Understanding changes in peripheral and central excitability following submaximal contractions

By

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Abstract

The objective of this thesis was to examine the effects of brief (2s), non-fatiguing, submaximal (50% of MVC) and intermittent (2s on, 2s off) contractions on measures of central and peripheral excitability. Nine resistance-trained males completed a contraction protocol consisting of 5 such contractions of the elbow flexors. Pre-, immediately post-, and 5 minutes post-contractions the participants received transcranial magnetic stimulation (TMS), transmastoid electrical stimulation (TMES), peripheral nerve stimulation, and motor point stimulation to elicit motor-evoked potentials (MEPs), cervicomedullary-evoked potentials (CMEPs), maximal muscle compound action potentials (Mmax), and peak twitch force (PT), respectively. All MEPs and CMEPs were normalized to Mmax. In addition, correlations between central and peripheral excitability were analyzed in order to determine if the two are separate entities or related. Finally, all measurements were taken both at rest, as well as during a slight (5% of MVC) contraction. This allowed us to determine if the changes in central and peripheral excitability, as well as the correlations between the two, were state-dependent. Results showed an increase in corticospinal excitability (CSE) that was state-dependent, a decrease in spinal excitability that was not state-dependent, and an increase in muscle excitability that was not state-dependent following the contraction protocol. There was a positive correlation between CSE and peripheral excitability that was state-dependent, and a negative correlation between spinal excitability and peripheral excitability that was not state-dependent. Since some of the trends observed were state-dependent, the major finding of this thesis is that results obtained at rest should not be generalized to movement situations.

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Table of Contents

List of Figures

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

MVC	Maximum voluntary contraction
TMES	Transmastoid electrical stimulation
CMEP	Cervicomedullary-evoked potential
PSI	Presynaptic inhibition
TMS	Transcranial magnetic stimulation
MEP	Motor-evoked potential
CSE	Corticospinal excitability
PED	Post-exercise depression
PEF	Post-exercise facilitation
TES	Transcranial electrical stimulation
PT	Peak twitch
PAP	Post-activation potentiation
ADM	Abductor digiti minimi
FDI	First dorsal interosseous
CMAP	Compound muscle action potential
Mmax	Maximal muscle compound potential
EMG	Electromyography
RFD	Rate of force development
RMS	Root mean square
SICI	Short latency intracortical inhibition
LICI	Long latency intracortical inhibition
CNS	Central nervous system
SICF	Short interval intracortical facilitation

List of Appendices

Chapter 1: Review of Literature

1.1: Introduction

Excitability of the central and peripheral nervous systems is malleable, and as such can be altered by a muscle's contractile history. In many cases, previous voluntary contraction of a target muscle can cause altered excitability lasting well beyond cessation of the contraction. The purpose of the present thesis was to examine the changes in both central and peripheral excitability (as defined below) following a contraction protocol consisting of five brief (2s), non-fatiguing, submaximal (50% of MVC) and intermittent (2s on, 2s off) contractions of the biceps brachii.

In addition to determining whether the contraction protocol has an effect on measures of central and peripheral excitability, the proposed study will also look at the effect of measurement state on these changes. All measurements will therefore be taken both at rest (condition 1), as well as during a slight contraction (condition 2), in order to determine if the observed changes in excitability are state-dependent. Existing literature supports the idea that measurements differ based on the state during which they are taken. However, there has not yet been a study conducted examining whether post-exercise changes in excitability are affected by measurement state, thus providing a rationale for the proposed study.

While there is a plethora of literature surrounding the effect of previous voluntary muscle contraction on central and peripheral excitability, there are currently no studies examining whether the two are separate entities or are connected. In order to fill this gap in the literature, analyses will be performed on the data obtained in this study to determine if there is a correlation between central and peripheral changes in excitability following the aforementioned contraction protocol.

The purpose of this chapter is to review relevant literature and formulate hypotheses for the proposed study. In addition, possible explanations for each hypothesis will be explored. Finally, this chapter will briefly describe how the outcomes will be assessed.

1.2: Central Excitability

For the purposes of this thesis, central excitability will refer to excitability of the central nervous system. The central nervous system is comprised of spinal and supraspinal (i.e. spinal cord and brain) segments. As such, the remainder of this section will henceforth be divided into spinal and supraspinal.

1.2.1: Spinal

Assessing spinal excitability

Transmastoid electrical stimulation (TMES) will be used as the measure of spinal excitability. TMES generates a single descending volley of stimulation by directly stimulating spinal motor neurons (Taylor 2006). This is done by placing stimulating adhesive electrodes over the two mastoid processes on the back of the skull. Electrical current is then passed between the two electrodes via a constant current stimulator. Cervicomedullary-evoked potentials (CMEPs) evoked via TMES provide a non-invasive, though transiently painful method of assessing spinal excitability (Taylor and Gandevia 2004). For example, if CMEP amplitude showed an increase post-exercise compared to the pre-intervention values, we could infer that there was an increase in spinal excitability. This method has been successfully used to asses spinal excitability in several similar studies within the field of neurophysiology (i.e. (Philpott et al. 2015) and in

conjunction with transcranial magnetic stimulation (TMS) to elucidate the location of facilitation or depression of central excitability (Aboodarda et al. 2015; Pearcey et al. 2014).

Effect of contraction protocol

Based on the available literature, it seems that CMEPs typically decrease in amplitude following exercise interventions similar to the one being used in the proposed study. Gandevia et al. (1999) reported a decrease in spinal motor neuron excitability following a sustained maximum voluntary contraction of the elbow flexors (10 seconds to two minutes in duration). CMEP amplitudes were, on average, about half of the pre-exercise values. This effect was transient, returning to baseline about two minutes post-contraction. Petersen et al. (2003) also showed decreased spinal excitability, in this case following short duration (10s) contractions of the elbow flexors at 50% of maximum voluntary contraction (MVC). CMEP amplitudes showed a post-exercise depression in the biceps brachii that was greater with maximal effort contractions but still present during submaximal effort contraction. Thus, changes in spinal excitability appear to be intensity-dependent.

Giesebrecht et al. (2011) showed depression of spinal excitability following maximum voluntary contraction (10s) of the first dorsal interosseous. While these contractions were of maximal effort, they were similar to our study in that they did not induce fatigue. Depression of CMEPs in the target muscle was transient in nature, having fully dissipated 15 minutes post-exercise.

Aboodarda et al. (2015) were the first to investigate the effects of brief (2s), non-fatiguing, submaximal (50% of MVC) and intermittent (2s on, 2s off) voluntary contractions on supraspinal and spinal excitability. Eight physically active and resistance trained males

performed the above contraction protocol using the biceps brachii. Results showed a transient depression of CMEPs post-exercise, indicating an immediate decrease in spinal excitability as a result of the exercise intervention.

Hypothesis

Based on the available literature, the first hypothesis is as follows: following the aforementioned contraction protocol, spinal excitability as assessed via TMES will decrease. The decrease will likely be transient in nature, eventually returning to baseline. It is likely that the greatest depression of CMEPs will therefore occur immediately post-exercise.

Potential mechanisms

The exact mechanism responsible for post-exercise depression of spinal excitability is not yet known. A depression of excitability at the spinal level could be the result of a decrease in excitability of the motor neuron itself (Taylor et al. 2006), with possible causes including an increased threshold or longer after-hyperpolarization time. Decreased spinal excitability could also originate at the motor neuron synapse, where a decrease in excitatory neurotransmitter or an increase in inhibitory neurotransmitter could be responsible for the depression.

Petersen et al. (2003) suggest that a depletion of readily available neurotransmitter stores at the synapse could be a potential explanation for the decrease in CMEP amplitude following voluntary contractions that was observed in their study. More recently, it has been suggested that post-contraction reductions in spinal excitability are due to activity-dependent changes at the soma (Khan et al. 2012) and/or changes to the intrinsic properties of spinal motor neurons

(McNeil et al. 2011). Aboodarda and colleagues (2015) speculate that the changes seen in their study likely involve some combination of these mechanisms.

1.2.2: Supraspinal

Assessing supraspinal excitability

Transcranial magnetic stimulation (TMS) will be used as the measure of supraspinal excitability, in conjunction with transmastoid electrical stimulation (TMES). TMS delivers a single pulse stimulus that evokes multiple descending volleys of stimulation in the motor cortex. This is done using a circular coil manually maintained on top of the head and connected to a magnetic stimulator. TMS evokes motor potentials in the contralateral target muscle by stimulating interneurons in the motor cortex, which project onto upper motor neurons that activate the corticospinal tract (Marsden et al. 1983; Rothwell et al. 1987; Rothwell et al. 1991). These motor-evoked potentials (MEPs) elicited via TMS provide a measure of excitability of the corticospinal pathway, which travels all the way from the brain, down the spinal cord, to the muscle in question (Gruet et al. 2014). For example, as corticospinal excitability (CSE) increases, so too does the amplitude of the elicited MEP. By measuring and comparing MEP amplitudes pre and post- intervention, this technique provides a non-invasive method of examining changes in CSE (Kiers et al. 1993).

Because TMES activates the same pathway as TMS subcortically, but is unaffected by altered excitability at the cortical level, CMEPs often offer the most appropriate comparison to allow for interpretation of changes in MEP amplitude (Taylor 2006). If MEP amplitudes remained the same while CMEP amplitudes increased, then it would be likely that facilitation occurred at the spinal rather than supraspinal level. Conversely, if CMEP amplitudes remained

the same while MEP amplitudes increased, then it would be likely that facilitation occurred at the supraspinal as opposed to spinal level. This interpretation should, however, be taken with caution. While we can speculate, we cannot directly compare MEP and CMEP data due to differences in how they activate motor neurons (directly versus indirectly and single versus multiple descending volleys). This method was selected as it has been successfully used in several similar studies within the field of exercise neurophysiology (Aboodarda et al. 2015; Pearcey et al. 2014; Philpott et al. 2015).

Effect of contraction protocol

A common consequence of previous voluntary contraction is a decrease in MEP amplitude elicited via TMS. This is referred to as post-exercise depression (PED), and typically occurs following a fatiguing contraction protocol (Gandevia 2001; Hallett et al. 1995). The vast majority of PED research tends to focus on elucidating the mechanisms of this central fatigue. A less commonly reported effect of exercise is its potential to *increase* excitability of the corticospinal pathway. This is a process known as post-exercise facilitation (PEF). PEF results in an increased response rate, shortened latency, and increased amplitude of MEPs (Norgaard et al. 2000). It occurs with minimal exercise and lasts beyond cessation of the contraction (Brasil-Neto et al. 1993; Samii et al. 1996). The facilitation is, however, transient in nature, with values returning to baseline as rapidly as 15.2 seconds post-exercise (Balbi et al. 2002).

PEF typically occurs following contraction protocols that are shorter in duration and non-fatiguing in nature. It is therefore likely that our contraction protocol will induce PEF of MEPs. The findings of similar research studies are consistent with this hypothesis. For example, Norgaard and colleagues (2000) studied post-exercise facilitation of MEPs using TMS in 15

healthy subjects ranging from 28 to 58 years of age. Their results showed PEF in the biceps brachii following standardized and controlled isometric contractions. Participants were asked to maintain six second contractions at differing intensities (25%, 50%, and 100% of MVC) to determine whether the PEF was dependent on the intensity of the contraction. The results showed no significant difference in PEF with differing contraction intensities. MEP amplitudes were also obtained at differing time delays post-contraction including 500ms, 1000ms, 2500ms, and 5000ms. The results showed that MEPs obtained at 1000ms, 2500ms, and 5000ms were significantly smaller than MEPs obtained at 500ms. So interestingly, PEF was independent of contraction intensity but highly dependent on the time delay from muscle relaxation to delivery of the magnetic stimulus. Additionally, though only to a minor degree, PEF was dependent on the duration of maintained muscular contraction. There was a significant difference between MEP amplitudes obtained at 2s and 6s, with the longer contraction showing greater PEF (time delay of 1000ms).

Samii et al. (1996) studied the effects of exercise on MEPs elicited via TMS and transcranial electrical stimulation (TES) in 18 healthy subjects aged 28 to 63. Participants performed 30 second voluntary isometric contractions of the extensor carpi radialis until fatigue was reached, which in this study was defined as the inability to maintain half maximum force. MEPs were elicited via TMS and recorded from the resting muscle following each exercise period. Their results showed that post-exercise MEPs were greater than twice the pre-exercise values, thus indicating PEF. However, once fatigue was reached, post-exercise MEP amplitudes were approximately 60% of the pre-exercise value, indicating PED. There was a gradual return to baseline over several minutes following the exercise intervention for both post-exercise facilitation and depression of MEPs. Exercise intensity ranged from 10% to 50% of MVC and,

consistent with the findings of Norgaard and colleagues (2000), Samii et al. (1996) found no change in post-exercise MEP facilitation with changes in exercise intensity. Additionally, they found no PEF of MEPs elicited via transcranial electrical stimulation (TES). This is likely due to the fact that the two methods of stimulation have different locations of action. TES acts preferentially on the axon hillock of corticospinal motor neurons, while TMS activates these neurons mostly presynaptically through intracortical connections (Amassian et al. 1987; Day et al. 1987).

Brasil-Neto and colleagues (1993) obtained MEP amplitudes elicited via TMS in six healthy male volunteers, with flexor carpi radialis being the target muscle. Like our contraction protocol, the 30s periods of wrist flexion were not constant, but intermittent contractions. Similar to Samii et al. (1996), the results showed a decrease in MEP amplitude evoked by TMS immediately following a fatiguing task. However, immediately following 30 second periods of wrist flexion that were not performed to fatigue, the amplitudes of the first one or two MEPs were increased, indicating PEF. Once the participant felt fatigued the MEP increase disappeared. Consistent with the findings of Samii et al. (1996), the same effects were not observed with TES.

The purpose of the study conducted by Balbi and colleagues (2002) was to determine whether the duration or intensity of voluntary muscle contractions influenced PEF in healthy subjects. MEPs from the thenar muscles were recorded following contractions of differing durations (5, 15, and 30s) and intensities (10%, 25%, and 50% of MVC). While every combination produced post-exercise MEP facilitation that was comparable, the maximal facilitation was observed with the shortest and strongest muscle contraction (i.e. 5s in duration at 50% of MVC). This combination is the most similar to what will be used in our study.

Aboodarda and colleagues (2015) showed PEF of MEPs assessed at rest following an identical contraction protocol to what will be used in the proposed study (5, 2s contractions at 50% of MVC, with 2s on 2s off), with their target muscle also being the biceps brachii. The results indicated that the observed facilitation was transient, with MEP amplitudes returning to baseline approximately 5 minutes post-exercise.

Hypothesis

Based on the available literature, the second hypothesis is as follows: following the aforementioned contraction protocol, CSE as assessed via TMS will increase. The increase will be transient in nature, returning to pre-exercise values within minutes. It is likely that the greatest facilitation of MEPs will therefore occur immediately post-exercise.

While we cannot directly compare CSE and spinal excitability, the suspected simultaneous decrease in spinal excitability following the contraction protocol indicates that any facilitation of CSE is more likely to be of supraspinal, rather than spinal origin.

Potential mechanisms

While research has yet to elucidate the exact cause of PEF, we can speculate on some potential mechanisms. Firstly, there are three basic physiological mechanisms that can influence the size of a MEP: the number of motor neurons recruited, the number of motor neurons discharging more than once, and the synchronization of TMS-induced motor neurone discharge (Rossini 1990). An increase in MEP amplitude could indicate facilitation anywhere along the corticospinal pathway. However, since a number of studies have shown a decrease in spinal excitability following non-fatiguing contraction protocols (Aboodarda et al. 2015; Gandevia et

al. 1999; Giesebrecht et al. 2011; Khan et al. 2012; Petersen et al. 2003) it is likely that the mechanisms responsible for any facilitation of MEPs observed in our study will be of supraspinal, rather than spinal origin.

Potential mechanisms for an increase in central nervous system excitability at the supraspinal level could include an increase in activation of excitatory neural pathways in the motor homunculus or brainstem, or a decrease in activation of inhibitory neural pathways at either of these sites (Brasil-Neto et al. 1993; Samii et al. 1996). Garry et al. (2004) postulate that post-exercise reductions in intracortical inhibition lead to enhanced input to the primary motor cortex and are therefore responsible for the increased supraspinal excitability.

1.3: Peripheral Excitability

For the purposes of this paper, peripheral excitability will refer to excitability of the muscle itself. In the proposed study the muscle in question will be the biceps brachii.

Assessing muscle excitability

Motor point stimulation will be the method used to assess peripheral excitability. This will be done by placing stimulating electrodes over the distal tendon of the biceps brachii and the motor point (just proximal and medial to the midpoint of the biceps brachii muscle belly). Evoked contractile properties will be measured by stimulating the muscle directly and recording the elicited force output (i.e. twitch force). By measuring and comparing peak twitch force (PT) pre and post- intervention, this technique provides a measure of change in the intrinsic excitability of the target muscle. For example, if the elicited PT is greater post-exercise compared to the baseline values, it would be reasonable to conclude that the intervention caused

an increase in excitability of the muscle itself. This method was selected as it has been successfully used for this purpose in several similar studies (Behm et al. 2004; Pearcey et al. 2016).

Effect of contraction protocol

Post-activation potentiation (PAP) is a phenomenon that typically occurs in the muscle following contractions that are non-fatiguing in nature. PAP is a process by which the contractile history of a muscle influences the mechanical performance of subsequent muscle contractions (Lorenz 2011). As a result, the force produced by a muscle is increased because of its previous contraction. In other terms, excitation of the peripheral nervous system produces an increase in contractile function during subsequent contractions (Rixon et al. 2007). PAP is customarily induced by non-fatiguing voluntary contractions of short duration, while fatiguing contractions have the opposite effect, impairing subsequent muscle performance (Stone et al. 2008). The most common indicator of PAP is an increase in evoked isometric twitch force (Mitchell and Sale 2011), as discussed above.

Vandervoort et al. (1983) were among the first to study PAP following voluntary contraction of the target muscle. They found that the extent of PAP was intensity-dependent, with the amount of potentiation and the exercise intensity increasing linearly until fatigue was reached. Their results also showed that brief and submaximal contractions were capable of inducing PAP, though to a lesser degree than maximal contractions. Consistent with these findings were those of Mitchell and Sale (2011) who concluded that while PAP is most commonly induced by maximum voluntary contractions, it can also occur following submaximal isometric contraction protocols.

Behm and colleagues (2004) investigated the effect of a warm-up consisting of maximal voluntary contractions of the leg extensors on force and activation of subsequent contractions in the same muscle. Both evoked and voluntary isometric contractions were tested. Nine healthy and physically active male subjects were tested for twitch, tetanic, submaximal (30% of MVC) and maximal voluntary contractile properties pre and post-contractions (1, 5, 10, and 15 minutes post). Each subject performed one, two, or three 10s MVCs as the warm-up protocol. The results showed that MVC force either did not change (as was the case with one or two MVCs) or was depressed (as was the case with the 10 and 15 minute measurements taken following 3 MVCs). MVC activation was decreased post-contractions, while submaximal contractions were minimally affected. Overall, twitches were potentiated post-contractions, but three MVCs had a significantly greater twitch potentiation than one or two MVCs at 5 and 10 minutes post-contractions. It was concluded that voluntary and evoked contractile properties respond differently to 10s MVCs. Voluntary performance was not enhanced following 1-3 10s MVCs, while evoked contractions were enhanced. While these results do indicate PAP following isometric contractions, their study differed from ours in that they used a lower limb as opposed to an upper limb muscle, and maximal instead of submaximal contractions. Similar to our study, however, was the fact that their exercise protocol was non-fatiguing in nature.

Hypothesis

To our knowledge, there are currently no other studies examining evoked contractile properties using brief, non-fatiguing, submaximal and intermittent contractions. However, based on the available literature using submaximal exercise interventions and non-fatiguing isometric contractions, the third hypothesis is as follows: PAP will be observed in the proposed study. In

other words, peripheral excitability as assed via motor point stimulation will increase following the aforementioned contraction protocol.

To summarize, with respect to changes in excitability following the contraction protocol, previous literature suggests that the results of the proposed study will show: 1) an increase in supraspinal excitability, 2) a decrease in spinal excitability, and 3) an increase in muscle excitability.

Potential mechanisms

Unlike mechanisms of central excitability, causes of PAP are known. PAP can be attributed to the following mechanisms: 1) calcium kinetics, 2) regulatory light chain myosin phosphorylation, and 3) muscle stiffness. Firstly, following a voluntary contraction there may be calcium that has yet to be sequestered. The following twitch will therefore have the calcium that is released as a result of the single stimulus, as well as the calcium that is left over from the previous contraction. More actin active sites will subsequently open up, thus producing a greater force (Ismailov et al. 2004). With respect to myosin phosphorylation, myosin regulatory light chains can remain phosphorylated for as long as ten minutes post-contraction (Houston and Grange 1990). This causes the actin-myosin to become more sensitive to the calcium released from the sarcoplasmic reticulum during the twitch, thus increasing the observed twitch force (Grange et al. 1993; Sweeney et al. 1993). Finally, stiffness of the muscle in question can contribute to PAP. Following a contraction, the target muscle becomes stiffer due to residual crossbridge attachments and to a lesser degree, greater blood perfusion of the tissues. A stiffer musculotendinous unit will allow for a more efficient transfer of force following a single pulse of stimulation (twitch) (Hodgson et al. 2005).

1.4: Measurement State

The third and final piece of our study is that all measurements will be taken at rest, as well as during a slight (5% of MVC) contraction. Hess and colleagues (1987) were among the first to look at the difference between taking measurements at rest versus during voluntary muscle contraction. Hess et al. (1987) used a coil producing a magnetic field of 0.9-1.6 Tesla to deliver a single scalp stimuli which produced a twitch in the right abductor digiti minimi (ADM), first dorsal interosseous (FDI), and adductor pollicis muscles. Compound muscle action potentials (CMAP) and single motor units from these muscles were recorded. The twitch force and corresponding CMAP were greatly enhanced by having the participant perform a voluntary background contraction of the muscle. When the ADM went from total relaxation to a slight contraction, the onset latency of the CMAP was shortened by approximately 3ms. The threshold for excitation of pathways to the ADM muscle was reduced by voluntary contraction of the contralateral ADM and by contraction of the ipsilateral FDI muscles. Using these contractions, the CMAP of totally relaxed ADM muscle showed a more than 2-fold amplitude increase, a shortening of onset latency that was similar to the measurements taken during a slight contraction of the muscle itself, and single motor units in the ADM muscle discharged earlier. These findings indicate that measurements differ significantly based on the state during which they are taken.

More recent studies have indirectly shown that measurements differ depending on the state during which they are recorded. For example, Pearcey et al. (2014) studied the effects of chronic resistance training on CSE of the biceps brachii during elbow flexion at various contraction intensities. Fifteen male subjects, 8 of whom were chronically resistance-trained and

the other 7 non-resistance trained, performed four sets of 5s elbow flexor contractions at 10 target intensities ranging from 10-100% of MVC. During each contraction subjects received TMS, TMES, and brachial plexus stimulation to determine MEP, CMEP, and Mmax amplitudes of the biceps brachii, respectively. Results showed that MEPs were similar in both groups up to 50% MVC, but MEP amplitudes were lower in the chronic resistance-trained group beyond 50% of MVC. CMEP amplitudes were similar for both groups. The ratios of MEP amplitude/absolute force and CMEP amplitude/absolute force were reduced at all intensities in the chronically resistance-trained subjects compared to the non-resistance trained subjects. It was therefore concluded that chronic resistance training alters supraspinal and spinal excitability, with supraspinal excitability being affected to a greater extent. While the results of this study are not directly relevant to the proposed study, graphs published in the paper do show that MEP amplitudes elicited via TMS vary during different conditions (i.e. 10% contraction vs. 100% contraction) in both groups, indicating that measurements are contraction intensity-dependent. This is not surprising as previous work has shown that MEPs elicited via TMS during a slight contraction will be enhanced compared to MEPs recorded at rest (Kischka et al. 1993).

Hypothesis

Examining research in which measurements were taken during differing states allows us to hypothesize that the measurements themselves will differ depending on the state during which they were taken. For example, MEPs recorded during the 5% contraction will be greater in amplitude than those taken at rest. Though it is less clear how they will differ, it is likely that CMEPs and evoked contractile properties will also show a difference when measured at rest

versus during a 5% contraction. This is because rest and contraction are two very different physiological states, and the neuromuscular system responds as such.

Perhaps the more interesting question is whether the changes in excitability following submaximal contractions will be state-dependent. In the PED, PEF, and PAP studies discussed above, all of the measurements were taken at rest. It is possible that the first to third hypotheses may hold true during the rest condition, but not during the 5% contraction. To our knowledge there is no available research examining the effect of measurement state on these phenomena.

1.5: Correlations between Central and Peripheral Excitability

While we can formulate hypotheses on how central and peripheral excitability will change in response to exercise, it is not yet known if the two are related. To our knowledge, there is no existing research looking at whether changes in CSE and excitability of the muscle itself are separate entities or if one is affected by the other. Our study will attempt to fill this gap in the literature by performing statistical analyses on the data to determine if there are any correlations between changes in excitability at the central and peripheral levels. Logically, it seems likely that the two would be directly proportional. If central excitability increased (i.e. an increase in central drive from the brain to the muscle), the muscle itself would subsequently become more excitable. This relationship could, however, be state-dependent. For example, central and peripheral excitability may show a positive correlation during the rest condition, but the opposite during a slight contraction. Hypothetically, a slight voluntary contraction could cause the muscle itself to become more excitable. If this were the case, we may see a subsequent decrease in central excitability as less central drive would be required to produce the same amount of force output. Behm and colleagues (2004) showed an increase in twitch force and a simultaneous decrease in

MVC following a contraction protocol intended to be a warm-up, and therefore non-fatiguing in nature. If the decrease in MVC could not be attributed to fatigue, then perhaps it was the result of a decrease in central drive. Applying the above logic to this situation, maybe central excitability decreased as a compensatory mechanism following the increase in peripheral excitability.

We hypothesize that central and peripheral excitability will be correlated, but the type of correlation will be state-dependent. Central excitability will directly influence peripheral excitability in the resting condition (i.e. if central excitability increases so too will peripheral excitability, and if central excitability decreases peripheral excitability will show a subsequent decrease as well). In the 5% contraction condition, however, the two will be negatively correlated. In an attempt to compensate for an increase in excitability within the muscle itself, excitability at the central level will decrease. This hypothesis should be taken with caution, as there is currently no available research looking directly at the link between central and peripheral excitability.

1.6: Conclusion

In summary, research shows that both central and peripheral excitability are malleable. One commonly studied method of inducing such changes in excitability is previous voluntary contraction of the target muscle. Contraction history can alter central excitability, both at the spinal and supraspinal levels. An increase or decrease in central excitability is referred to as PEF and PED, respectively, and is assed using TMS. By analyzing MEPs elicited via TMS before and after an exercise intervention, we can assess changes in CSE. Analyzing CMEPs elicited via TMES allows us to further categorize the changes into spinal versus supraspinal. Contraction history can also alter peripheral excitability. Enhancement of peripheral excitability following

voluntary muscle contraction is referred to as PAP. However, research shows that previous contraction can also inhibit peripheral excitability. Motor point stimulation allows us to assess changes in peripheral excitability by analyzing the evoked twitch force before and after an intervention. It remains unclear whether changes in excitability at the central and peripheral levels are related. Furthermore, while research tells us that individual measurements are state-dependent, we do not yet know the effect of state-dependency on phenomena such as correlations between central and peripheral excitability, PEF, PED, and PAP.

Based on the reviewed literature, the following hypotheses were made: 1) spinal excitability will decrease following the contraction protocol, 2) supraspinal excitability will increase following the contraction protocol, and 3) muscle excitability will increase following the contraction protocol. However, the extent (i.e. duration and amount of depression, facilitation, and potentiation) to which these changes occur remains unclear.

While we can reasonably assume that individual measurements will differ based on the state during which they are taken, it is difficult to say whether any observed changes in central or peripheral excitability following the contraction protocol will also be state-dependent. It is also difficult to hypothesize, based on the lack of available research, whether central and peripheral changes will be correlated, and if so will the correlations be state-dependent. While we did speculate that one would influence the other, and that state-dependency may also play a role, the lack of existing literature makes it difficult to formulate hypotheses. Our study is therefore needed to determine the relationship (or lack thereof) between changes in central and peripheral excitability as a result of brief, non-fatiguing, submaximal and intermittent contractions.

1.7: References

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Chapter 2: Co-authorship Statement

My contributions to this thesis are outlined below:

i) I recruited all participants and analyzed all data collected for this thesis, with the help of my fellow masters student Mr. Brandon Collins

ii) With the assistance of Mr. Brandon Collins (masters student) and Miss. Natasha Buckle (masters student), I collected the experimental data for this thesis.

iii) I prepared the manuscript and thesis with the help and guidance of my supervisor, Dr. Duane Button.

iv) Dr. Duane Button provided constructive feedback on the manuscript and thesis.

Chapter 3: Changes in central and peripheral excitability during rest and a 5% MVC of the elbow flexors following an intermittent submaximal isometric contraction protocol.

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3.1: Abstract

The purpose of the current study was 1) to assess the effects of repeated, submaximal voluntary contractions of the elbow flexors on measures of central (i.e. corticospinal and spinal) and peripheral (i.e. muscle contractile properties) excitability and 2) to determine if post-exercise measures of central and peripheral excitability are state-dependent (i.e. rest versus active conditions). Nine and 6 university age males participated in *experiments A* and *B*, respectively and completed two sessions (rest and 5% MVC of the elbow flexors) for each experiment. During each session, participants received 3 stimulation protocols: 1) transcranial magnetic stimulation (TMS: *experiment A*) of the motor cortex or transmastoid electrical stimulation (TMES: *experiment B*) to examine corticospinal excitability (CSE) and spinal excitability, respectively of the biceps brachii, 2) electrical stimulation of the biceps brachii motor point to examine evoked contractile properties of the elbow flexors and 3) electrical stimulation of Erb's point to determine maximal muscle compound action potential (M_{max}). TMS-induced motor-evoked potentials (MEPs) or TMES-induced cervicomedullary evoked potentials (CMEPs), potentiated twitch (PT), rate of force development (RFD), and M_{max} were recorded prior to, immediately, and 5 minutes following a submaximal (50% of MVC) intermittent (2s on, 2s off) elbow flexor contraction protocol. In *experiment A* MEP amplitudes increased (215% and 165% of maximal M wave, $p \leq 0.03$), at 1 and 5s post-contraction protocol, respectively, but did not differ at any other time points thereafter during rest. There was no change in MEPs during the 5% MVC. In *experiment B*, CMEP amplitudes decreased during rest and 5% MVC (21% to 58% of maximal M wave, $p \leq 0.04$) for up to 36s post-contraction protocol but did not differ at any other time points thereafter during rest or 5% of MVC. There was no change in MEPs during the 5% MVC. In *experiments A* and *B*, PT and RFD increased immediately post-contraction protocol

and remained elevated for two minutes (PT: range 122 to 147% increase; RFD: range 118 to 145% increase, $p < 0.05$). Both PT and RFD did not differ between pre- and 5 minutes post-contraction protocol for either the rest or 5% MVC in either experiment. In conclusion, the changes in post-contraction protocol corticospinal and spinal excitability are state- (rest versus 5% MVC) and time- (immediate-post but not at 5 minutes post-contraction protocol) dependent, whereas peripheral excitability is only time-dependent. Corticospinal and spinal excitability are positively and negatively correlated, respectively, with changes in peripheral excitability.

3.2: Key Words: Corticospinal excitability, transcranial magnetic stimulation, post-activation potentiation, post-exercise facilitation

3.3: Introduction

Excitability of the central and peripheral nervous systems is malleable, and as such can be altered by a muscle's contractile history. In many cases, previous voluntary contraction of a target muscle can cause altered central and peripheral excitability lasting well beyond cessation of the contraction. When contraction protocols are prolonged and fatiguing in nature, a depression of excitability is typically observed (Gandevia 2001; Hallett et al. 1995; Stone et al. 2008).

A less commonly reported consequence of exercise is its potential to increase excitability, both at the central (corticospinal pathway) and peripheral (muscle) levels (Samii et al. 1996; Norgaard et al. 2000; Balbi et al. 2002; Vandervoort et al. 1983; Behm et al. 2004; Rixon et al. 2007). One such example is post-exercise facilitation (PEF), which refers to an increase in excitability of the corticospinal pathway following voluntary contractions (Norgaard et al. 2000). A commonly used method of assessing changes in corticospinal excitability (CSE) is to measure motor-evoked potentials (MEPs) elicited via transcranial magnetic stimulation (TMS) of the human motor cortex (Aboodarda et al. 2015). Using this technique, several studies have shown PEF following contraction protocols that are non-fatiguing in nature (Samii et al. 1996; Norgaard et al. 2000; Balbi et al. 2002).

Samii et al. (1996) showed PEF of MEP amplitude that was, on average, greater than twice the pre-exercise value following 30 second periods of voluntary isometric contraction (50% of MVC) of the extensor carpi radialis. Contractions were performed to the point at which participants could no longer maintain half of their maximum force. Norgaard and colleagues (2000) showed PEF in the biceps brachii following standardized and controlled isometric contractions at differing intensities (25%, 50%, and 100% of MVC) and durations (2, 4, and 6s).

PEF was not significantly affected by intensity or duration, but was transient, having dissipated 15.2s post-contraction. Unlike Norgaard et al. (2000), Balbi and colleagues (2002) found that PEF was intensity- and duration-dependent. While PEF was comparable for all intensity (10%, 25%, and 50% of MVC) and duration (5, 15, and 30s) combinations, the shortest and strongest muscle contraction yielded the greatest amount of PEF (5s and 50% of MVC).

While we do not know the exact location of PEF, we do know that it can occur concurrent with decreased cervicomedullary MEP (CMEP) amplitudes, which assess excitability of the corticospinal tract at the spinal level. Several studies have shown depressed CMEPs following non-fatiguing contraction protocols (Aboodarda et al. 2015; Gandevia et al. 1999; Giesebrecht et al. 2011; Khan et al. 2012; Petersen et al. 2003). As such, it is likely that the facilitation of MEPs is of supraspinal, rather than spinal origin.

Excitability of the muscle itself can also be enhanced following non-fatiguing exercise interventions. This is referred to as post-activation potentiation (PAP), a process by which excitation of the peripheral nervous system produces an increase in contractile function during subsequent contractions (Rixon et al. 2007). Vandervoort and colleagues (1983) were among the first to study PAP, showing that brief and submaximal contractions were capable of inducing PAP. Behm et al. (2004) showed PAP, as indicated by an increase in evoked isometric twitch force, following a warm-up consisting of maximal voluntary contractions of the knee extensors.

Though there is a plethora of research to support the fact that central and peripheral excitability can be altered by exercise, there are currently no studies examining the relationship (or lack thereof) between the two. Additionally, it is not yet known if the changes in central and peripheral excitability, and potential correlations between the two, are state-dependent. While we do know that measures of excitability can differ depending on the state during which they are

taken (Hess et al. 1987), we do not know whether phenomena such as PEF, PED, and PAP are state-dependent. This is because all of the measurements in the studies discussed above were taken at rest.

While it is clear that central and peripheral excitability can be altered by exercise, our study will be the first to investigate the effect of brief (2s), non-fatiguing, submaximal (50% of MVC) and intermittent (2s on, 2s off) contractions of the biceps brachii on measures of central and peripheral excitability. In addition, by performing statistical analyses on the data to determine if there are any correlations between changes in excitability at the central and peripheral levels, this study will be the first to assess whether the two are separate entities or somehow related. Finally, all measurements will be taken at rest, as well as during a low intensity contraction (5% of MVC) in order to determine if the changes in central and peripheral excitability, and/or the correlations between the two are state-dependent.

3.4: Materials and Methods

3.4.1: Participants

Nine university age males (178.65 ± 7.43 cm, 82.47 ± 12.38 kg, 24.11 ± 5.25 years) from the university population were recruited for *experiment A*. Six of the nine participants were also recruited for *experiment B*. All participants were instructed to refrain from heavy exercise 24 hours before testing and to follow the Canadian Society for Exercise Physiology (CSEP 2003) preliminary instructions (no eating, drinking caffeine, smoking, or drinking alcohol for 2, 2, 2, or 6 hours, respectively) prior to the start of testing. Participants completed a magnetic stimulation safety checklist prior to participation in order to screen for potential contraindications with magnetic stimulation procedures (Rossi et al. 2009). Participants were verbally informed of the

procedures to be used during testing, and all gave informed written consent. 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.

3.4.2: 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˚. 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 contractions.

3.4.3: Electromyography

Electromyography (EMG) activity was recorded from the biceps brachii muscle 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 2 cm apart (center to center) over the midpoint of the muscle belly of the participant's biceps brachii. 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 remove dry epithelial cells. An inter-electrode impedance of <5 k was obtained 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 cut off 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.

3.4.4: Stimulation conditions

Transcranial magnetic stimulation (TMS)

TMS was used to measure corticospinal excitability. MEPs were recorded from the relaxed biceps brachii muscle as well as during a 5% contraction, using a transcranial magnetic stimulator (Magstim 200, maximal output 2.0 Tesla) with a circular coil (13 cm outside diameter) directly placed over the vertex (Forman et al. 2014). 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 for the activation of the left or right biceps brachii (depending on arm dominance), respectively. The vertex was located by marking the measured halfway points between the nasion and inion and tragus to tragus. The intersection of these halfway points was defined as the vertex. Stimulation intensity was adjusted to elicit either a resting threshold response (≥50μV in ≥ 4 out of 8 trials) or an active threshold response (signal clearly discernable from background EMG in ≥ 4 out of 8 trials) in the biceps brachii depending on the experimental session. Stimulator intensity was then increased by 20%, and this was the stimulation intensity used for the remainder of the experiment (Forman et al. 2014).

Transmastoid electrical stimulation (TMES)

Stimulation was applied via surface electrodes placed over the mastoid processes and current was passed between them (100μs duration, 100-350 mA; model DS7AH, Digitimer Ltd, Welwyn Garden City, UK). Stimulation intensity was adjusted to prevent ventral root activation. To ensure the ventral roots were not activated during the experiment, CMEP responses were closely monitored for any decrease in onset latency (~2ms), which would be indicative of cervical ventral root activation (Taylor, 2006). Stimulation intensity was adjusted to elicit a resting response, in 50% of the trials (i.e. 4 out of 8 trials) in the biceps brachii either during rest or during 5% MVC. Stimulator intensity was then increased by 10% for the remainder of the experiment.

Brachial plexus stimulation

Stimulation of the brachial plexus was used to measure maximal compound muscle action potential (M_{max}). Erb's point was electrically stimulated by placing stimulating adhesive electrodes (Ag-AgCl, 10mm in diameter) on the skin over the supraclavicular fossa (cathode) and the acromion process (anode) (Pearcey et al. 2014). Current pulses were delivered as a singlet (200 μs duration, 150-350 mA) via a constant current stimulator. 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., 10% greater than that required to elicit M_{max}) was used.

Motor point stimulation

Biceps brachii motor point stimulation was used to measure evoked contractile properties. Electrical stimulation was delivered via stimulating adhesive electrodes (Ag-AgCl, 10mm in diameter) placed on the skin over the biceps brachii distal tendon (anode) and the motor point (cathode). The motor point electrode was placed just proximal and medial to the midpoint of the biceps brachii muscle belly (Pearcey et al. 2016). Current pulses were delivered as a doublet (10 ms apart, 100 μs duration, 175-300 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., 10% greater than that required to elicit maximum twitch force) was used for the remainder of the experiment.

3.4.5: Experimental Set-up

Experiment A

Participants completed three experimental sessions, with session 1 being used as a familiarization period and sessions 2 and 3 being the experimental sessions (completed in random order). All three experimental sessions took place on separate days, with 24-48 hrs between sessions.

Submaximal contraction protocol

The exercise protocol consisted of 5 submaximal and intermittent contractions of the elbow flexors. The contractions were performed at 50% of elbow flexor MVC, with 2s of contraction followed by 2s of rest. The 50% target force was displayed on a computer screen and

participants were asked to match their force output to the target force. Participants were fairly successful at matching this intensity, as they were given the opportunity to practice and adjust accordingly during the familiarization session.

Experimental session 1

This session was used for experimental familiarization. Participants completed maximum voluntary contractions (MVCs) and the submaximal contraction protocol. Participants performed two 5 second MVCs of the dominant elbow flexors, with 2 minutes of rest between contractions. If the difference between the two MVCs was greater than 5%, a third MVC was performed. Following completion of the MVCs, participants completed the submaximal contraction protocol. Participants also received the three different types of stimulations to ensure that they were comfortable with each and would be able to complete the experimental sessions.

Experimental session 2

Participants were prepared with EMG electrodes and asked to perform an initial pre-contraction protocol elbow flexor MVC. Thereafter, the location and stimulation intensities for MEP, M_{max} and evoked contractile properties were determined (approximately 15 minutes) during rest. The experimental procedures then began. Pre- and post-contraction protocol measurements consisted of three sets of 30s duration trials, with each 30s trial consisting of a twice repeated sequence of stimulations in the same order (TMS, TMS, and motor point). Each stimulus was separated by 5 seconds. Erb's Point stimulation occurred at 28s within each 30s trial. Each series of neuromuscular testing lasted 90s and included 12 transcranial, 6 motor point and 3 Erb's point stimulations. This sequence was performed pre-, immediately post-, and 5

minutes post-contraction protocol. Approximately 6.5 minutes following the contraction protocol another elbow flexor MVC was performed to assess maximum force production capability.

Experimental session 3

Participants performed the exact same protocol as outlined during session 2, however, during session 3 participants performed a contraction at 5% of their MVC during all three, 90s blocks of testing, as well as during determination of stimulation intensities.

Experiment B

Participants completed only two sessions in *experiment B*, both of which were experimental sessions, as all six participants had completed *experiment A thus negating the need for* a familiarization session. The experimental sessions took place on separate days and in random order, with 24-48 hrs between sessions. Experimental sessions 1 and 2 were identical to the experimental sessions 2 and 3 outlined in *experiment A*, with one major difference. In *experiment B*, TMS was replaced with TMES to assess spinal excitability. All other portions of the protocol remained the same. See Figure 2 A and B for experimental set-up.

3.5: Data and Statistical Analysis

3.5.1: Data Analysis

The peak-to-peak amplitudes and onset latency (i.e. stimulus artefact to the onset of the evoked potential) were measured for MEP, CMEP and M_{max} responses. Onset of MEP, CMEP and M_{max} were defined as the point at which the voltage trace became tangent to baseline in either the positive or negative direction and were taken from the averaged unrectified traces.

Changes in MEP and/or CMEP amplitude or latency could be due to changes at the peripheral level (i.e. muscle). Thus, all MEPs and CMEPs were normalized to the recorded M_{max} within the same 30s trial.

Evoked contractile properties of the biceps brachii [peak twitch force (PT) and rate of force development (RFD)] elicited via motor point stimulation were assessed as a measure of peripheral excitability. . PT was defined as the peak-to-peak amplitude of the twitch response and RFD was defined as the amplitude of the twitch response divided by the time it took to reach the maximum amplitude from baseline.

MEP, CMEP, PT and RFD responses were compared two different ways: 1) the overall mean values for all MEPs, CMEPs, PT and RFD (i.e. average of all responses for each variable recorded during each 90s block) was calculated for pre-, immediately post- and 5 minutes post-contraction protocol and 2) each individual MEP, CMEP, PT and RFD response recorded immediately post- and 5 minutes post-contraction protocol were compared to the average (i.e. average of all responses for each variable recorded during the pre-contraction protocol 90s block) of all MEP, CMEP, PT and RFD responses recorded pre-contraction protocol. The second comparison was made because there was no significant main effect for time on pre-contraction protocol MEP (% of M_{max}, $p = 0.558$ and $p = .297$), CMEP (% of M_{max}, $p = 0.073$ and $p = .555$), PT ($p = 0.285$ and $p = 0.14$, *experiment A* and $p = .348$ and $p = .14$, *experiment B*) or RFD ($p = 0.418$ and $p = 0.234$, *experiment A* and $p = .348$ and $p = .343$, *experiment B*) responses during rest and 5% MVC, respectively. Furthermore, we wanted to compare our data similar to that previously reported from our lab (Aboodarda et al. 2015).

For correlations, 2 MEP and 2 CMEP amplitudes (% of M_{max}) were averaged from every 15 second block during the 90s of recording immediately post-contraction protocol and

correlated to the following PT amplitude that was recorded in that given 15s. MEP and CMEP correlations to PT were based on MEP, CMEP and PT amplitude percentage change (at the time points immediately post-contraction protocol) from the pre-contraction protocol average.

The MVC was defined as peak-to-peak amplitude of the force trace. During the MVCs, root mean square (RMS) EMG mean amplitude was measured for 1s at peak force and during each contraction of the contraction protocol RMS EMG mean amplitude was measured for 2s.

3.5.2: Statistical Analysis

Statistical analyses were computed using SPSS software (SPSS 18.0, IBM Corporation, Armonk, New York, USA). Assumptions of sphericity (Mauchley test) and normality (Shapiro-Wilk test) were tested for all of the 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 was performed on all dependent variables to examine within group differences. Because all measures were recorded at rest in one experimental session and during a 5% MVC in the other experimental session and that corticospinal excitability is different between resting and muscle contraction conditions, a two way ANOVA was not performed. A Bonferroni *post hoc* test was performed to test for significant differences between time points. *F*-ratios were considered statistically significant at the $p < 0.05$ levels. Pearson product-moment correlation coefficients between changes in central (i.e. MEPs and CMEPs) and peripheral (i.e. PT) excitability were also performed. Descriptive statistics in text is reported as absolute values whereas in the figures it is reported as mean percentage change. All data are reported as means ± SE.

3.6: Results

3.6.1: Experiment A

Corticospinal excitability

During rest, there was no significant main effect (p=0.360) for time on average MEP amplitude. The overall average MEP amplitude was 4.75±1.22 % of M_{max}, 5.36±1.64 % of M_{max} and 4.60±1.04 % of M_{max} during pre-, immediately-post and 5 minutes post-contraction protocol, respectively. During rest, there was a significant main effect (p<0.01) for time on individual MEP amplitudes (Figure 2A). MEP amplitudes were significantly (p=0.030 and p<0.01) higher at 1s (11.06±6.77, % of M_{max}) and 6s (7.45±3.31, % of M_{max}) post-contraction protocol than the averaged pre-contraction protocol MEP amplitude (4.75±1.22, % of M_{max}).

During 5% MVC, there was a significant main effect (p=0.034) for time on average MEP amplitude. There was a trend (p=0.066) for MEP amplitude immediately post-contraction protocol (14.97±2.99 % of M_{max}) to decrease compared to pre-contraction protocol (16.88±3.07 % of M_{max}). MEP amplitude significantly (p=0.048) increased from immediately post-contraction protocol (14.97±2.99 % of M_{max}) to 5 minutes post-contraction protocol (19.07±4.46 % of M_{max}). During the 5% MVC, there was a significant main effect (p<0.01) for time on individual MEP amplitudes (Figure 2B). MEP amplitude was significantly (p=0.029) lower and significantly (p=0.045) higher at 51s (15.32±3.98, % of M_{max}) and 361s (29.17±8.68, % of M_{max}), respectively, post-contraction protocol than the averaged pre-contraction protocol MEP amplitude (23.15±6.83, % of M_{max}).

During rest, there was no significant main effect (p=0.401) for time on average MEP latency. The overall average MEP latency was 15.0±1.45ms, 15.9±3.62ms and 14.6±0.82ms during pre-, immediately-post and 5 minutes post-contraction protocol, respectively. During 5% MVC, there was no significant main effect (p=0.856) for time on average MEP latency. The overall average MEP latency was 12.4±0.74ms, 12.5±0.61ms and 12.4±0.82ms during pre-, immediately-post and 5 minutes post-contraction protocol, respectively.

Peripheral excitability

During rest, there was a significant main effect (p<0.01) for time on average PT amplitudes. PT amplitude at pre- (64.45±5.98 N), immediately post- (84.56±7.94 N) and 5 minutes post-contraction (69.55±7.26 N) protocol were significantly (p<0.02) different from one another. During rest, there was a significant main effect (p<0.01) for time for individual PT amplitudes (Figure 2C). The PT amplitudes at 11, 26, 41, 56, 71, 86, 311, 326, 341 and 356s (ranging from 91.82±8.63 – 68.96±7.25 N) post-contraction protocol were significantly (p<0.04) higher than the averaged pre-contraction protocol PT amplitude (63.86±5.98 N). The PT amplitudes recorded 11, 26, 41, 56, 71 and 86s (ranging from 91.82±8.63 – 80.93±8.43 N) post-contraction protocol were all significantly (p<0.01) higher than those recorded at 311, 311, 326, 341, 356, 371 and 386s (ranging from 77.12±6.96 – 68.08±8.14 N) post-contraction protocol.

During the 5% MVC, there was a significant main effect (p<0.01) for time on average PT amplitudes. PT amplitude significantly (p<0.01) increased immediately post-contraction protocol (89.16±5.85 N) compared to pre-contraction protocol (71.02±5.90 N) and 5 minutes post-contraction protocol (73.76±6.66 N). During 5% MVC, there was a significant main effect (p<0.01) for time on individual PT amplitudes (Figure 2D). The PT amplitudes at 11, 26, 41, 56,

71 and 86s (ranging from 93.52±5.50 – 86.25±6.42 N) post-contraction protocol were significantly (p<0.01) higher than the averaged pre-contraction protocol PT amplitude (71.02±5.90 N) and than those recorded at 311, 326, 341, 356, 371 and 386s (ranging from 70.96±5.73 – 76.05±6.48 N) minutes post-contraction protocol.

During rest, there was a significant main effect (p<0.01) for time on average RFD. RFD at pre- (131.59±17.46 N/s), immediately post- (169.31±20.71 N/s) and 5 minutes post-contraction protocol (141.11±18.59 N/s) were significantly (p<0.03) different from one another. During rest, there was a significant main effect (p<0.01) for time on individual RFD (Figure 2E). RFD at 11, 26, 41, 56, 71, and 86 and 311s (ranging from 181.83±20.68 – 147.22±19.01 N/s) post-contraction protocol were significantly (p<0.04) higher than the averaged pre-contraction protocol RFD (131.59±17.46 N/s). RFD recorded at 11, 26, 41, 56, 71 and 86s (ranging from 181.83±20.68 – 156.03±22.42 N/s) post-contraction protocol was all significantly (p<0.01) higher than those recorded at 326, 341, 356, 371s (ranging from 137.51±18.22 – 142.03±18.75 N/s) post-contraction protocol.

During 5% MVC, there was a significant main effect (p<0.01) for time average RFD. RFD significantly (p<0.01) increased immediately post-contraction protocol (177.53±14.91 N/s) compared to pre-contraction protocol (136.61±13.57 N/s) and 5 minutes post-contraction protocol (139.58±14.72 N/s). During 5% MVC, there was a significant main effect (p<0.01) for time on individual RFD (Figure 2F). RFD at 11, 26, 41, 56, 71, and 86s (ranging from 186.50±18.70 – 162.86±15.76 N/s) post-contraction protocol were significantly (p<0.04) higher than the averaged pre-contraction protocol RFD (131.59±17.46 N/s) and than those at 311, 326, 341, 356, 371 and 386s (139.11±14.14 – 119.07±12.45 N/s) post-contraction protocol.

There was no significant main effect (p=0.859 and p=0.674) for the contraction protocol on Mwave amplitudes during rest and 5% MVC, respectively. Mwave amplitudes ranged from 12.98±1.86 - 13.52±2.08ms and 8.42±1.43 – 9.40±1.90ms during rest and 5 % MVC, respectively.

Correlations between central and peripheral measures of excitability

Changes in central and peripheral excitability (i.e. CSE and PT) showed a significant correlation immediately post-contraction protocol during resting (r=0.883, p=0.01) (Figure 4A) but not 5% MVC (r=0.395, p=0.219) (Figure 4B).

MVC and EMG

There was no significant main effect (p=0.136 and p=0.287) for the contraction protocol on MVC force at rest and 5% MVC, respectively. During rest, MVC values were 470.12±33.28 N and 461.40±25.54 N pre- and post-50% MVC contractions. During 5% MVC, MVC values were 473.28±28.12 N and 449.73±30.10 N pre- and post-contraction protocol.

There was a significant main effect (p=0.013) for contraction number during the contraction protocol on RMS EMG. During rest, RMS EMG was significantly (p<0.01) higher at contraction 5 (0.67±0.14) than contraction 1 (0.57±0.13). During 5% MVC, there was a trend (p=0.059) for RMS EMG to be higher at contraction 5 (0.62±0.11) than contraction 1(0.52±0.09).

3.6.2: Experiment B

Spinal excitability

During rest, there was a significant main effect (p=0.013) for time on average CMEP amplitude. CMEP amplitude significantly (p=0.026) decreased and had a trend (p=0.061) to decrease from pre-contraction protocol (22.54±6.15 % of M_{max}) to immediately post-contraction protocol (13.79±3.67 % of M_{max}) and 5 minutes post-contraction protocol (20.93±5.95 % of M_{max}), respectively. During rest, there was a significant main effect (p<0.01) for time on individual CMEP amplitudes (Figure 3A). The CMEP amplitudes at 1, 5, 16, 21, 31, 36, 46, 51, 61s (ranging from 10.06±3.02 - 15.32±3.79 % of M_{max}) post-contraction protocol were significantly (p<0.05) lower than the averaged pre-contraction protocol CMEP amplitude (22.54±6.15 % of M_{max}).

During 5% MVC, there was a significant main effect (p=0.010) for time on average CMEP amplitude. CMEP amplitude significantly (p=0.020 and p=0.011) decreased immediately post-contraction protocol (15.47±1.72 % of M_{max}) compared to pre-contraction protocol (20.57±2.53 % of M_{max}) and 5 minutes post-contraction protocol (19.55±1.96 % of M_{max}). During 5% MVC, there was a significant main effect (p<0.01) for time on individual CMEP amplitudes (Figure 3B). The CMEP amplitudes at 1, 5, 21, 36, 76 and 81s (ranging from 10.26±1.58 - 17.62±3.10 % of M_{max}) post-contraction protocol were significantly (p<0.03) lower than the averaged pre-contraction protocol CMEP amplitude (20.57±2.53 % of M_{max}).

During rest, there was no significant main effect (p=0.357) for time on average CMEP latency. The overall average CMEP latency was 7.5±0.57 ms, 7.8±0.66 ms and 7.6±0.60 ms during pre-, immediately-post and 5 minutes post-contraction protocol, respectively. During 5% MVC, there was no significant main effect (p=0.988) for time on the average of all CMEP

latencies. The overall average CMEP latency was 8.2±0.68 ms, 8.3±0.63 ms and 8.2±0.51 ms during pre-, immediately-post and 5 minutes post-contraction protocol, respectively.

Peripheral excitability

During rest, there was a significant main effect (p<0.01) for time on average PT amplitude. PT amplitude significantly (p<0.01) increased immediately post-contraction protocol (91.30±6.38 N) compared to pre-contraction protocol (69.16±6.69 N) and 5 minutes post-contraction protocol (73.41±8.04 N). During rest, there was a significant main effect (p<0.01) for time on individual PT amplitudes (Figure 3C). The PT amplitudes at 11, 26, 41, 56, 71 and 86s (ranging from 99.77±4.59 – 86.08±7.65 N) were significantly (p<0.05) higher than the averaged pre-contraction protocol PT amplitude (69.16±6.69 N) and than those at 311, 326, 341, 356, 371 and 386s (ranging from 74.68±5.44 - 72.25±8.34 N) post-contraction protocol.

During 5% MVC, there was a significant main effect (p<0.01) for time on average PT amplitude. PT at pre- (70.89±4.36 N), immediately post- (92.97±4.75 N) and 5 minutes post-contraction protocol (77.10±4.99 N) were significantly (p<0.04) different from one another. During 5% MVC, there was a significant main effect (p<0.01) for time on individual PT amplitudes (Figure 3D). The PT amplitudes at 11, 26, 41, 56, 71 and 86s (ranging from 97.58±2.22 – 87.40±6.43 N) were significantly (p<0.05) higher than the averaged pre-contraction protocol PT amplitude (70.89±4.36 N) and than those at 311, 326, 341, 356, 371 and 386s (ranging from 78.38±4.22 – 75.34±3.81 N) post-contraction protocol.

During rest, there was a significant main effect (p<0.02) for time on average RFD. RFD significantly increased (p<0.01) and had a trend (p=0.053) to increase immediately post-contraction protocol (181.27±18.43 N/s) compared to pre-contraction protocol (147.43±17.61

N/s) and 5 minutes post-contraction protocol (145.09±18.92 N/s). During rest, there was a significant main effect (p<0.01) for time on individual RFD (Figure 3E). RFD at 11, 26, 41, 56, 71 and 86s (ranging from 195.42±12.13 – 170.11±24.98 N/s) post-contraction protocol were significantly (p<0.05) higher than the averaged pre-contraction protocol RFD (147.43±17.61 N/s) and than those at 311, 326, 341, 356, 371 and 386s (ranging from 130.22±21.59 – 158.75±20.40 N/s) post-contraction protocol.

During 5% MVC, there was a significant main effect (p<0.01) for time on average RFD. RFD at pre- (132.96±9.23 N/s), immediately post- (185.17±11.76 N/s) and 5 minutes post-contraction protocol (148.81±12.24 N/s) were significantly (p<0.04) different from one another. During 5% MVC, there was a significant main effect (p<0.01) for time on individual RFD (Figure 3F). RFD at 11, 26, 41, 56, 71 and 86s (ranging from 195.42±12.13 – 170.11±24.98 N/s) post-contraction protocol were significantly (p<0.05) higher than the averaged pre-contraction protocol RFD (147.43±17.61 N/s) and than those at 311, 326, 341, 356, 371 and 386s (ranging from 130.22±21.59 – 158.75±20.40 N/s) post-contraction protocol.

There was no significant main effect (p=0.113 and p=0.297) for the contraction protocol on Mwave amplitudes during rest and 5% MVC, respectively. Mwave amplitudes ranged from 10.14±2.7 – 12.6±2.70ms and 11.24±2.67 – 11.85±2.73ms during rest and 5 % MVC, respectively.

Correlations between central and peripheral measures of excitability

Changes in central and peripheral excitability (i.e. spinal and PT) were correlated immediately post-contraction protocol during rest (r =-0.848, p=0.016) (Figure 4C) and 5% MVC (r =-0.955, p<0.01) (Figure 4D).

MVC and EMG

There was no significant main effect (p=0.363 and p=0.471) for contraction protocol on MVC force at rest and 5% MVC, respectively. During rest, MVC values were 466.82±82.06 N and 453.36±70.87 N pre- and post-contraction protocol, respectively. During 5% MVC, MVC values were 446.07±72.05 N and 442.95±71.82 N pre- and post-contraction protocol, respectively.

There was no significant main effect (p=0.969) for contraction number during the contraction protocol on RMS EMG. During rest, RMS EMG values for contraction 1 and 5 were 0.59±0.16 and 0.58±0.13, respectively and during 5% MVC, RMS EMG values for contractions 1 and 5 were 0.62±0.15 and 0.64±0.16, respectively.

3.7: Discussion

To our knowledge, this study is the first to examine the effects of brief, non-fatiguing, submaximal and intermittent contractions on measures of central and peripheral excitability. This report is also the first to examine the relationship between the two, and whether or not the observed trends are state-dependent. Since there was no change in MVC force output from pre- to post-contractions during rest or 5% MVC we can assume that the changes in excitability discussed below were not influenced by fatigue.

With respect to spinal excitability, the general trend was a decrease in excitability immediately post-contractions that was observed in both the rest and 5% contraction condition. Thus, changes in spinal excitability following the contraction protocol were not state-dependent. While the exact mechanisms of decreased spinal excitability are not yet known, we can speculate on why we may have seen this PED. For example, the contraction protocol could have caused an

increase in PSI which caused a subsequent decrease in spinal excitability (Iles, 1996). Another explanation could be a depletion of readily available neurotransmitter stores at the motor neuron synapse, as was suggested by Petersen and colleagues (2003) following the PED of spinal excitability that was observed in their study. More recent work suggests that PED of spinal excitability is due to activity-dependent changes at the soma (Khan et al. 2012) and/or changes to the intrinsic properties of spinal motor neurons (McNeil et al. 2011). Aboodarda and colleagues (2015) speculate that the PED seen in their study was likely some combination of the two. In the case of our study, the decrease in spinal excitability at rest following the contraction protocol occurred in conjunction with an increase in CSE. Perhaps the decrease, then, was simply a compensatory mechanism.

As alluded to above, the general trend for CSE was a significant increase in the first one or two MEPs immediately post-contractions (Figure 2). This trend was observed in the rest condition but not during the 5% contraction, thus indicating that changes in CSE following the contraction protocol were state-dependent. Since the increase in CSE occurred in conjunction with a decrease in spinal excitability, the facilitation was likely of supraspinal, rather than spinal origin. As mentioned above, this increase in supraspinal drive to the muscle could lead to a decrease in spinal excitability (i.e. less would be needed for the same amount of central output). While this could be the mechanism responsible for the decrease in spinal excitability, it does not explain the increase in supraspinal excitability following the contraction protocol. Similar to spinal excitability, research has not yet elucidated the exact mechanism responsible for PEF of supraspinal excitability. Garry et al. (2004) postulate that post-exercise reductions in intracortical inhibition lead to enhanced input to the primary motor cortex and are therefore responsible for increases in supraspinal excitability. While this could be the cause of the increased supraspinal

excitability observed in our study, further tests would be needed to know for sure (i.e. short latency intracortical inhibition and long latency intracortical inhibition).

The general trend observed for PT force was an increase immediately post-contractions in both conditions. Thus, changes in PT following the contraction protocol were not state-dependent. Unlike with changes in central excitability, the exact mechanisms responsible for this PAP are known. The increase in PT force that was observed in our study following the contraction protocol was likely attributable to some combination of the following mechanisms: 1) calcium kinetics, 2) myosin phosphorylation, and 3) muscle stiffness. Firstly, following a voluntary contraction there may be calcium that has yet to be sequestered. The following twitch will therefore have the calcium that is released from the single stimulus, as well as the calcium that is left over from the previous contraction. As a result, more active sites will be opened, subsequently producing a greater force (Ismailov et al. 2004). With respect to myosin phosphorylation, myosin regulatory light chains can remain phosphorylated for as long as ten minutes following a voluntary contraction (Houston and Grange 1990). This causes the actin-myosin complex to become more sensitive to the calcium released from the sarcoplasmic reticulum during the twitch, thus increasing the observed twitch force (Grange et al. 1993; Sweeney et al. 1993). Finally, stiffness of the target muscle can contribute to PAP since a stiffer musculotendinous unit will allow for a more efficient transfer of force following a single pulse of stimulation (twitch) (Hodgson et al. 2005). The muscle in question becomes stiffer post-contraction due to residual crossbridge attachments and, to a lesser degree, greater blood perfusion of the tissues.

Correlations between central and peripheral excitability showed that there was a strong positive correlation between CSE and PT in the rest condition but not in the 5% contraction

condition, while spinal excitability and PT showed a strong negative correlation that was present in both conditions. This indicates that, at least at rest, central and peripheral excitability are not two separate entities, but are in fact related. Since, to our knowledge, this is the first study to look at the relationship between the two, the mechanisms responsible for these correlations are not yet known. In the case of CSE and PT, perhaps when CSE increases (i.e. an increase in central drive) there is a subsequent increase in excitability at the muscle. Looking at it from the perspective of MEPs, there are three basic physiological mechanisms that can influence the size of a MEP: the number of motor neurons recruited, the number of motor neurons discharging more than once, and the synchronization of TMS-induced motor neurone discharge (Rossini 1990). Any one of these would result in a greater amount of central drive from the brain to the muscle. It seems logical that if there were more input from the CNS, the muscle itself would become more excitable as a result. With respect to spinal excitability and PT, perhaps the negative correlation is a compensatory mechanism. Since spinal excitability decreases post-contractions, maybe peripheral excitability responds by increasing in order to maintain the same amount of output. This idea is consistent with the work of Sale (2002) who suggested that PAP offsets decreases in central excitability.

Interestingly, several of these phenomena seem to be state-dependent. For example, PEF of CSE excitability was present in the rest condition but not during the 5% contraction condition. The PED of spinal excitability, however, was present during both conditions. Similarly, the correlation between CSE and PT occurred only at rest and not during the 5% contraction, while the correlation between spinal excitability and PT occurred during both. Thus, PEF of CSE and the correlation between CSE and PT seem to be state-dependent, while the PED of spinal excitability and the correlation between spinal excitability and PT do not. This could indicate

that there is something occurring at the supraspinal level but not the spinal level during the 5% contraction that causes the observed changes. Perhaps in the 5% contraction condition there is a greater number of motor neurons activated in the corticospinal tract. If more motor neurons were recruited voluntarily, then there would be less remaining available for involuntary activation. As a result, less motor neurons would be activated via TMS, and the size of the MEP would go down. We would therefore see an increase in MEP amplitude following the contraction protocol in the rest condition, but not in the 5% contraction condition. While we can speculate, it should be noted that we cannot comment with any certainty on the cause of the observed differences between the rest and 5% contraction conditions. Though the exact mechanism of the state-dependency remains unknown, it is clear that results obtained from PEF studies should not be generalized to movement situations if the measurements were taken at rest. The results of this study strongly indicate that the phenomenon is state-dependent.

There were some limitations in our study. Firstly, we were unable to use a combination of all stimulation paradigms. Thus, TMS and TMES induced MEPs and CMEPs, respectively were not recorded within the same time frame. As such, we could not directly compare the two. We could only speculate on what the results of each might mean in relation to each other from the patterns shown in the figures. Furthermore, the stimulation techniques used are gross measurements of CSE. It is therefore difficult to draw specific conclusions regarding the exact location of facilitation or depression. Stimulus response curves may be a more reliable and robust measure of CSE to consider for future research (Cirillo et al. 2010). In addition, changes in supraspinal excitability were assessed by measuring excitability of the entire corticospinal pathway and comparing it against excitability of the spinal segment. In order to fully understand changes that are presumed to have occurred at the supraspinal level, short latency intracortical

inhibition (SICI), long latency intracortical inhibition (LICI), and short interval intracortical facilitation (SICF) should be used. This would provide information on specific changes in inhibitory and excitatory pathways within the brain, allowing us to better interpret and understand changes occurring at the supraspinal level.

3.8: Conclusion

Immediately following a bout of 5 brief, non-fatiguing, submaximal and intermittent contractions, CSE (likely suprapsinal excitability) and muscle excitability increase, while spinal excitability decreases. Statistical analyses revealed that these changes in central and peripheral excitability are, in fact, related. While these results hold true in the rest condition, this was not always the case in the 5% contraction condition. It can therefore be concluded that some of these phenomena are state-dependent. As such, researchers should be cautious when generalizing results that were obtained at rest to movement situations.

Future research should focus on the mechanisms responsible for the relationships observed between central and peripheral excitability, as this was the first study to combine the two. The causes of the state-dependency observed in this study are also unknown, and would therefore be a worthwhile direction for future research.

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3.10: References

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3.11: Figure Legends

Figure 1. Experimental set-up and general procedure. **A)** During a rest or elbow flexion at 5% MVC, transcranial magnetic stimulation (TMS) was applied over vertex to activate the motor cortex of the contralateral hemisphere. Transmastoid electrical stimulation (TMES) was applied between the mastoid processes, nerve stimulation at Erb's point and muscle stimulation at biceps brachii motor point. Evoked potentials were recorded from the biceps brachii. **B)** The protocol consisted of TMS (experiment A), TMES (experiment B) and motor point stimulation at 5s intervals and Erb's point stimulation at 28s during a 30s duration time frame. The frame was then repeated 3 times; pre-, immediately post- and 5 minutes post-contractions (top panel). The exercise itself consisted of 5, 50% MVC contractions with 2s rest between contractions (bottom panel).

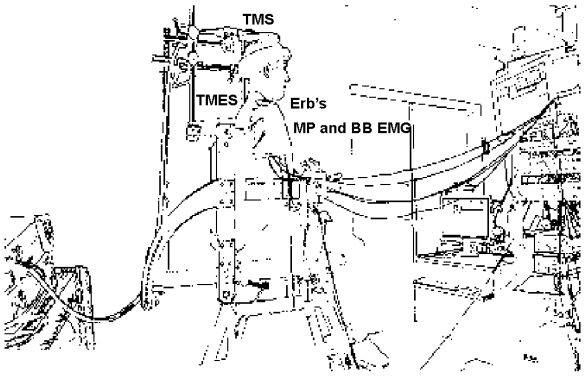
Figure 2. Changes in corticospinal and peripheral excitability following 5, 50% MVCs of the elbow flexors. Percentage change from pre-contractions for motor evoked potentials (MEPs) during **A)** rest and **B)** 5% MVC of the elbow flexors, twitch forces during **C)** rest and **D)** 5% MVC of the elbow flexors and rate of force development (RFD) for twitch force during **E)** rest and **F)** 5% MVC of the elbow flexors. The horizontal dashed line in each panel represents the average of all pre-contraction values. Pairs of diagonal lines represent the 3.5-minute time period between data recording. Each data point represents the group mean ± SE for all time points immediately post- and 5 minutes post-contractions. Asterisk (*) indicates a significant difference ($p < 0.05$) from pre-contraction protocol and (#) indicates a significant difference ($p < 0.05$) from immediately post-contraction protocol.

Figure 3. Changes in spinal and peripheral excitability following 5, 50% MVCs of the elbow flexors. Percentage change from pre-contractions for cervicomedullary motor evoked potentials (CMEPs) during **A)** rest and **B)** 5% MVC of the elbow flexors, twitch forces during **C)** rest and **D)** 5% MVC of the elbow flexors and rate of force development (RFD) for twitch force during **E)** rest and **F)** 5% MVC of the elbow flexors. The horizontal dashed line in each panel represents the average of all pre-contraction values. Pairs of diagonal lines represent the 3.5-minute time period between data recording. Each data point represents the group mean ± SE for all time points immediately post- and 5 minutes post-contractions. Asterisk (*) indicates a significant difference ($p < 0.05$) from pre-contraction protocol and (#) indicates a significant difference ($p < 0.05$) from immediately post-contraction protocol.

Figure 4. Correlations between central and peripheral excitability following 5, 50% MVCs of the elbow flexors. Relationships between motor evoked potentials (MEPs) and peak twitch forces during **A)** rest and **B)** 5% MVC of the elbow flexors. Relationships between cervicomedullary motor evoked potentials (CMEPs) and peak twitch forces during **C)** rest and **D)** 5% MVC of the elbow flexors. The slopes and R^2 values are illustrated for each group. Asterisk (*) indicates a significant correlation.

Figure 1

A



B

Pre-Contraction Protocol MVC

Pre-Contraction Protocol

TMS or TMES	TMS or TMES	MP	TMS or TMES	TMS or TMES	MP	Erb's	
↓ 5s	↓ 5s	↓ 5s	↓ 5s	↓ 5s	↓ 3s	↓	X 3

Contraction Protocol

	2s		2s		2s		2s	
	50% MVC		50% MVC		50% MVC		50% MVC	50% MVC
Contraction #	1		2		3		4	5

Immediately Post-Contraction Protocol

	TMS or TMES	TMS or TMES	MP	TMS or TMES	TMS or TMES	MP	Erb's	
	↓ 5s	↓ 5s	↓ 5s	↓ 5s	↓ 5s	↓ 3s	↓	X 3
Block 1 (s)	1	5	11	16	21	26	28	
Block 2 (s)	31	36	41	46	51	56	58	
Block 3 (s)	61	66	71	76	81	86	88	

5 Minutes Post-Contraction Protocol

	TMS or TMES	TMS or TMES	MP	TMS or TMES	TMS or TMES	MP	Erb's	
	↓ 5s	↓ 5s	↓ 5s	↓ 5s	↓ 5s	↓ 3s	↓	X 3
Block 1 (s)	301	305	311	316	321	326	328	
Block 2 (s)	331	336	341	346	351	356	358	
Block 3 (s)	361	366	371	376	381	386	388	

MVC at ~390s Post-Contraction Protocol

Figure 2

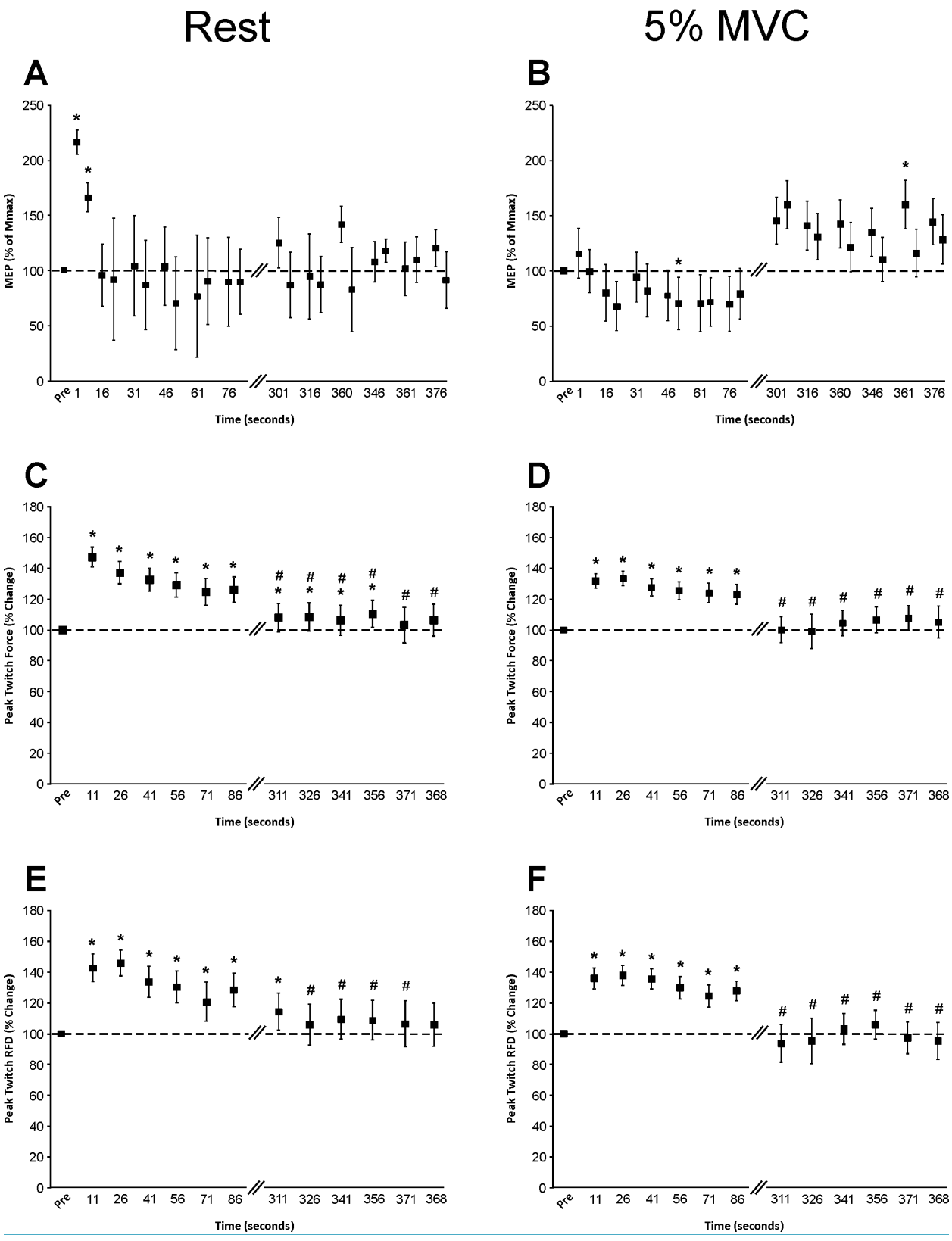

Rest
5% MVC
A
B
C
D
E
F
MEP (% of Mmax)
Peak Twitch Force (% Change)
Peak Twitch RFD (% Change)
Time (seconds)
Pre
1
16
31
46
61
76
301
316
360
346
361
376
11
26
41
56
71
86
311
326
341
356
371
368

Figure 3

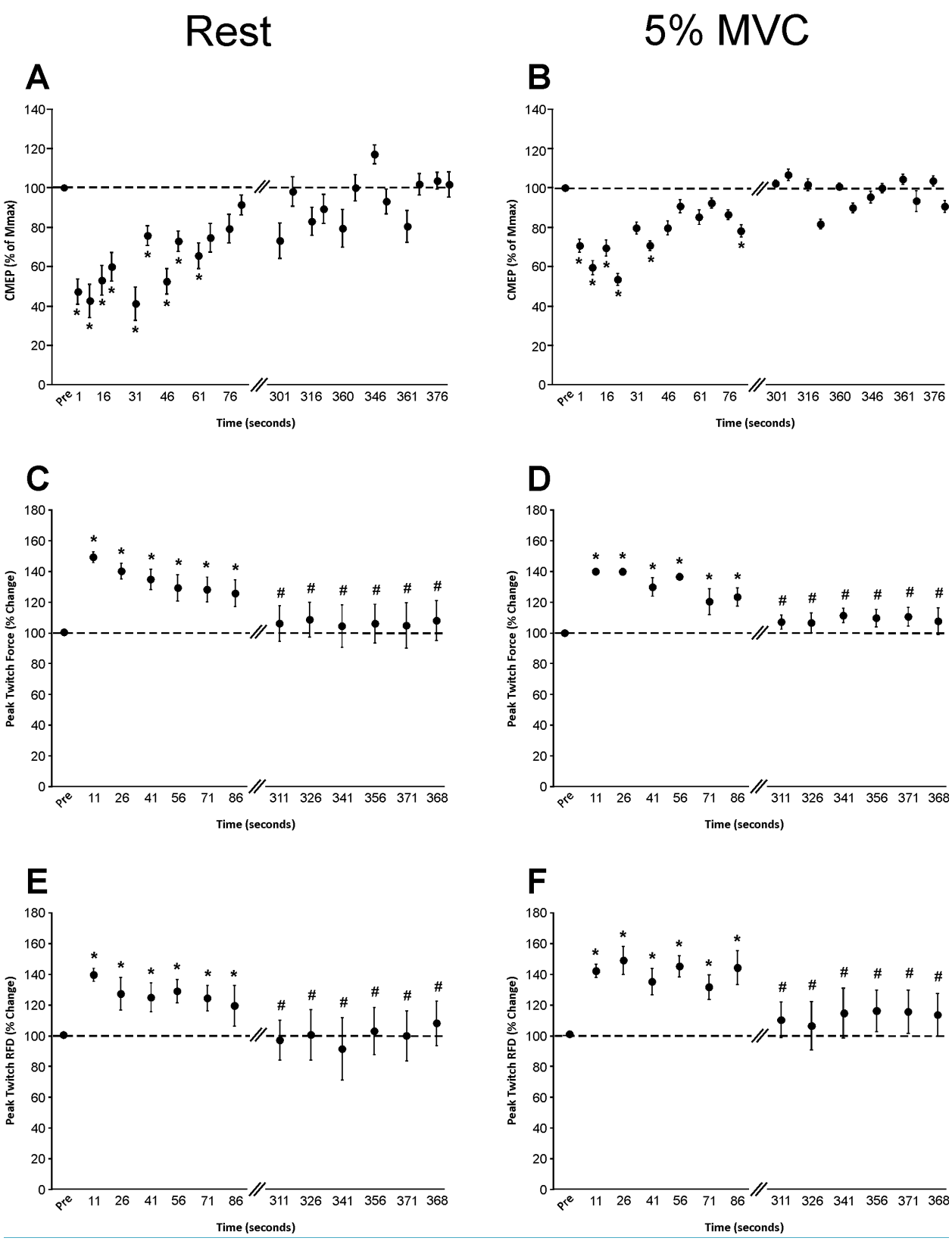
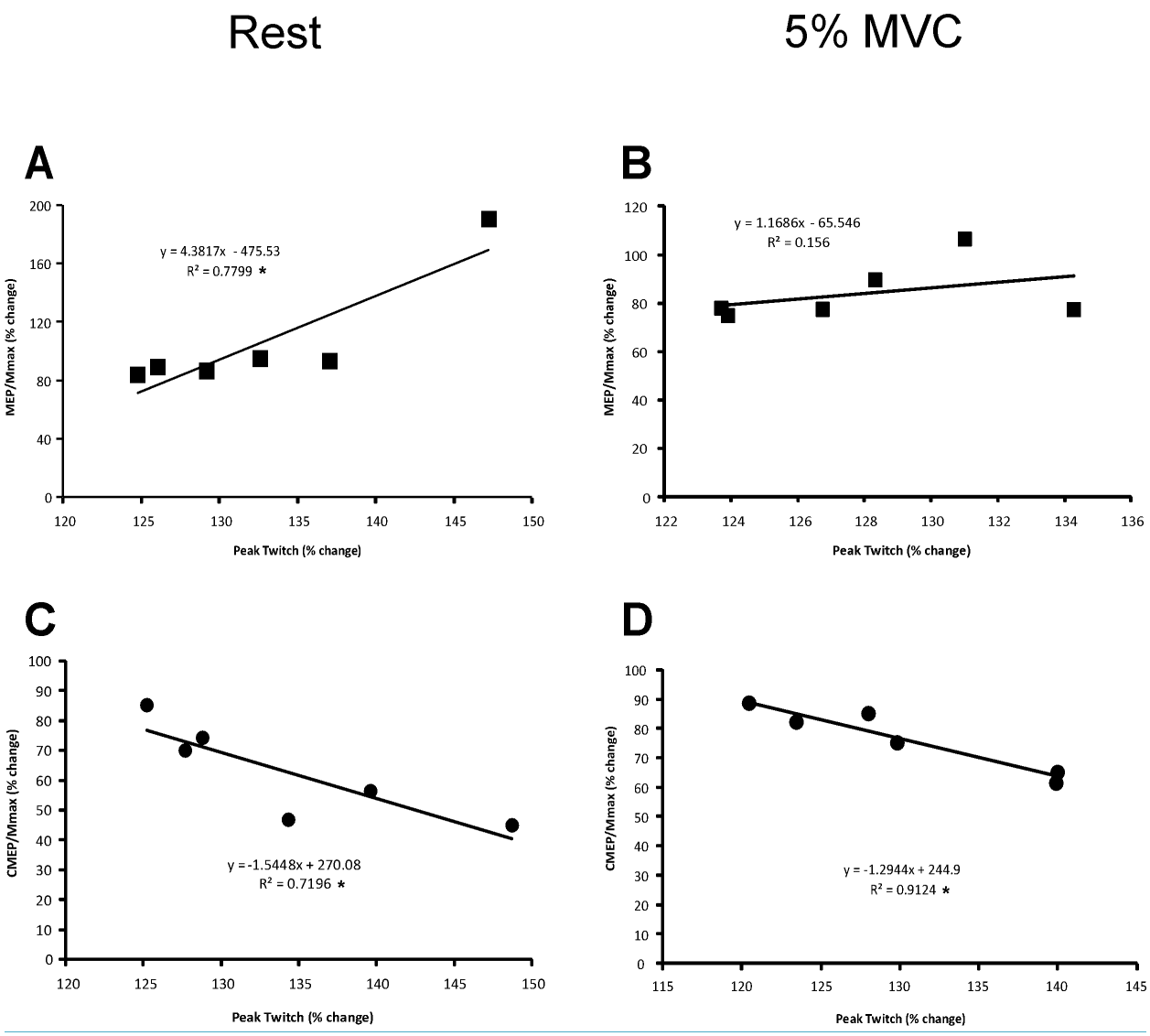

Rest
5% MVC
A
B
C
D
E
F
CMEP (% of Mmax)
Peak Twitch Force (% Change)
Peak Twitch RFD (% Change)
Time (seconds)
Pre
1
16
31
46
61
76
301
316
360
346
361
376
11
26
41
56
71
86
311
326
341
356
371
368

Figure 4


Rest
5% MVC
A
y = 4.3817x - 475.53
R² = 0.7799 *
MEP/Mmax (% change)
Peak Twitch (% change)
B
y = 1.1686x - 65.546
R² = 0.156
MEP/Mmax (% change)
Peak Twitch (% change)
C
y = -1.5448x + 270.08
R² = 0.7196 *
CMEP/Mmax (% change)
Peak Twitch (% change)
D
y = -1.2944x + 244.9
R² = 0.9124 *
CMEP/Mmax (% change)
Peak Twitch (% change)

Appendix A: TMS Safety Checklist

The safety of TMS continues to be supported by recent metaanalyses of published research (i.e. Machii et al., 2006; Loo et al., 2008; Janicak et al., 2008; Rossi et al., 2009). To ensure participant's safety, they were required to complete the following questionnaire prior to receiving TMS.

Magnetic Stimulation safety checklist

Please answer the following questions by circling yes or no.

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:

- Chlorpromazine (Thorazine) ○ Chlorprothixene
- Fluphenazine (Prolixin) ○ Flupenthixol (Depixol and Fluanxol)
- Perphenazine (Trilafon) ○ Thiothixene (Navane)
- Prochlorperazine (Compazine) ○ Zuclopenthixol (Clopixol and Acuphase)
- Thioridazine (Mellaril) • Butyrophenones:
- Trifluoperazine (Stelazine) ○ Haloperidol (Haldol)
- Mesoridazine ○ Droperidol
- Promazine ○ Pimozide (Orap)
- Triflupromazine (Vesprin) ○ Melperone
- 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:	The effect of training status and muscle activity level on supraspinal and spinal excitability of the biceps brachii following brief, non-fatiguing submaximal contractions of the elbow flexors.
Researcher(s):	Laura Gale; School of Human Kinetics and Recreation; lhg163@mun.ca Brandon Collins; SHKR; bwc568@mun.ca Michael Monks; SHKR; mmonks@mun.ca
Supervisor(s):	Dr. Duane Button; SHKR; dbutton@mun.ca Dr. Kevin Power; SHKR; kevinp@mun.ca

You are invited to take part in a research project entitled "The effect of training status and muscle activity level on supraspinal and spinal excitability of the biceps brachii following brief, non-fatiguing submaximal contractions of the elbow flexors."

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. 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, Laura Gale, if you have any questions about the study or would like more information 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:

My name is Laura Gale, and I am a Masters Student within the School of Human Kinetics and Recreation. As part of my Master's thesis I am conducting research under the supervision of Dr. Duane Button.

Purpose of study:

The neuromuscular system is capable of undergoing both acute and chronic adaptations in response to increases or decreases in physical activity levels. Aboodarda, Copithorne, Pearcey, Button, and Power (2015) looked at the effects of repeated muscle contractions on supraspinal (brain) and spinal responsiveness or excitability in physically active, resistance-trained males and found that brief, non-fatiguing intermittent submaximal voluntary contractions transiently enhanced supraspinal excitability while decreasing spinal excitability. The purpose of this study is to assess the effects of repeated muscular contractions on supraspinal and spinal responsiveness or excitability in trained compared to untrained males.This study is important because until we understand the basic mechanisms controlling motoneurone and muscle excitability 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 both clinical and non-clinical populations.

What you will do in this study:

For this study you will be asked to come to the lab on 3 separate occasions. The study involves the use of stimulation techniques which can cause some discomfort but are generally not found to be painful. Below is a brief description of these techniques. They will be discussed in further detail during your first lab session, and you are free to withdraw after that session if you do not feel comfortable with the techniques in question.

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. In order to do this, it will be necessary to place recording electrodes over the muscles being tested. Magnetic stimulation will be applied to the motor cortex and electrical stimulation to, (1) the back of the neck close to the base of your skull (2) the nerve located just above the collar bone and (3) the muscle.

DAY 1 will be used to familiarize you with the testing procedures (i.e. Cervicomedullary motor-evoked potentials (CMEPs), maximal voluntary contraction (MVC), electrical nerve stimulation (Mwave) and muscle stimulation. If you feel comfortable with the stimulation techniques and are willing to be a participant, you will return for two additional testing sessions.

DAY 2 will be used to assess the neuromuscular system before and after five submaximal isometric elbow flexion contractions. You will be seated on a custom-made chair with the elbow flexed to 90° and upper arm fixed to the chair makes 0° with the trunk. The Electromyographic (EMG) activity is recorded form biceps brachii and triceps muscles using self-adhesive electrodes. Your maximal voluntary elbow flexion will first be measured followed by a rest

period of 45 minutes. At the 45 minute mark the stimulation protocols will be administered and measurements made. This is followed by five contractions at ~50% of your maximal effort. You will be asked to hold each contraction for two seconds with a two second rest period. The timing will be maintained via an audible metronome. Post contraction measurements will be made at time points 0s, 30s, 60s, 90s, 120s, 180s and 300s.

DAY 3 is the same as Day 2 with one exception – the post-contraction measurements will be made while you perform a 5-20% MVC as opposed to at rest. This is due to the well-known state-dependent effects of spinal motoneurone excitability. Essentially, measurements taken at rest may be very different when taken during a contraction, even one as small as 5% MVC.

Length of time:
The total length of time required for participation in this study is approximately 3.5 hours (session 1: 30 minutes, sessions 2&3: 1.5 hours).

Withdrawal from the study:

You are free to stop your participation at any point during the data collection, and any data collected up until the point of withdrawal will be destroyed. To withdraw from the study you must simply verbally indicate that you wish to end your participation. There will be no consequences for those choosing to withdraw.

After participation has ended the data can no longer be removed from the study as it will be in aggregate form.

Possible benefits:

The primary benefit of this study to participants is that it will expose them to the research environment and a number of different research technologies. This is of importance given that most of the participants will be students who will be learning about these techniques in the classroom.

This research benefits the scientific community as well as society as a whole because it has the potential to build on our understanding of how the nervous system responds to movement. These findings will be used for guiding rehabilitation strategies designed to maximize the residual motor function of individuals with motor impairments following, for example, spinal cord injury or stroke.

Possible risks:

A possible physical risk of this study is redness or irritation on the skin in the area where the electrodes are attached. This is a very normal reaction to these electrodes. It does not leave a permanent mark, with redness disappearing in 1-2 days.

Course professors will not know who does or does not participate in this study, and it can in no way affect your course grades.

Confidentiality:

The ethical duty of confidentiality includes safeguarding participants' identities, personal information, and data from unauthorized access, use, or disclosure. All data and personal information will be stored on a password protected computer in a lab within the School of Human Kinetics and Recreation. No personal information or participant identities will be included should the findings be released to the public. All data and information collected during this study will be kept for a period of 5 years. Computer files will be stored on a password protected computer and paper records will be kept in a locked filing cabinet in the lab.

Anonymity:

Anonymity refers to protecting participants' identifying characteristics, such as name or description of physical appearance. We will ensure anonymity in this study by assigning each participant a participant code when they are first recruited for the study. In all documents and computer files containing personal information or data from participants this code will be used as the means of identification. The only individuals who will know the link between participant name and code are those directly involved in conducting the research. There will only be one document that links participants to their code. This document will be an information sheet used when the participant is contacted to set-up a time to come to the lab. This sheet will remain in a locked cabinet within the School of Human Kinetics and Recreation. Upon completion of the study this sheet will be destroyed.

A potential limitation to ensuring your anonymity is the fact that the study will be conducted in the School of Human Kinetics and Recreation during normal business hours. As a result, fellow students and other researchers may see you entering the lab and therefore know that you are participating in the study.

Every reasonable effort will be made to ensure your anonymity; and you will not be identified in any publications coming from this study.

Storage of Data:

The only individuals who will have access to and ownership of the data will be the principal investigator, co-investigators and supervisors. Electronic data will be stored on a password protected computer, while hardcopy data will be stored in a locked filing cabinet located in the School of Human Kinetics and Recreation. Data will be kept for a minimum of five years, as required by Memorial University's policy on Integrity in Scholarly Research. After the 5 year period electronic data will be deleted and hardcopy data physically destroyed. The data will not have archival value.

Reporting of Results:

The goal is to publish the results of this study as an article in an academic journal, and as such the results would be publically available online or in print. The findings will be published in my thesis and publically available at the QEII Library. All data will be reported only in an aggregated form.

Sharing of Results with Participants:
This research will be fairly technical in nature and as such the results are likely to be of little interest to the general population. Therefore, we will not be providing participants with results unless they explicitly indicate a desire to have the results communicated to them. Please indicate to the researcher at this time if you wish to be provided with the results of this study.

Questions:
You are welcome to ask questions at any time before, during, or after your participation in this research. If you would like more information about this study, please contact: Laura Gale (lhg163@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 participation in the study without having to give a reason, and that doing so will not affect you now or in the future.
- You understand that if you choose to end participation **during** data collection, any data collected from you up to that point will be destroyed.
- You understand that your data cannot be removed once data collection has ended.

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

Your signature confirms:

☐ I have read what this study is about and understood 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.

☐ A copy of this Informed Consent Form has been given to me for my records.

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