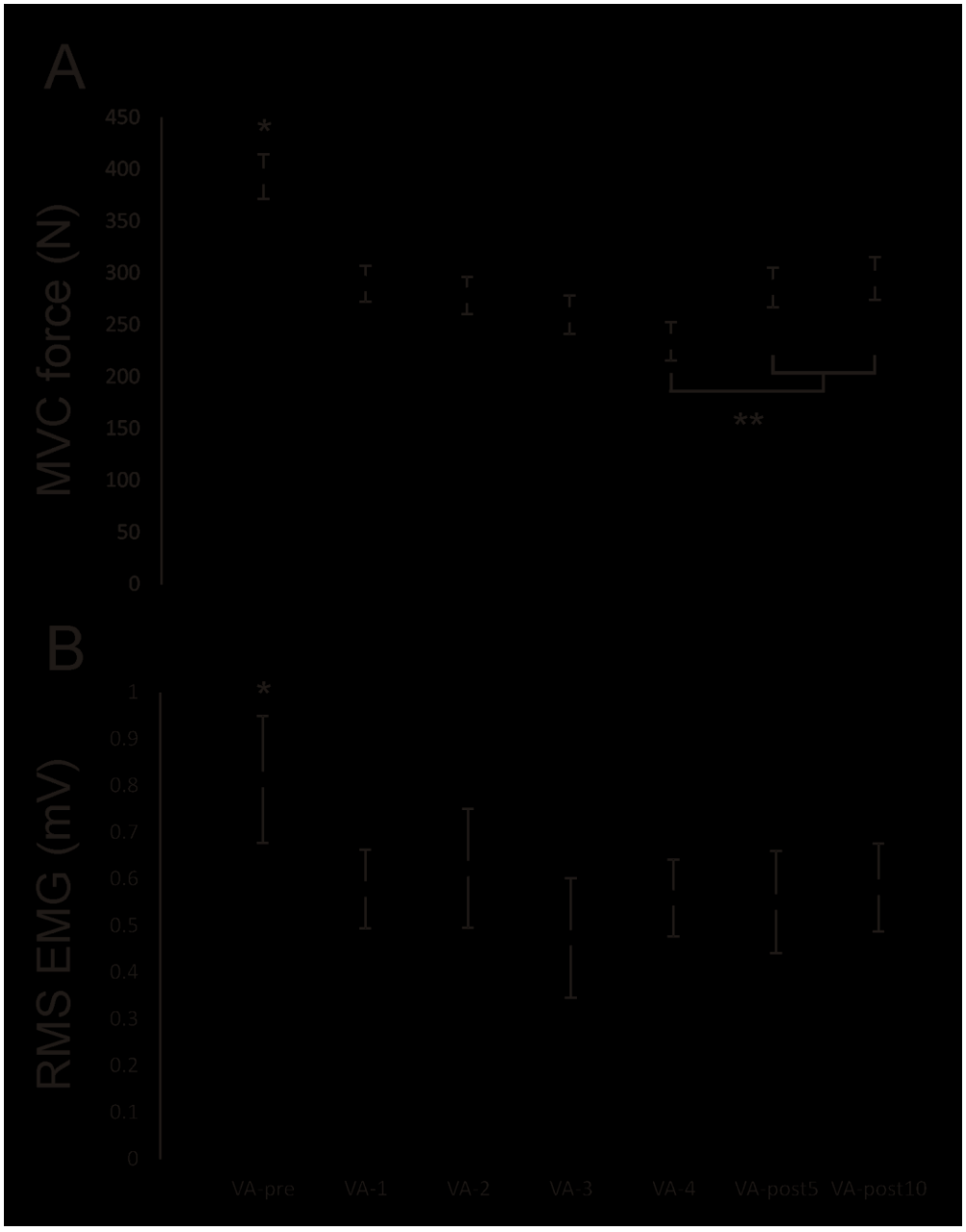
A
MVC force (N)
450
400
350
300
250
200
150
100
50
0
*
**
B
RMS EMG (mV)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
*
VA-pre
VA-1
VA-2
VA-3
VA-4
VA-post5
VA-post10

Figure 3

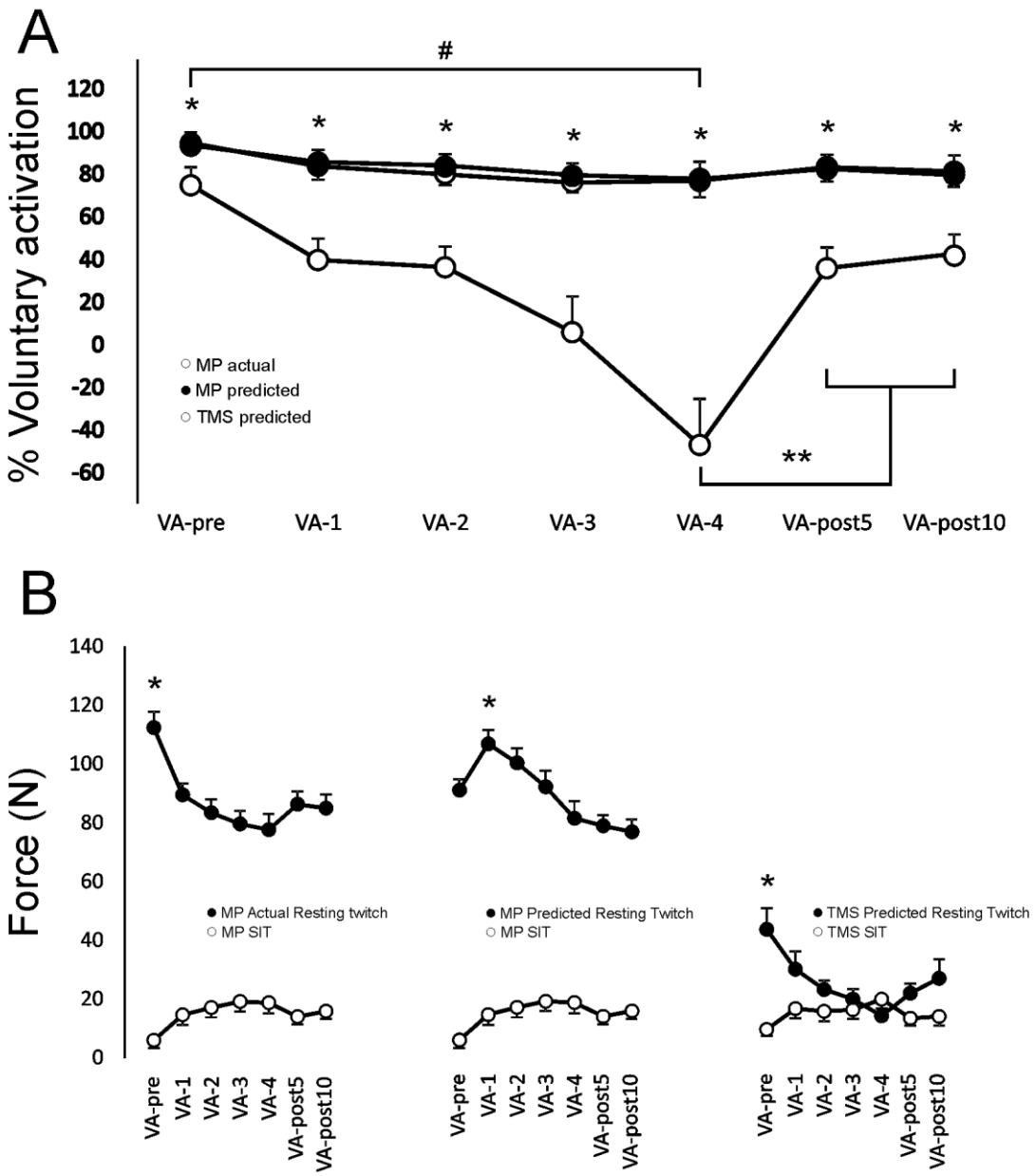
A
#
*
% Voluntary activation
120
100
80
60
40
20
0
-20
-40
-60
MP actual
MP predicted
TMS predicted
**
VA-pre
VA-1
VA-2
VA-3
VA-4
VA-post5
VA-post10
B
Force (N)
140
120
100
80
60
40
20
0
MP Actual Resting twitch
MP SIT
MP Predicted Resting Twitch
MP SIT
TMS Predicted Resting Twitch
TMS SIT

Figure 4

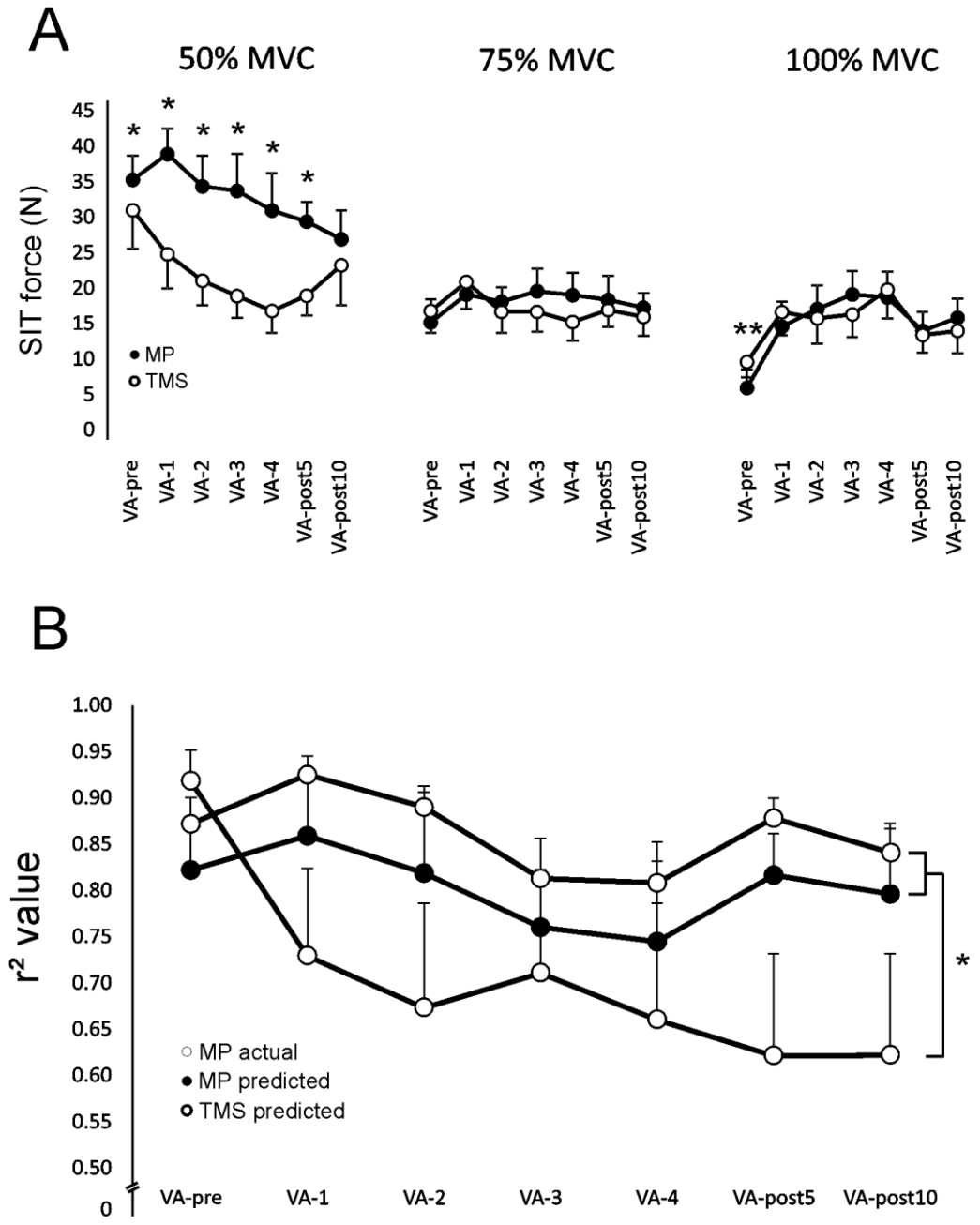
A
50% MVC
75% MVC
100% MVC
SIT force (N)
45
40
35
30
25
20
15
10
5
0
MP
TMS
VA-pre
VA-1
VA-2
VA-3
VA-4
VA-post5
VA-post10
B
r² value
1.00
0.95
0.90
0.85
0.80
0.75
0.70
0.65
0.60
0.55
0.50
0
MP actual
MP predicted
TMS predicted

Figure 5

**Appendix A: TMS Safety Checklist**

The safety of TMS continues to be supported by recent metaanalyses of the published literature (see Machii et al., 2006; Loo et al., 2008; Janicak et al., 2008, Rossi et al. 2009). To ensure safety of the participants they will have to fill out the following questionnaire prior to TMS.

## Magnetic Stimulation safety checklist

*Please answer the following questions by circling yes or no.*

*If your answer to any of the following questions is YES, you are deemed ineligible to participate in this study. At any time you may inform the researcher that a nonspecific aspect of the checklist applies to you, therefore you cannot continue. Please do not elaborate on any personal medical history*

1. Do you suffer from epilepsy, or have you ever had an epileptic seizure? **YES/NO**

2. Does anyone in your family suffer from epilepsy? **YES/NO**

3. Do you have any metal implant(s) in any part of your body or head? (Excluding tooth fillings) **YES/NO**

4. Do you have an implanted medication pump? **YES/NO**

5. Do you wear a pacemaker? **YES/NO**

6. Do you suffer any form of heart disease? **YES/NO**

7. Do you suffer from reoccurring headaches? **YES/NO**

8. Have you ever had a skull fracture or serious head injury? **YES/NO**

9. Have you ever had any head surgery? **YES/NO**

10. Are you pregnant? **YES/NO**

11. Do you take any medication? **YES/NO**
    a. Note if taking medication, check list for contraindicated medication on next page.

12. Do you suffer from any known neurological or medical conditions? **YES/NO**

Comments:
_____
_____
_____

Name: _____

Signature: _____

Date: _____

**Medications contraindicated with magnetic stimulation: 1) Tricyclic antidepressants**

| Name | Brand name |
|---|---|
| amitriptyline (& butriptyline) | Elavil, Endep, Tryptanol, Trepiline |
| desipramine | Norpramin, Pertofrane |
| dothiepin hydrochloride | Prothiaden, Thaden |
| imipramine (& dibenzepin) | Tofranil |
| iprindole | - |
| nortriptyline | Pamelor |
| opipramol | Opipramol-neuraxpharm, Insidon |
| protriptyline | Vivactil |
| trimipramine | Surmontil |
| amoxapine | Asendin, Asendis, Defanyl, Demolox, Moxadil |
| doxepin | Adapin, Sinequan |
| clomipramine | Anafranil |

## 2) Neuroleptic or Antipsychotic drugs

### A) Typical antipsychotics

• Phenothiazines: • Thioxanthenes:
- o Chlorpromazine (Thorazine) o Chlorprothixene
- o Fluphenazine (Prolixin) o Flupenthixol (Depixol and Fluanxol)
- o Perphenazine (Trilafon) o Thiothixene (Navane)
- o Prochlorperazine (Compazine) o Zuclopenthixol (Clopixol and Acuphase)
- o Thioridazine (Mellaril) • Butyrophenones:
- o Trifluoperazine (Stelazine) o Haloperidol (Haldol)
- o Mesoridazine o Droperidol
- o Promazine o Pimozide (Orap)
- o Triflupromazine (Vesprin) o Melperone
- o Levomepromazine (Nozinan)

### B) Atypical antipsychotics

- Clozapine (Clozaril)
- Olanzapine (Zyprexa)
- Risperidone (Risperdal)
- Quetiapine (Seroquel)
- Ziprasidone (Geodon)
- Amisulpride (Solian)
- Paliperidone (Invega)

### C) Dopamine partial agonists:

Aripiprazole (Abilify)

### D) Others

Symbyax -A combination of olanzapine and fluoxetine used in the treatment of bipolar depression. Tetrabenazine (Nitoman in Canada and Xenazine in New Zealand and some parts of Europe Cannabidiol One of the main psychoactive components of cannabis.

**Appendix B: Free and Informed Consent Form**

**Informed Consent Form**

| | |
|---|---|
| Title: | Corticospinal excitability and force relationship comparing between 90 degree flexion at the shoulder joint versus 0 degree flexion when assessing biceps differing muscles in sedentary and endurance trained individuals |


| | |
|---|---|
| Researcher(s): | Ted Cadigan<br>School of Human Kinetics and Recreation<br>Email: ewjc63@mun.ca |
| Supervisor(s): | Dr. Duane Button<br>School of Human Kinetics and Recreation<br>Email: dbutton@mun.cs |


You are invited to take part in a research project entitled *"*Corticospinal excitability and force relationship comparing between 90 degree flexion at the shoulder joint versus 0 degree flexion when assessing biceps differing muscles in sedentary and endurance trained individuals*"*

This form is part of the process of informed consent. It should give you the basic idea of what the research is about and what your participation will involve. It also describes your right to withdraw from the study at any time. In order to decide whether you wish to participate in this research study, you should understand enough about its risks and benefits to be able to make an informed decision. This is the informed consent process. Take time to read this carefully and to understand the information given to you. Please contact the researcher, Mr. Cadigan, if you have any questions about the study or for more information not included here before you consent.

It is entirely up to you to decide whether to take part in this research. If you choose not to take part in this research or if you decide to withdraw from the research once it has started, there will be no negative consequences for you, now or in the future.

**Introduction**

This research is being conducted by Ted Cadigan, a master student in the School of Human Kinetics and Recreation at Memorial University of Newfoundland. As a part of my Masters I am conducting research under the supervision of Dr. Duane Button. This research is aimed at measuring the changes in corticospinal neurone activity during submaximal and maximal muscular contractions in both fresh and fatigued muscles. To initiate purposeful movements, corticoneurons in the brain sends signals to the spinal cord to activate cells called motoneurones, which in turn send electrical signals to the muscles for contraction. Previous work has shown that differing intensities of muscle

contractions can alter the responsiveness of corticoneurons, spinal motoneurones and muscles. For example, maximal effort muscular contractions cause a reduction in spinal motoneurone excitability; while, very low-level repeated contractions increase the responsiveness of spinal motoneurones which would mean that the amount of effort required initiating and maintaining muscle contraction is reduced, making movement easier. It is currently unknown how the corticospinal excitability/force relationship differs across muscles or if this relationship is affected by change in joint angle or a fatigued state at the Biceps.

**Purpose of study:**
The sole purpose of this study is to determine the central and peripheral nervous systems role of the biceps brachii during changes in joint angle and fatigue at the shoulder.

**What you will do in this study:**
This study will consist of three different testing sessions conducted on separate days. The following is a brief description of the techniques being utilized and the protocol for each individual testing session.

**TESTING SESSION 1:**
This session will be used to introduce you to the experimental procedures and to familiarize with the stimulation protocols. We will also us this time to gather data that will be needed for the second testing session.

**TESTING SESSION 2:**
When you arrive at the lab you will be asked to do a 5-minute warm-up on a Monark ergometer at an intensity of ~50rpm and 50 watts. After completing the warm-up, electrodes will be fixed to your biceps brachii, triceps brachii muscles as well as over the mastoid processes (on the skull) and supraclavicular space (just above the collar bone). The vertex on the skull will also be marked. Once electrodes have been attached, you will be asked to perform a maximal voluntary contraction (MVC) for the biceps brachii. A series of submaximal, maximal, and fatiguing contractions. elbow extensor contractions will be completed with stimulations being administered pre, during, and post contraction. Each muscle contraction will be separated by 20 seconds to reduce fatigue effects unless fatigue is intentionally being introduced at a specific component of the session. These contractions and stimulations will take place with the shoulder flexed at 0 degrees (with your arm by your side)

**TESTING SESSION 3:**
Will begin with a 5-minute warm-up on a Monark ergometer at an intensity of ~50rpm and 50 watts. After warm-up, participants will be positioned as described above for an elbow extension MVC. Following the MVC the same protocol as testing session will be administered consisting of maximal and submaximal contractions. Immediately (within 5s) post contraction participants will receive the stimulation protocol. The alteration from

testing session 2 is that now the shoulder will be flexed at 90 degrees ( with the elbow pointing forward and the hand pointed to the ceiling

*General stimulation procedures:* Corticoneuron, spinal motoneurone and muscle excitability will be assessed by recording muscle activity in response to stimulation of the brain, spinal cord, nerve and muscle. To do this, it will be necessary to place recording electrodes over the muscle and also to apply magnetic stimulation to the motor cortex and electrical stimulation to, (1) the back of the neck close to the bottom of your skull electrical stimulation of the nerve (2) to nerve, located just above the collar bone and (3) the muscle. Measurements will be taken during each muscle contraction.

**Length of time:**
Participation in this study will require you to come to a lab located in the School of Human Kinetics and Recreation at Memorial for three testing sessions. The total time commitment will be approximately 5 hours (session 1: 1 hour, session 2 & 3: 2 hours each). You will be asked to not engage in weight training or vigorous exercise prior to all sessions. The following table outlines the testing schedule:

| TESTING SESSION | PROCEDURE |
|---|---|
| **1** | Familiarization |
| **2** | Corticospinal excitability/force relationship measurements with the shoulder flexed at 0 degrees |
| **3** | Corticospinal excitability/force relationship measurements with the shoulder flexed at 90 degrees |

**Withdrawal from the study:**
You will be free to withdraw from this study at any point. To do so you simply need to inform the researchers and you will be free to leave. Any data collected up to this point will not be used in the study and will be destroyed. In addition, you may request for the removal of your data at any time up to one year later. If you are a student, your participation in and/or withdrawal from this study will not in any way, now or ever, negatively impact either your grade in a course, performance in a lab, reference letter recommendations and/or thesis evaluation.

**Possible benefits:**
The benefit of participating in his study is that you will learn about the functioning of your nervous system. You will also be aiding our basic understanding of how the nervous system responds to repeated submaximal contractions. This investigation is important because until we understand the basic mechanisms controlling motoneurone and muscle excitability at altrered shoulder positions we cannot fully understand mechanisms of

impaired motor function. The findings of this research may be used for guiding rehabilitation strategies and exercise interventions for clinical and non-clinical populations.

**Possible risks:**
There are several minor risks associated with participating in this study:

1) You will have electrodes placed on the front and back of your arm. These electrodes have an adhesive that has a tendency cause redness and minor irritation of the skin. This mark is temporary (usually fades within 1-2 days) and is not generally associated with any discomfort or itching.

2) The electrical stimulations will cause twitching of the muscles and mild discomfort, but is not painful. The sensation has been described as if you flicked your neck and arm muscles firmly with a finger. The sensation will be very brief (less than a second) and will in no way result in any harm to either muscles or skin.

3) Electrical stimulation used to assess spinal excitabliy is applied at the base of the skull between the mastoid processes. This will cause twitching of the neck musculature resulting in head movement and a transient unpleasant sensation (some participants do not experience any discomfort, myself included).

4) Transcranial magnetic stimulation used to assess motor cortex excitability is applied at ~ the apex of the skull. This will cause activation of the motor cortex resulting in small muscle contraction (most individuals do not experience any discomfort).

5) Post experiment muscle soreness, simlilar to that following an acute bout of exercise may also be experienced by some participants.

6) The stimulators used for the experiment are designed for human research, are completely safe and have been used extensively by Drs Power and Button for many years.

**7)** Participants will undergo multiple types of stimulation (top of head and above collarbone) which may cause nervousness and/or anxiety. Participants will be familiarized with the procedure on Day 1 and will be reminded that they are free to withdraw from the study, without prejudice, if they are uncomfortable for any reason.

If for any reason medical attention is needed Mr. Cadigan and Dr. Button are both certified in first aid. If further help is needed the counselling center at MUN is located at UC-5000 which can be reached at 864-8874.Or available for walk-ins on Monday-Thursdays from 10am-1:00pm, and Monday- Friday from 2:00pm-5:00pm.

**Confidentiality and Storage of Data:**

a. Your identity will be guarded by maintaining data in a confidential manner and in protecting anonymity in the presentation of results (see below)

*b.* Results of this study will be reported in written (scientific article) and spoken (local and national conferences and lectures) forms. For both forms of communication only group average data will be presented. In cases where individual data needs to be communicated it will be done in such a manner that your confidentiality will be protected (i.e. data will be presented as coming from a representative subject).

*c.* All data collected for this study will be kept in a secured location for 5 years, at which time it will be destroyed. Paper based records will be kept in a locked cabinet in the office of supervisors Dr. Power or Button while computer based records will be stored on a password protected computer in the office of Dr. Power or Button. The only individuals who will access to this data are those directly involved in this study.

*d.* Data will be retained for a minimum of five years, as per Memorial University policy on Integrity in Scholarly Research after which time it will be destroyed.

*e.* The data collected as a result of your participation can be withdrawn from the study at your request up until the point at which the results of the study have been accepted for publication (~1year post study).

**Anonymity:**

Your participation in this study will not be made known to anyone except researchers who are directly involved in this study. In addition to Dr. Button and I, the other researchers, who are all master students, required with acquiring data collection are:

1. Laura Gale
2. Michael Monks
3. Brandon Collins
4. Garreth Kippenhuck
5. Mitchell Brenton

**Recording of Data:**

There will be no video or audio recordings made during testing.

**Reporting of Results:**

The thesis will be publically available at the QEII library. Results of this study will be reported in written (scientific article) and spoken (local and national conferences and lectures). Generally, all results will be presented as group averages. In cases where individual data needs to be communicated it will be done in such a manner that your confidentiality will be protected (i.e. data will be presented as coming from a representative subject).

**Sharing of Results with Participants:**
Following completion of this study please feel free to ask any specific questions you may have about the activities you were just asked to partake in. Also if you wish to receive a brief summary of the results then please indicate this when asked at the end of the form.

**Questions:**
You are welcome to ask questions at any time during your participation in this research. If you would like more information about this study, please contact: Ted Cadigan (ewjc63@mun.ca) or Dr. Duane Button (dbutton@mun.ca)

The proposal for this research has been reviewed by the Interdisciplinary Committee on Ethics in Human Research and found to be in compliance with Memorial University's ethics policy. If you have ethical concerns about the research (such as the way you have been treated or your rights as a participant), you may contact the Chairperson of the ICEHR at icehr@mun.ca or by telephone at 709-864-2861.

**Consent:**
Your signature on this form means that:

- You have read the information about the research.
- You have been able to ask questions about this study.
- You are satisfied with the answers to all your questions.
- You understand what the study is about and what you will be doing.
- You understand that you are free to withdraw from the study at any time, without having to give a reason, and that doing so will not affect you now or in the future.
- You understand that any data collected from you up to the point of your withdrawal will be destroyed.

If you sign this form, you do not give up your legal rights and do not release the researchers from their professional responsibilities.

**Your signature:**
I have read and understood what this study is about and appreciate the risks and benefits. I have had adequate time to think about this and had the opportunity to ask questions and my questions have been answered.

☐ I agree to participate in the research project understanding the risks and contributions of my participation, that my participation is voluntary, and that I may end my participation at any time.

☐ I wish to receive a summary of the results of this study Please provide an e-mail address where this summary can be sent:

_____

Signature of participant Date

**Researcher's Signature:**
I have explained this study to the best of my ability. I invited questions and gave answers. I believe that the participant fully understands what is involved in being in the study, any potential risks of the study and that he or she has freely chosen to be in the study.

Signature of Principal Investigator Date