Modulation of corticospinal excitability and short intracortical inhibition during submaximal force outputs of the biceps brachii in chronic resistance trained and non-resistance trained individuals

By:

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A Thesis submitted to the

School of Graduate Studies

In partial fulfillment of the requirements for the degree of

Master of Science (Kinesiology)

School of Human Kinetics and Recreation

Memorial University of Newfoundland

February 2019

St. John's, Newfoundland and Labrador

Thesis Abstract

Most of studies investigating neural mechanisms during isometric voluntary contractions have focused mainly on the corticospinal tract. Little is known about the modulation of the intracortical inhibitory and facilitatory circuits during different levels of muscle activation. Also, studies using a transcranial magnetic stimulation (TMS) to examine neural adaptations have shown that excitability of the corticospinal tract is modulated following chronic resistance training. But, the effects of a long period of resistance training on the modulation of intracortical interactions has not examined yet. The current study was designed to assess corticospinal excitability and short intracortical inhibition (SICI) modulation using two different TMS protocols during different target forces. Using these techniques, we sought to determine whether a central nervous system excitability and SICI system changes as a function of contraction intensity, as well as determine whether these probable changes were similar in chronic resistance trained (RT) and non-RT subjects.

Acknowledgements

Firstly, I would like to express my sincere gratitude to my supervisor Dr. Duane Button for providing me with such an incredible opportunity to pursue my master's degree here at Memorial university. I am grateful for his patience, motivation, knowledge and for always helping me complete my goals. Without Dr. Button this work would not have been possible nor would I be where I am in my career. His efforts go above and beyond what is required of a supervisor.

Secondly, I would like to thank my wife, Mahsa Saeeda, for all her patience, support and for always having my back throughout this process. Thank you so much for helping me to pursue my education away from home.

Thirdly, I would like to thank the faculty of Human Kinetics and Recreation, Memorial University of Newfoundland, for their assistance in knowledge translation in all aspects of my Masters education.

Lastly, I would also like to acknowledge Dr. Kevin Power for all his expertise and guidance, my fellow graduate students Mr. Shawn Wiseman for his assistance throughout the data collection process, and all those who volunteered their time to participate in this study. Without them my thesis would not be possible.

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ABBREVIATIONS LIST

CMEP	- cervicomedullary motor evoked potential
CNS	- central nervous system
CS	- conditioning stimulus
EMG	- electromyography
FDI	- first dorsal interosseous
ICF	- intracortical facilitation
ISI	- inter stimulus interval
LICI	- long intracortical inhibition
MEP	- motor evoked potential
M_{max}	- maximum amplitude of the compound muscle action potential
MSO	- maximum stimulator output
mV	- millivolt
μV	- microvolt
MVC	- maximum voluntary contraction
M-wave	- compound muscle action potential
RT	- resistance trained
s	- seconds

SE - standard error

SICF - short intracortical facilitation

SICI - short intracortical inhibition

TES - transcranial electrical stimulation

TMS - transcranial magnetic stimulation

TMES - transmastoid electrical stimulation

TS - test stimulus

CHAPTER 1:

INTRODUCTION

1.1 Background of the study

Improving muscle strength and performance are two critical goals for rehabilitation and athletic training. Because skeletal muscles are under voluntary control, the modulation of the central and peripheral nervous system during voluntary contraction plays a crucial role in the control of muscle function. Accordingly, the evaluation of the nervous system modulation during different target forces will help us to understand muscle activation. Furthermore, detecting the long-term adaptations of the central nervous system (CNS) to resistance training is another important factor when planning for muscle function improvement.

To find potential mechanisms to understand the modulation of the nervous system, it is necessary to understand how cortical and spinal levels interact to produce voluntary force. Multiple brain regions and neuronal pathways generate movements. One of the principal areas in the brain involved in motor function is the primary motor cortex or M1. This area is traditionally known as a key region to generate neural impulses for the planning and execution of voluntary movements. The secondary motor cortex is another cortex region involved in motor function including the premotor cortex, the posterior parietal cortex and the supplementary motor area (SMA). The only direct pathway from the cortex to the spinal cord is called the corticospinal tract and includes million fibers. This tract is generated by neurons in M1, SMA and premotor cortex. Most of the fibers of the corticospinal tract cross over to the opposite side of the body at the brainstem. After crossing, the fibers continue to descend through the spinal cord, terminating at the appropriate spinal levels. The corticospinal tract is the main pathway contributing to the transmission of central commands leading to activation of spinal motoneurons and consequently, the control of voluntary movement in humans. These fibers synapse onto motoneurons and interneurons in the ventral horn

of the spinal cord. Motoneurons in the spinal cord, or lower motoneurons stimulate muscle contraction (Chouinard & Paus, 2006; Perrey 2013).

With the recent advance in human neurophysiology research techniques since early 19th, especially in stimulation techniques, studies have been conducted to investigate how the different parts of the CNS, from corticoneurones in the brain to the motoneurones in the spinal cord, are modulated during or following a specific task. However, the aim of this study is to focus on intracortical interactions to evaluate how the activity elicited by cortical stimulation may affected by the intracortical circuitry of the M1 area.

1.2 Purpose of the study

To investigate how corticospinal excitability and short intracortical inhibition (SICI) of the biceps brachii will change during different force outputs (15, 25 and 40% of maximum voluntary contraction (MVC)) in chronic resistance trained (RT) and non-RT participants.

1.3 Significance of the study

This work will help us understand how corticospinal excitability and short intracortical inhibition is modulated during various force outputs. Investigating intracortical networks' mechanisms as well as intracortical outputs adaptation to chronic resistance training may, in part, help us understand why individuals who are chronically trained have improved performance during varying motor output intensities compared to untrained individuals. This can be helpful for both rehabilitative and athletic training purposes.

1.4 References

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CHAPTER 2:
LITERATURE REVIEW

2.1 Introduction

The role of nervous system during different tasks have been extensively investigated. Of the studies that have examined the modulation of the nervous system during various target forces, the majority have focused on the corticospinal tract and motoneuron pool. Researchers apply a combination of multiple stimulation techniques to detect the modulation at the supraspinal vs. spinal level. However, very little is known regarding the modulation of intracortical interactions. To the best of my knowledge, only two studies evaluated changes at the cortical network facilitation and inhibition during different target forces, by focusing on the brain areas projecting to small hand muscles like the dorsal interosseous. It has been shown that various strategies are involved in the activation of different muscle groups, especially during higher force outputs. For example, large muscle groups like biceps rely on motor unit recruitment, whereas small hand muscles increase the voluntary force by increasing motor unit firing rate (Martin, Gandevia, & Taylor, 2006). The examination of distal muscles of the upper limb cannot be generalised to all muscle groups, thus further studies are needed. Furthermore, since the motor control function is different between various muscle groups, the intracortical inhibitory and facilitatory circuits projecting to the intrinsic hand muscles are organized differently from those projecting to the proximal arm muscles (Giovanni Abbruzzese, Assini, Buccolieri, Schieppati, & Trompetto, 1999).

Another important consideration for the study design in this field is the training background of the participants. While it is clear that acute and chronic resistance training can change the CNS excitability at the supraspinal and/or the spinal level, it remains unclear how training status affects the modulation of intracortical interactions. A few studies have been conducted to examine the effects of short-term resistance training on the intracortical interactions, however, there is no

investigation regarding the effects of long-term (chronic) resistance training on the intracortical inhibitory and facilitatory circuits.

2.2 Corticospinal Stimulation Technique

While there are a broad range of stimulation techniques/protocols that have been developed to assess corticospinal excitability in humans, for the purpose of this review, only two different TMS protocols will be discussed.

2.2.1 Single-pulse TMS

Applying high-voltage electric stimuli via electrodes on the scalp was the early approach for investigating the CNS excitability modulation. This uncomfortable method is called transcranial electrical stimulation (TES) (Merton & Morton, 1980). However, the neurophysiologic assessment of the central and peripheral nervous system has been dramatically developed by introducing a non-invasive and practically painless technique which is called transcranial magnetic stimulation (TMS) (A. T. Barker, Jalinous, & Freeston, 1985). This technique delivers a magnetic field that can penetrate the cranium virtually unimpeded. The changing magnetic fields in the cortex cause the creation of a current and depolarize cerebral neurons by generating an excitatory or inhibitory neural response (Terao & Ugawa, 2002). The ability of TMS to stimulate deep neural structures, such as the motor cortex, has enabled researchers to assess the integrity of the brain to muscle pathway and the functionality of cortical networks. As mentioned in the introduction, neurons connecting to muscles have their geographical location across the motor cortex. As such, it would be possible to deliver magnetic stimuli to discrete collections of neurons relating to specific muscle groups (Goodall, Howatson,

Romer, & Ross, 2014). Typically, TMS stimulates trans-synaptic pyramidal neurons of the corticospinal tract eliciting the creation of indirect waves (I-waves) which occur approximately 1.5 ms following a direct wave (D-wave) evoked by anodal transcranial electrical stimulation (Chen, 2000; V Di Lazzaro, Oliviero, et al., 1998). This is the most important features that distinguish TES from TMS. TMS-evoked responses are usually recorded from the target muscle group as compound muscle action potentials in the EMG trace and are referred to as motor evoked potentials (MEP) (J. L. Taylor, Petersen, Butler, & Gandevia, 2002). The following properties of the MEP can be used to assess the CNS excitability:

MEP amplitude. The MEP peak-to-peak amplitude is one of the most common measures of corticospinal excitability. The amplitude of a MEP is an essential index because it provides a direct measure of the excitability of cortical and spinal motoneurons (Taube et al., 2006).

The MEP latency. The time interval between the TMS delivery and the MEP onset is noticeably affected by conduction velocities in fast descending corticospinal fibers. Thus, it provides another index of the efficiency of the corticospinal projections (Rossini et al., 2015).

The motor threshold (MT). The MT is a common measure of cortical excitability and refers to the minimum required stimulation intensity to the motor cortex to elicit a reliable and discernable MEP in the target muscle EMG. There are different techniques to determine MT. However, the most common one is to measure the minimum intensity to elicit MEPs of at least 50μV in 50% of a series of consecutive trials. The MT can be measured either at rest (resting motor threshold, RMT) or with minimal tonic contraction (active motor threshold, AMT) (Rossini et al., 2015).

Contralateral silent period (cSP). When a single TMS pulse is delivered during a tonic

contraction of the corresponding muscle, the MEP will be facilitated followed by a period of near-silence in the EMG signal, called the contralateral silent period (cSP). This period of EMG suppression is thought to be mediated primarily by $GABA_B$ receptors at the cortical level (Michelle N McDonnell, Orekhov, & Ziemann, 2006; Werhahn, Kunesch, Noachtar, Benecke, & Classen, 1999). Although the previous findings of the initial and the later parts of the silent period have changed recently, it is still well-known that cortical mechanisms can considerably influence the duration of cSP (Schnitzler & Benecke, 1994).

Similar to MEP, the cSP increases rapidly in response to stimulation intensity increase, until they eventually plateau. However, different neurophysiological mechanisms are responsible for the changes. The MEP is affected by both changes of the membrane and trans-synaptic excitability while the CSP reflects $GABA_B$-mediated inhibitory processes (Groppa et al., 2012).

2.2.2 Paired-pulse TMS

In an attempt to overcome the single-pulse TMS limitations regarding the understanding of the intracortical interactions and modulation of motor cortex output, paired-pulse TMS was developed by Kujirai et al. (1993). This approach allows authors to evaluate intracortical inhibition and facilitation. In this technique, a conditioning stimulus (CS), before a test pulse stimulus (TS), is delivered to the motor cortex via the same coil. By varying the inter-stimulus interval (ISI), the MEP response can be inhibited or facilitated compared to the single-pulse response. It was observed that ISIs between 1 and 5 ms, inhibited the response, while ISIs between 7 and 20 ms facilitated the response (Kujirai et al., 1993). However, a different type of facilitation, SICF, may also occur at ISI of 1-5 ms (Ziemann, Rothwell, & Ridding, 1996). After delivering a paired-pulse

(conditional pulse), the MEP peak-to-peak amplitudes are compared to those produced by the TS alone as a reference condition.

This method is a valuable tool to investigate inhibitory and excitatory circuitry of the human motor cortex (Eldaief, Press, & Pascual-Leone, 2013; Kobayashi & Pascual-Leone, 2003) and has been extensively used to test the modulation of intra-hemispheric interactions within M1 during and/or following voluntary contraction (Ortu, Deriu, Suppa, Tolu, & Rothwell, 2008; Ridding, Taylor, & Rothwell, 1995; Roshan, Paradiso, & Chen, 2003), muscle fatigue (Benwell, Mastaglia, & Thickbroom, 2007; McNeil, Giesebrecht, Gandevia, & Taylor, 2011; Tergau et al., 2000), age-related changes (Kossev, Schrader, Däuper, Dengler, & Rollnik, 2002; McGinley, Hoffman, Russ, Thomas, & Clark, 2010), short-term resistance training (Kidgell, Stokes, Castricum, & Pearce, 2010), and many neurological and psychiatric disorders such as Parkinsons (Ridding, Rothwell, & Inzelberg, 1995), Migraine (Siniatchkin, Kröner-Herwig, Kocabiyik, & Rothenberger, 2007), Dystonia (Ridding, Sheean, Rothwell, Inzelberg, & Kujirai, 1995), Tourette's syndrome (Ziemann, Paulus, & Rothenberger, 1997), Schizophrenia (Daskalakis, Christensen, Chen, et al., 2002), and Huntington's disease (G Abbruzzese et al., 1997).

Some of the intracortical circuits which can modulate the primary motor cortex (M1) output, and can be evaluated by the application of paired-pulse TMS, are as follows:

Short intracortical inhibition (SICI): SICI is elicited when a subthreshold CS delivered prior to a suprathreshold TS at an ISI of ~ 2 ms (Kujirai et al., 1993). It has been shown that SICI is originated from the cortical level (V Di Lazzaro, Restuccia, et al., 1998; Nakamura, Kitagawa, Kawaguchi, & Tsuji, 1997). Although SICI is widely known as a main inhibitory system in the M1 (Kujirai et al., 1993; Nakamura et al., 1997), it does not represent a single inhibitory mechanism. There are two main phases of SICI, at ISI of 1 ms and 2.5 ms. The initial phase is

believed to be related to the neuronal refractoriness (Fisher, Nakamura, Bestmann, Rothwell, & Bostock, 2002), yet, SICI at 2.5 ms, or true SICI, is directly related to the activation of the intracortical inhibitory $GABA_A$ network (Fisher et al., 2002; Roshan et al., 2003). This result was also confirmed by pharmacological studies (V Di Lazzaro et al., 2005; Ilić et al., 2002; Ziemann, Lönnecker, Steinhoff, & Paulus, 1996). Although multiple inhibitory mechanisms are involved to form SICI, it is well known that SICI reflects a balance between intracortical facilitation and inhibition (Ilić et al., 2002; Roshan et al., 2003).

Long intracortical inhibition (LICI): If a suprathreshold CS and TS conditioning stimuli are delivered at an ISI of 50-200 ms to the motor cortex, the MEP response will also be inhibited which is called LICI (Valls-Solé, Pascual-Leone, Wassermann, & Hallett, 1992). While SICI is a well-known standard of TMS application, much less is known about the inhibitory mechanisms at longer ISIs (Reis et al., 2008). It has been shown that unlike SICI, LICI and cSP are mediated by $GABA_B$ intracortical inhibitory activity. However, a $GABA_B$ increase has different effects on LICI and cSP. Accordingly, LICI could be considered as the magnitude of the inhibition whereas cSP is regarded as an estimate of inhibition duration (Michelle N McDonnell et al., 2006).

Intracortical facilitation (ICF): Like SICI, ICF can be elicited by a subthreshold CS. But at a different ISI (between 6 to 25 ms) (Kujirai et al., 1993). Although the physiological mechanisms of ICF are not well understood (Vincenzo Di Lazzaro et al., 2006), some researchers are of the opinion that ICF is mediated by a separate neural population than those related to inhibitory circuits, and could be considered as a separate phenomenon (Ziemann, Rothwell, et al., 1996). Likewise, Liepert, Classen, Cohen, and Hallett (1998) further supports the idea that ICF and intracortical inhibitory mechanisms are independent of one another. Moreover, it has been

suggested that excitatory glutamatergic interneurons within M1 (Ziemann, 2003) and $GABA_A$ activity (Ziemann, 2004; Ziemann, Lönnecker, et al., 1996) may influence ICF.

Short intracortical facilitation (SICF): SICF is a different type of facilitatory reaction within M1 and can be elicited by two suprathreshold stimului at three different ISIs: 1.5, 2.9, 4.5 ms (Ziemann, Tergau, Wassermann, et al., 1998). Since SICF is the summation of different I-waves at corticospinal neurons, it is also known as I-wave facilitation (Hanajima et al., 2002; Ilić et al., 2002; Ziemann & Rothwell, 2000). The pulse intensity and the ISI suitable to elicit SICF partly overlap with those of SICI. This is a reason for SICI reduction at higher intensities of CS (Peurala, Müller-Dahlhaus, Arai, & Ziemann, 2008).

Although the aforementioned physiological interactions are separated into inhibitory and excitatory mechanisms, they are likely to overlap. Accordingly, what is measured by paired-pulse protocol is a net effect. To discuss the influence and the modulation of these interactions separately, stimulus parameters should be considered (Reis et al., 2008).

2.3 The effects of Stimulation Intensity on Corticospinal Excitability Modulation

Motor threshold (MT) intensity to evoke such MEP responses via EMG of various muscles (A. Barker, Freeston, Jalinous, & Jarratt, 1986) have been evaluated in healthy subjects as well as patients with various neurological disorders (Dolberg, Dannon, Schreiber, & Grunhaus, 2002; P. Fitzgerald, T. Brown, Z. Daskalakis, & J. Kulkarni, 2002; Pennisi et al., 2002). Also, it has been shown that MT changes with aging (Kozel et al., 2000) and following the ingestions of various medications (Maeda, Keenan, & Pascual-Leone, 2000). Although there are studies examining MT required to elicit MEP, we lack knowledge regarding the threshold intensity to evoke cortical

activity, and regarding the method to evaluate the spread of the cortical activity to other areas of the brain. It is believed that MT is highly variable between individuals and between sessions (Dolberg, 2002). However, as discussed previously, applying paired-pulse TMS protocol is a useful and reliable method to elicit cortical activity at subthreshold intensities (subthreshold CS delivered at different ISIs prior to TS) (Awiszus, Feistner, Urbach, & Bostock, 1999; Boroojerdi et al., 2000). Other stimulation techniques, like rTMS (Fitzgerald, Fountain, & Daskalakis, 2006) and triple-pulse TMS (Ni, Gunraj, & Chen, 2007) could also be used to evaluate spread of TMS evoked activity within and between hemispheres, inter- and intra-cortical facilitation and inhibition (Cicinelli et al., 2000; Ferbert et al., 1992; P. B. Fitzgerald, T. L. Brown, J. Z. Daskalakis, & J. Kulkarni, 2002).

Komssi, Kähkönen, and Ilmoniemi (2004) studied the effects of amplitude intensity on the TMS evoked neural activity, using electroencephalographic (EEG) responses to TMS. They suggested that the amplitude of the responses increases with stimulus intensity, but, scalp distribution of the cortical activation is similar for different intensities. They concluded that TMS can evoke measurable brain activity at the stimulation activity even below 60% of MT.

Several parameters including the CS intensity, the TS intensity, and the duration of ISI should be controlled when applying a paired-pulse protocol. These parameters affect the magnitude of SICI in healthy participants (Bütefisch et al., 2000; Ilić et al., 2002).

SICI is usually assessed by a CS intensity of 80-90% AMT or 90% RMT, followed by a TS intensity of 120-130% AMT (Kujirai et al., 1993; Ziemann, Tergau, Wischer, Hildebrandt, & Paulus, 1998). AMT is also defined as the lowest stimulus intensity (% MSO) required to elicit a MEP with 1mv peak to peak amplitude (Kujirai et al., 1993; Roshan et al., 2003; Sanger, Garg, & Chen, 2001), or 50 μv peak to peak amplitude (Hunter, McNeil, Butler, Gandevia, & Taylor, 2016).

In addition, it is well known that the relationship between the SICI and CS intensity is a U-shape curve (Chen et al., 1998; Ilić et al., 2002; Kujirai et al., 1993; Ziemann, Rothwell, et al., 1996). At low CS intensities, increasing the CS intensity results in greater SICI. This is likely because of the recruitment of the inhibitory interneurons. However, a further increase in CS intensity results in a reduction of inhibition and eventually facilitation, probably due to involvement of SICF (Chen & Garg, 2000; Tokimura, Ridding, Tokimura, Amassian, & Rothwell, 1996; Ziemann, Tergau, Wassermann, et al., 1998), and ICF (Kujirai et al., 1993; Ziemann, Lönnecker, et al., 1996).

Peurala et al. (2008) investigated the relationship between SICI and SICF by applying different CS intensities at various ISIs. They showed that at high intensities of approximately 90% of AMT, there is possibility of activating both SICI and SICF, yet, SICI is strongest at the ISI of 2 ms. They also reported that at ISI of 2.6 ms, which is the second peak of SICF and because of the contamination by SICF, SICI reduces. Accordingly, they could interpret the U-shape curve relationship of the SICI and CS intensities. At low CS intensities where increasing intensity causes greater inhibition, probably cortical inhibition is altered. But, the reduction of SICI at higher intensities (right half of the U-shape curve) is likely because of the altered facilitation. They also found that ISI is a very important detriment of SICI and SICF interaction. According to the studies mentioned above, it has been suggested to measure use a range of CS intensities to detect whether the changes are because of the altered inhibition or facilitation (Ni & Chen, 2008).

Similar to CS intensity, there is a U-shape curve for the relationship between SICI and TS intensity with maximum inhibition at a TS intensity adjusted to elicit a 1mv MEP (Daskalakis, Christensen, Fitzgerald, Roshan, & Chen, 2002; Sanger et al., 2001). However, the intensity of TS to evoked maximum inhibition may vary according to the examined muscle group. For example, Chen et al. (1998) applied a TS intensity adjusted to produce MEPs of ~300 μV peak-to-peak

amplitude to study SICI changes in rectus abdominus, biceps brachii and quadriceps femoris. Also, Weier, Pearce, and Kidgell (2012) used a TS intensity of 120% of AMT to study SICI reduction in rectus femoris following a short-term resistance training. They determined AMT as the minimum stimulus intensity required to elicit a MEP of at least 200 μV in three of five consecutive trials. McGinley et al. (2010) tried a different approach and defined AMT as the lowest TMS intensity required to evoke MEPs with a peak-to-peak amplitude ≥ two times that present in at least three of six of the voluntary trials. Then, they used TS intensity of 130% of the recorded AMT.

Garry and Thomson (2009) examined the effects of different TS intensities on SICI and observed that, regardless of the excitability state, the estimates of SICI are systematically affected by TS intensity, suggesting the factors that change corticospinal excitability, and consequent MEP size, may confound the interpretation of SICI. The authors also suggested that SICI should be tested by a constant TS intensity, regardless of any changes in corticospinal excitability due to the experiment.

Stimulation intensity is also affected by a large variability between individuals in terms of biological differences (Kozel et al., 2000; McConnell et al., 2001), skull thickness or the pattern of cortical sulcation (Wassermann, 2002). In a comprehensive study by Wassermann (2002), one hundred fifty-one subjects were evaluated for observing the variability and other characteristics of TMS-induced MEP. According to the results of this study: MEP threshold and the paired-pulse ratio (CS/TS) varied widely in healthy subjects, subjects showed inhibition and/or facilitation at all ISIs, there was no correlation with the age and sex, and there was a significant effect of genetics on MEP amplitude. In another attempt to assess the variability of paired-pulse TMS measurements between subjects and between sessions, different intensities of CS were tested on 16 subjects. The

results indicated that the variability is significant if a single CS intensity is used to compare SICI and ICF between subjects. The authors suggested that it is possible to improve the reliability of between-subject comparisons by expressing the CS intensity as a percentage of individual's threshold for SICI and ICF (Orth, Snijders, & Rothwell, 2003). Although a part of the reported variability in the last study could be due to factors such as changes in coil position (they applied figure-of-8 coil which may move during the experiment introduce variability), inherent differences between the electrophysiological properties of the neuronal population and differences in the synaptic efficiency between individuals are probably some of the other underlying mechanisms.

In summary, it would be worthful to mention that regardless of the applied TMS protocol, one of the most important components of the stimulation protocol is determining the right TMS intensity. As mentioned above, it has been shown that the result of the same intervention could be completely different by using different CS and TS intensities. The main reason for this variability is that by applying different stimulation intensities, various components of the corticospinal volley will be activated. By increasing the intensity, early I waves, late I waves and D waves are evoked, respectively. Also, it is important to keep the stimulation protocol and intensity unchanged when testing an intervention. Because, the I waves' population is controlled by intracortical inhibitory and facilitatory circuits and consequently, any minor changes in the stimulation intensity and/or protocol during the experiment, can drastically affect the recorded responses (Di Lazzaro, 2012). It is suggested to determine the best TMS intensity to see maximum inhibition and/or facilitation by considering the following factors: 1) the examined muscle group, 2) the type of intervention, 3) the type of TMS protocol and 4) the research question.

Finally, in most of the reviewed studies, SICI and ICF were assessed during the relaxation of the target muscle. However, SICI reduces markedly during muscle contraction (Fisher et al.,

2002; Orth et al., 2003; Ridding, Taylor, et al., 1995; Roshan et al., 2003). This effect will be extensively discussed in the next part.

2.4 The effects of voluntary contraction on Corticospinal Excitability Modulation

It has been shown that voluntary muscle contraction increases the motor cortex and motoneuron pool excitability (Martin et al., 2006). During contraction, TMS is able to evoke more components of the corticospinal volley in humans compared to the rest position (Hess, Mills, & Murray, 1986). Similarly, the total amplitude of descending epidural volleys in conscious humans was observed to increase by 50% during maximal voluntary contractions (MVCs) compared with rest (Lazzaro et al., 1998). The increase in MEP size from relaxation to weak contraction occurs regardless of the stimulation intensity (McNeil et al., 2011). Moreover, the MEP response to TMS stimulation recorded from the biceps brachii, brachioradialis and adductor pollicis muscle increases significantly as the level of background voluntary contraction increases. However, the increase in MEP has been recorded only during weak contractions (≤50% MVC) (J. Taylor et al., 1997), followed by a plateau and subsequent decrease in both MEP and CMEP responses at particularly high contraction intensities (Martin et al., 2006; Todd, Taylor, & Gandevia, 2003; Pearcey, G. E., Power, K. E., & Button, D. C., 2014).

During strong voluntary contractions, the MEP size decreases with increasing contraction strength. During contractions of 50% MVC, TMS elicited large MEPs in biceps brachii (>90% M_{max}) which decreased in size (to ~70% M_{max}) with maximal effort. The authors suggested that this decrease in MEP amplitude was probably because of the motoneurons inability to fire in response to the excitatory input of the TMS (Todd et al., 2003). In conclusion, the excitability of

the motor cortex and/or motoneuron pool does not continue to increase across the entire contraction range.

Since the amplitude of TMS-induced MEP could be affected by mechanisms which are located anywhere along the corticospinal pathway, using TMS alone is not able to detect the exact site of modulation. Accordingly, a combination of stimulations and/or protocols should be used to differentiate cortical and spinal excitability changes.

Martin, Gandevia, and Taylor (2006) conducted a research to detect the central nervous system site of modulation during voluntary contraction. They investigated MEP responses elicited by stimulation of motor cortex and CMEP responses elicited by trans mastoid electrical stimulation (TMES), that stimulate the descending corticospinal pathway. According to their results, MEP and CMEP responses from the elbow flexors increased from weak contractions to about 50 % MVC and then decreased by about 25% M max from 50% to 100% MVC. Also, MEPs recorded from the first dorsal interosseous (FDI) decreased by about 35% M max during strong contractions. Since no difference was observed between the MEP and the CMEP amplitudes, the authors concluded that the change in corticospinal pathway excitability was due to a spinal mechanism, probably the modulation of the motoneuron pool. Similarly, an investigation about corticospinal-evoked responses in soleus and medial gastrocnemius during plantar flexion at varying contraction intensities (from rest to 100 % MVC) revealed that for both muscles, MEP and CMEP peak-to-peak amplitude increased, followed by a plateau, from weak to very strong contraction intensities (Oya, Hoffman, & Cresswell, 2008). While similarities between the trends of corticospinal excitability modulation in different muscle groups exist, there are differences between the intensity of muscle contraction in which CNS excitability modulation begins, suggesting differences in the

pattern of motor unit recruitment and rate coding when producing voluntary force (Martin et al., 2006).

Investigating the changes of the neural circuitry of the motor cortex is another important assessment that may help us to interpret the result of the cortically initiated MEP. It has been shown that in subjects with focal isolated ischemic lesion of primary motor cortex associated with the arm and leg, that even when the MEP is preserved, loss of cSP induced by TMS can occur. Also, in these cases, spinal SP was normal, suggesting that the origin of cSP is cortical and elicited by the primary motor cortex and probably reflects the activity of inhibitory interneurons within the cortex (Schnitzler & Benecke, 1994).

As previously mentioned, there are several measurements of intracortical inhibition and excitation including SICI, LICI, ICF and SICF. By applying paired-pulse TMS protocol over the cortical motor area of FDI muscle, it was observed that the SICI and the ICF were significantly weaker during the maintenance of a slight contraction of the FDI muscle compared to the rest (Fisher et al., 2002; Ridding, Taylor, et al., 1995; Roshan et al., 2003). However, other studies confirmed the previous result only with the CS ≥ 80% AMT. At lower CS intensities, SICI at resting muscle was not significantly different from SICI during weak muscle contraction (~ 10% MVC) (Ortu et al., 2008; Zoghi, Pearce, & Nordstrom, 2003). As these authors discussed, these differences in these results due to increasing the CS intensity could be interpreted by the activation of the interneurons responsible for intracortical facilitatory circuits. By applying a CS intensity great enough to activate various intracortical circuits, it would be possible to examine the effect of voluntary contraction on SICI system.

Ortu et al. (2008) evaluated SICI and SICF during various target forces (10%, 25% and 50% of MVC) and showed that SICI reduced during voluntary contraction from 10% to 25% of

MVC. The authors hypothesized that intracortical excitability reflects a balance between activation of SICI and SICF system. Accordingly, these two systems are able to influence corticospinal neurons by producing Inhibitory postsynaptic potential (IPSP) and Excitatory postsynaptic potential (EPSP). But, the contribution of each system to the descending volley depends on the condition of the target muscle (rest vs. contracted). At rest, the threshold activation of the SICI system is lower than the SICF system. Therefore, a CS intensity as low as 80% or 90% AMT can activate SICI system. But, this intensity is not great enough to activate the SICF system which has an activation threshold equal to around 100% AMT. In this condition, by applying the classical SICI protocol during the rest, only the SICI system can exert its influence. However, during a weak isometric contraction, the activation threshold of the SICF system is lower and consequently, a CS stimulus of 80–90% AMT is now able to activate both SICI and SICF systems. As a result, the reduction of SICI is seen. As a result, SICI effects on corticospinal neurons reduces progressively at higher forces, which is largely restricted to corticospinal neurons controlling the muscle targeted for activation over the range of forces tested (up to 25% MVC) (Zoghi & Nordstrom, 2007). Also, no significant inhibition of the conditioned MEP is observed at higher force outputs (≥ 25% of MVC) of the FDI muscle (Ortu et al., 2008).

Finally, while most studies of SICI and ICF have been conducted using small muscle groups such as intrinsic hand muscles, it has been reported that relatively similar phenomena occurs across larger muscles such as biceps brachii (Giovanni Abbruzzese et al., 1999), digastric muscles (Jaberzadeh, Pearce, Miles, Türker, & Nordstrom, 2007), quadriceps femoris, and rectus abdominus (Chen et al., 1998). Chen et al. (1998) observed that although the resting and active motor threshold vary between different muscle groups, the CS intensity required to elicit ICI and ICF seems to depend on the strength of corticospinal projection. Also, there was no significant

differences in ISIs between various muscle groups and all showed a relatively high inhibition at ISI of 2ms.

In summary, although the discussed evidence suggests that the stimulation intensity and ISI used for eliciting SICI in different muscle groups are relatively the same, the other specifications of different muscle groups when exerting higher force outputs may play an important role in the qualification of SICI. It is well known that the neural strategies to recruit muscle during higher target forces vary between muscles. This may cause a significant difference between the responses recorded from various muscle groups.

2.5 Neural Adaptation

Although the characteristics and the mechanisms of CNS modulation in response to resistance training is not currently well-known, the existence of neural adaptation following resistance training is accepted (Enoka & Fuglevand, 2001; Sale, 1988). Several studies have been conducted to examine the exercise-induced neural adaptation. Prior to reviewing these studies, it is important to outline neural adaptation. The most accepted explanation for the neural adaptation for changes in muscle activation patterns are changes in motor unit recruitment and/or discharge rate (Carolan & Cafarelli, 1992; Zehr & Sale, 1994).

In 2010, Kidgell and his colleagues conducted two studies to determine the sites of neural adaptation following a short-term strength training of FDI (Kidgell & Pearce, 2010), and biceps brachii (Kidgell et al., 2010). They reported that the corticospinal excitability following an acute resistance training was altered for both muscle groups. Similarly, a significant increase in muscle strength along with MEP amplitude has been reported for the tibialis anterior muscle, following a short-term resistance training (Griffin & Cafarelli, 2007) and the soleus muscle (Beck et al., 2007).

Likewise, M1 excitability modulation, specific MEP amplitude increase, following a simple motor task for as little as 30 min of training, has been well documented (Bütefisch et al., 2000; Muellbacher, Ziemann, Boroojerdi, Cohen, & Hallett, 2001).

In another attempt to detect whether the origin of neural adaptation to resistance training is of supraspinal or spinal, Carroll, Riek, and Carson (2002) carried out a study using TMS and TES and recorded MEP responses during a range of target forces (5 to 60% MVC). Accordingly, the authors suggested the existence of spinal cord properties modulation rather that supraspinal changes, following 4 weeks of resistance training of the index finger.

To interpret the inconsistent result of studies investigating neural adaptation, many authors are of the opinion that the training task and tested muscle group are two key factors. However, T. Carroll, Selvanayagam, Riek, and Semmler (2011) claimed that the neural adaptation to resistance training should have some general applicable principles among various types of resistance training which is the result of performing a repetitive task through the same neural drive.

To the best of my knowledge, only one study utilized paired-pulse TMS protocol to evaluated the effects of neural adaptation on the intracortical interactions following a short-term resistance training. According to the result of this study, corticospinal excitability increased, yet SICI reduced after 4 weeks of heavy load strengthen training of the quadriceps muscle compared to pre-training condition (Weier, Pearce, & Kidgell, 2012). The authors hypothesized that the effects of neural adaptation after an intervention may cause decreased inhibition of the cortical projection to the trained muscle.

Although ICF and fMRI activation of cM1 is significantly increased after a 30-min simple task training period of the wrist flexors (Lotze, Braun, Birbaumer, Anders, & Cohen, 2003), no change in intracortical inhibition and excitation has been detected following repeated performance

of a complex sensorimotor task (Michelle Nadine McDonnell & Ridding, 2006). Moreover, SICI was reduced following repetitive thumb movements and the changes in SICI was muscle and task-specific (Liepert et al., 1998). Likewise, a significant reduction of SICI has been reported after a short-term strength training in leg muscles (Perez, Lungholt, Nyborg, & Nielsen, 2004), and rectus femoris muscle (Weier et al., 2012).

Long-term changes in the CNS excitability following repeated resistance training (over a year) has also been extensively examined. Although some authors are of the opinion that there is no correlation between the increase in muscle strength and changes in corticospinal excitability following the long-term resistance training (Del Olmo, Reimunde, Viana, Acero, & Cudeiro, 2006), others reported significant CNS excitability modulation after chronic resistance training. It has been suggested that the discrepancy between the findings might be due to the methodology and the stimulation technique utilized by different authors.

It has been proposed that during strong contractions (≥ 50% MVC), MEP amplitude is smaller in the chronic-resistance compared to non-resistance trained group, yet, cervicomedullary evoked potential (CMEP) does not significantly change. Since a combination of potential mechanisms could change the evoked potential induced by TMS and TMES, there is a possibility of both supraspinal and spinal modulation in chronically trained individuals (Pearcey, Power, & Button, 2014).

Several studies evaluated the long-term effects of motor training on intracortical interactions modulation in musicians, who have undergone chronic training of their fingers. Some of these studies report that both SICI and ICF are weaker in musicians (Nordstrom & Butler, 2002). However, when SICI is evaluated across a range of CS intensities, it has been revealed that at

higher intensities of CS, musicians have stronger SICI compared to non-musicians participants (Rosenkranz, Williamon, & Rothwell, 2007).

Moreover, many studies have reported the modulation of interhemispheric interactions between the motor cortices in response to long-term resistance training of hand muscles. This modulation is believed to improve interhemispheric coordination (Shim et al., 2005). However, the chronic effects of resistance training on the intracortical interaction modulation of the areas projecting to relatively large muscles like biceps brachii, remain unknown.

2.6 Conclusion

The assessment of CNS excitability modulation during different force outputs in trained and untrained subjects suggests that CNS excitability is modulated through a complex combination of descending inputs from the motor cortex to the spinal motoneuron pool. Besides, this modulation appears to be dependent on the muscle group, target force and participant's training background. While the modulation of supraspinal and spinal parts of the corticospinal volley during voluntary contraction is well-known, it remains unclear how intracortical inhibition is modulated as the force output increases. Moreover, it is not clear how neural adaptations following chronic resistance training affects intracortical interactions. Thus, the following thesis will explore this idea by investigating the effects of chronic resistance training on corticospinal excitability and short intracortical inhibition modulation of the biceps brachii. In other words, we will compare the changes in these two measurements between chronic resistance trained and untrained participants. We decided to examine SICI modulation because 1)It is a reliable measurement of intracortical interactions, 2)The mechanisms of SICI modulation is currently well-known, 3)It reflects a balance between intracortical inhibitory and facilitatory mechanisms, 3)It is easy to record SICI of the

biceps brachii, and 4)It is less variable between subjects and sessions compared to other TMS protocols.

The findings of this research may have functional application for clinical settings and designing appropriate training plans for individuals with CNS and musculoskeletal impairments.

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CHAPTER 3:
STATEMENT OF CONTRIBUTIONS

3 Co-authorship Statement

The main idea of this project was conceived from the previous studies conducted by Dr. Button and Dr. Power. They investigated chronic resistance training adaptation during different tasks. Based on their experience, Dr. Button encouraged me to examine intracortical interactions' modulation during various isometric force outputs of the biceps brachii. I reviewed the literature and then, Dr. Button and I together wrote the project outline and planed the experiment.

Dr. Power contributed to the project by developing the theories and advised me on technical details and stimulation protocol.

Shawn Wiseman and myself carried out the planned experiments and collected the raw data. Then, I performed all data analysis procedures with the guidance of Dr. Button.

Finally, Dr. Button and I discussed the findings and I wrote the thesis under his supervision.

CHAPTER 4:
THESIS MANUSCRIPT

Title: Modulation of corticospinal excitability and short intracortical inhibition during submaximal force outputs of the biceps brachii in chronic resistance trained and non-resistance trained individuals.

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4.1 Abstract

The purpose of this study was to investigate the effects of chronic resistance training on corticospinal excitability and short intracortical inhibition of the biceps brachii. Eight chronic resistance trained (RT) and eight non-RT participants completed one experimental session including a total of 30 briefs (7s) elbow flexors isometric contractions at various force outputs (15, 25 and 40 % of maximum voluntary contraction (MVC)). Before the contractions, MVC, maximal compound muscle action potential (Mmax) during 5% MVC and active motor threshold (AMT) at the three various force outputs were recorded. MVC force of the chronic-RT group was 24 % higher than the non-RT group ($p \leq 0.001$; $\omega^2 = 0.72$). The chronic-RT group had lower AMTs at all targeted forces ($p = 0.022$, $p = 0.012$ and $p = 0.079$ for the 15, 25 and 40 % of MVC, respectively) compared to the non-RT group. During 25 and 40% of MVC, chronic-RT group had decreased SICI in comparison to the non-RT group ($p = 0.008$; $\omega^2 = 0.35$ and $p = 0.03$; $\omega^2 = 0.21$, respectively). However, SICI did not differ between groups at 15 % MVC ($p = 0.62$). In conclusion, chronic resistance training significantly reduces SICI. This suggests the presence of an adaptive process of inhibitory and facilitatory network activation, which may cancel out the SICI, allowing for increased corticomotor drive to the exercised muscle following a long period of resistance training.

4.2 Keywords

Transcranial magnetic stimulation, resistance training, inhibition, facilitation, voluntary contraction

4.3 Introduction

Paired-pulse transcranial magnetic stimulation (TMS) protocols are useful methods for the non-invasive assessment of inhibitory and facilitatory circuits in the human motor cortex (Hallett 2000; Kobayashi and Pascual-Leone 2003). When pairing a subthreshold conditioning stimulus (CS) with a suprathreshold test stimulus (TS) at short interstimulus intervals (ISIs) of 1 to 5 ms, low-threshold intracortical inhibitory circuits are activated and the motor evoked potential (MEP) amplitude is reduced compared to that elicited by the suprathreshold TS alone (Kujirai et al. 1993). This phenomenon is called short-interval intracortical inhibition (SICI) and can be represented by the ratio of the conditioned MEP amplitude over the test MEP amplitude. There is ample evidence suggesting that SICI is mediated by inhibitory neural mechanisms located at the cortical level (Fuhr et al. 1991; Nakamura et al. 1997; Di Lazzaro et al. 1998; Chen 2000). Although multiple inhibitory mechanisms are involved in forming SICI, it has been shown that SICI reflects a balance between intracortical facilitation and inhibition (Ilic et al. 2002; Roshan et al. 2003).

Evidence from work using tonic contractions as a motor output indicates that a reduction in SICI is thought to be important for enhancing the excitability of corticospinal cells via reduced intracortical inhibitory input to the corticospinal pathway. Additionally, it appears that the magnitude of SICI is highly task-dependent. For example, SICI is reduced during voluntary muscle contraction compared to rest (Fisher et al. 2002; Roshan et al. 2003; Zoghi et al. 2003). Furthermore, SICI reduction also occurs as force output increases in the first dorsal interosseous (FDI) (Ortu et al. 2008) and abductor pollicis brevis (APB) (Zoghi and Nordstrom 2007) muscles during submaximal contraction intensities. However, none of the aforementioned studies have assessed SICI during various force outputs from a larger gross motor control muscle such as the

biceps brachii. Moreover, there have been very few studies illustrating the effect of resistance training on modulation of SICI.

Existing evidence from single pulse TMS studies have reported inconsistent results regarding the central nervous system (CNS) adaptations to strength training. Carroll, Riek, and Carson (2002) reported a significant reduction in CNS excitability after short-term resistance training of FDI (Carroll et al. 2002), while others observed a significant increase in MEP size following short-term resistance training of tibialis anterior (Griffin and Cafarelli 2007) and the soleus (Beck et al. 2007) muscles. Indeed, the discrepancy in these results may be attributable to a number of factors, most notably the examined muscle group, the strength training protocols used, and/or the stimulation protocols employed (Carroll et al. 2011). In order to investigate potential mechanisms underlying changes in supraspinal excitability due to resistance training, Weier and colleagues (2012) applied paired-pulse TMS protocol to investigate SICI following a short-term resistance training protocol of the quadriceps femoris muscle (Weier et al. 2012). They observed that 4-weeks of heavy load squat strength training can lead to an increase in CNS excitability while significantly reducing SICI. In addition, acute motor skill training has been shown to decrease SICI in tibialis anterior (Perez et al. 2004) and the FDI (Perez et al. 2007) muscles.

Changes in the CNS excitability following chronic resistance training (over a year) has also been examined. Some authors have shown no correlation between increased muscle strength and changes in corticospinal excitability following chronic resistance training (del Olmo et al. 2006; Tallent et al. 2013) while others reported significant CNS excitability modulation. For example, Pearcey, Power, and Button (2014) reported smaller MEP amplitudes from the biceps brachii muscle in a chronic resistance trained (RT) group compared to a non-RT group during strong force outputs (≥ 50% MVC) (Pearcey et al. 2014). Also, Philpott et al. (2015) observed a significantly

increased spinal excitability of the non-dominant biceps brachii during high force outputs (50 and 70 % MVC) in chronic-RT group compared to non-RT group (Philpott et al. 2015). However, it remains unknown how SICI is altered in individuals who have been chronically resistance training. Several studies evaluated the long-term effects of motor training on intracortical excitability modulation in musicians who have undergone chronic training of their fingers. SICI and intracortical facilitation (ICF) were weaker in musicians than non-musicians (Nordstrom and Butler 2002). However, when SICI was evaluated across a range of CS intensities, it revealed that at higher intensities of CS, musicians have stronger SICI compared to non-musician participants (Rosenkranz et al. 2007). The differences in these results could be due to activation of other interneurons belonging to the ICF network. It has been shown that higher CS intensities may be able to activate these facilitatory interneurons (Ziemann et al. 1998). Thus, to avoid activation of the ICF network, a single sub-threshold CS intensity, instead of applying a range of CS intensities, may better reflect overall changes in SICI.

To date, no study has investigated the effects of chronic resistance training on SICI. Since the SICI system influences corticospinal neurons by producing inhibitory postsynaptic potentials (IPSPs) (Ortu et al. 2008), it could change corticomotor drive to the exercised muscle and can be subjected to long-term neural adaptation. Therefore, the purpose of this study was to examine corticospinal excitability and SICI in chronically resistance trained (chronic-RT) and non-resistance trained (non-RT) individuals utilizing single-pulse and paired-pulse TMS protocols. There were two hypotheses for this study: 1) SICI of the biceps brachii will decrease as force output increases from weak to moderate elbow flexors contractions and 2) chronic resistance training will differently modulate the SICI of the biceps brachii during weak to moderate elbow flexors contractions compared to no resistance training.

4.4 Methods

4.4.1 Participants

Sixteen healthy, university-aged, male individuals without a history of neurological disease volunteered for this study. The 16 participants were divided into two groups consisting of 8 chronic-RT (height 177.2 ± 10.5 cm, weight 84.6 ± 6.0 kg, age 27.5 ± 7.6 years) and 8 non-RT (height 174.5 ± 6.1 cm, weight 77.3 ± 10.0 kg, age 29.1 ± 3.2 years) individuals. For the chronic-RT group, participants were required to have had more than 2 continuous years of resistance training experience (at least 3 times per week) including a variety of multi-jointed weight training exercises. The participants in the non-RT group did not resistance train. Participants were verbally informed of the procedures being used for the experiment and signed a written consent form if they accepted. To detect any potential contraindications with magnetic stimulation procedures, all participants were asked to complete a magnetic stimulation safety checklist (Rossi et al. 2011) before participation. The University's Interdisciplinary Committee on Ethics in Human Research approved the study (#20190061-HK), which was in accordance with the Tri-Council guidelines in Canada with full disclosure of potential risks to participants.

4.4.2 Experimental set-up and recordings

Elbow Flexor Force

Participants were seated in a custom-built chair (Technical Services, Memorial University of Newfoundland, St. John's, NL, Canada) in an upright position, with the chest and head strapped in place to minimize movement, and the hips and knees flexed 90º. The shoulder was placed at 0º and the elbow was flexed 90º. At the 0º position, both arms were slightly abducted and rested on a padded support. The forearm was held horizontal, positioned midway between neutral and supinated positions, and placed in a custom-made orthosis that was connected to a load cell

(Omegadyne Inc., Sunbury, OH, USA). The load cell detected force output, which was amplified × 1000 (CED 1902, Cambridge Electronic Design Ltd., Cambridge, UK) and displayed on a computer screen. Data were sampled at 2000 Hz (Signal 4.0 software, Cambridge Electronic Design Ltd., Cambridge, UK). Participants were instructed to maintain an upright position with their head in a neutral position during contractions of the elbow flexors. Verbal encouragement and visual feedback were given to all participants during elbow flexor contractions (Figure 1A).

Electromyography (EMG)

EMG activity of the biceps brachii muscle was recorded using 10 mm diameter MediTrace Pellet Ag/AgCl electrodes (disc shape, Graphic Controls Ltd., Buffalo, NY). The electrodes were placed 2 cm apart (centre to centre) over the mid-muscle belly of the participant's biceps brachii. A ground electrode was placed on the lateral epicondyle of the opposite upper limb. Before the electrode placement, skin was prepared for all electrodes including shaving hair off the desired area, using abrasive sand paper to remove dead epithelial cells from the desired area, followed by cleansing with an isopropyl alcohol swab. Before the recording, we obtained an inter-electrode impedance of < 5 kOhms to check the ratio of the signal-to-noise. EMG signals were amplified (x1000) (CED 1902) and filtered using a 3-pole Butterworth with cutoff frequencies of 10-1000 Hz. Analog to digital conversion of the signals was performed at a sample rate of 5 KHz using a CED 1401 interface and Signal 4 software (Cambridge Electronic Design Ltd., Cambridge, UK).

Stimulation conditions

Brachial plexus electrical stimulation (Erb's Point Stimulation): Stimulation of the brachial plexus was used to measure participants' maximal compound motor unit action potential (M_{max}). Erb's point was electrically stimulated via a cathode and anode (Meditrace Ag-AgCl pellet electrode, disc-shaped 10 mm diameter, Graphic Controls Ltd., Buffalo, NY, USA) positioned on

the skin overlying the supraclavicular fossa and over the acromion process, respectively. Current pulses were delivered as a singlet using a constant-current electrical stimulator (square wave pulse, 200 μs duration at 100-300 mA; model DS7AH, Digitimer Ltd, Welwyn Garden City, UK). The electrical current was gradually increased until M_{max} of the biceps brachii was reached during 5% MVC. M_{max} was measured during 15, 25 and 40% MVC using the stimulator intensity used to elicit M_{max} during 5% MVC.

Transcranial magnetic stimulation (TMS): TMS was delivered using a circular coil (13 cm outside diameter) attached to a BiStim module connected to two magnetic stimulators (Magstim 200, Dyfed, United Kingdom).The stimulating coil was positioned directly over the vertex of participants' head. The vertex was located by marking the measured halfway points between the nasion and inion and the tragus to tragus. The intersection of these two points was defined as the vertex and was clearly marked with a felt-tipped permanent marker. 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 primary motor cortex ("A" side up for right side, "B" side up for left side), for the elicitation of current in the dominant biceps brachii motor cortical representation. Two stimulation protocols were used during various force outputs of the biceps brachii: 1) a single-pulse TMS protocol (to elicit test MEP) and 2) a paired-pulse TMS protocol (to elicit conditioned MEP). For the paired pulse protocol, a subthreshold stimulus (conditioned pulse) was delivered 2.5 ms prior to a suprathreshold stimulus (test pulse) to produce maximum SICI (Fisher et al. 2002). Also, the intensities of the conditioned and test pulse were set relative to the active motor threshold (AMT) of the MEP during each contraction intensity. AMT was defined as the lowest TMS intensity required to elicit a discernible MEP (≥ 100 μV) in at least 50 percent of the trials (Rossini et al.

2015) for each contraction intensity. To find the intensity of the conditioned stimulus and the test stimulus, the mean stimulator output was decreased and increased, respectively by 20% to determine each stimulation intensity for the remainder of the experiment (80% of each AMT for CS, and 120 % of each AMT for TS) (Ortu et al. 2008; Hunter et al. 2016).

4.4.3 Experimental protocol

Participants completed a single experimental session (~1.5 hrs). The procedure involved performing isometric contractions of the dominant elbow flexors at different intensities of MVC. The participants first performed isometric contractions for 5 s at various low intensities to get accustomed to producing varying force outputs. Participants then completed two elbow flexors MVCs, which were required to have force measurements (N) within 5% of one another to ensure maximal force output; if not, a third MVC was performed. The MVCs were proceeded by a 10-minute rest period where the participants were prepped for EMG and stimulation conditions. Following 10 minutes of rest, the intensities for each stimulation type were set. M_{max} was recorded during 5% MVC by gradually increasing stimulus intensity until the M-wave of the biceps brachii reached a plateau. The stimulator intensity used to determine M_{max} at 5% MVC was used to evoke M_{max} for the remainder of the experiment. AMT was then determined at the three different force outputs (15, 25 and 40% MVC) of the dominant biceps brachii. After determining the stimulation intensities, the participants began the isometric contraction protocol. Three blocks of voluntary isometric contractions of the elbow flexors were performed at 3 different force outputs (15, 25 and 40% of MVC). Each block included ten contractions for 7 s duration. Participants were given 20 s rest between contractions and 5 min rest between contraction blocks. For each contraction, the target force for the participants was displayed on a computer screen. Participants were required to contract their elbow flexors and match the target force line and maintain it for 7 s. During each

contraction, participants received 2 TMS pulses at two different time points (1.5 and 5.5 s) (Figure 1 B). The order of target forces and the type of TMS protocol were randomized. Following the isometric contraction protocol, participants performed three isometric contractions (one at each intensity) during which two M_{max} were recorded.

4.5 Data analysis and statistics

Average biceps brachii force during MVC performance was measured. Peak-to-peak amplitudes of test MEPs, conditioned MEPs and M-waves were recorded from the biceps brachii and then averaged for each target force. A total of 60 MEP responses were recorded (10 test and 10 conditioned MEPs at each of the three force outputs). Test MEPs peak-to-peak amplitudes were normalized to M_{max} (during 5% MVC) amplitude. To determine SICI, the mean amplitude of each conditioned MEP was measured and expressed as a percentage of the mean test MEP evoked by the suprathreshold pulse alone during the same contraction intensity. All data were analyzed off-line using Signal 4.0 software (CED, UK) and averages and ratios were calculated using Office Excel 2016 (Microsoft Corporation, Redmond, WA, USA).

Statistical analyses were completed using SPSS (SPSS 18.0 for Macintosh, IBM Corporation, Armonk, New York, USA). Normality of the data was assessed using both Shapiro-Wilk and Kolmogorov–Smirnov tests and was found to be normally distributed. In the event of a violation of the assumption of sphericity, *p*-values were adjusted using the Greenhouse-Geisser correction. First, a two-way ANOVA was applied to test the main effect of force output (15, 25 or 40% MVC) and resistance training background (chronic-RT vs. non-RT) on each of the dependent variables (AMT, MEP size, AMT and SICI). Then, a series of between group one-way ANOVAs were used to compare between-group differences during each target force separately. Data in text,

table and figures are reported as means ± SD and significance was set at $p < 0.05$. To determine the effect size of dependent variables, ω^2 was calculated. This measurement is shown to be more appropriate for one- and two-way ANOVA (Yigit & Mendes, 2018). ω^2 values were set at small (0.01), moderate (0.06) or large (0.14). Pearson correlations were used to determine the relationship between AMT and SICI during the various force intensities.

4.6 Results

4.6.1 Elbow flexors force output

MVC force in the chronic-RT group was 24% higher than the non-RT group ($p \leq 0.001$; $\omega^2 = 0.72$) (Figure 3).

4.6.2 Active motor threshold

For the TMS intensity required to elicit AMT, there was a main effect of resistance training background ($F_{1,42} = 17.657$; $p \leq 0.001$). However, there was no main effect of force output ($F_{1,42} = 0.819$; $p = 0.44$), or the interaction between force output and resistance training background ($F_{2,42} = 0.146$; $p = 0.86$). AMT for the chronic-RT group was 7% ($p = 0.022$, $\omega^2 = 0.26$), 6.5% ($p = 0.012$, $\omega^2 = 0.31$) and 7% ($p = 0.079$, $\omega^2 = 0.13$) lower than the non-RT group at 15, 25 and 40% MVC, respectively (Figure 4).

4.6.3 Short-interval intracortical inhibition

Figure 2 shows the raw data of the test and conditioned MEP recorded from two participants, one non-RT (top row) and one chronic-RT (bottom row), during the three various force outputs. Mean absolute values for SICI expressed as the ratio between the conditioned MEP over the test MEP is illustrated in Figure 5. During the 15% MVC condition, SICI was observed in all subjects, irrespective of resistance training background ($p = 0.62$, $\omega^2 = 0.04$). However, the

non-RT group exhibited higher SICI than the chronic-RT group at 25 (SICI: 78 ± 13% vs. 97 ± 9% of test pulse; $p = 0.008$; $\omega^2 = 0.35$, respectively) and 40% MVC (SICI: 86 ± 14% vs. 102 ± 11% of the test pulse; $p = 0.03$; $\omega^2 = 0.21$, respectively).

4.6.4 Corticospinal excitability

Since the M_{max} amplitudes were not significantly different during different levels of force outputs, we normalized test MEP responses by M_{max} values during 5% MVC. Two-way ANOVA results showed that there was a main effect for force output ($F_{2,42} = 3.840$; $p = 0.02$), yet, no main effect for resistance training background ($F_{1,42} = 0.002$; $p = 0.96$) or the interaction between resistance training background and force output ($F_{1,42} = 0.030$; $p = 0.97$). Although MEP responses increased as a function of force output, there were no significant differences in MEP amplitudes between the chronic-RT and non-RT group at 15 (Normalized MEP: 0.22 ± 0.28 vs. 0.24 ± 0.11; $p = 0.87$; $\omega^2 = 0.06$, respectively), 25 (Normalized MEP: 0.37 ± 0.16 vs. 0.36 ± 0.22; $p = 0.94$; $\omega^2 = 0.06$, respectively) and 40% MVC (Normalized MEP: 0.45 ± 0.13 vs. 0.43 ± 0.28; $p = 0.86$; $\omega^2 = 0.06$, respectively) (Figure 6).

4.6.5 Compound muscle action potential

There was no significant difference in M_{max} amplitudes between the chronic-RT group (11.9 ± 6.53 mV) and non-RT group (7.4 ± 2.49 mV) during 5% MVC ($p = 0.09$, $\omega^2 = 0.12$). Similarly, M_{max} amplitude was not significantly different at each of the contraction strengths ($p = 0.20$, $\omega^2 = 0.04$, $p = 0.17$, $\omega^2 = 0.06$, $p = 0.19$, $\omega^2 = 0.04$ during 15, 25 and 40% MVC, respectively).

4.6.6 Correlation between SICI and AMT

Pearson correlations were run to investigate the relationship between AMT and SICI during various force outputs, regardless of resistance training background. During the 15% MVC condition, no correlation was observed between AMT and SICI ($r = -0.13$, $p = 0.63$). However,

there was a strong linear relationship between these two variables during the 25% (r = -0.57, p = 0.02) and 40% (r = -0.58, p = 0.02) MVC conditions.

4.7 Discussion

This is the first study to directly examine the effects of resistance training background on changes in SICI during various force outputs of the biceps brachii. The main findings of our study showed that regardless of resistance training background, SICI is reduced as force output increases. However, the magnitude of the reduction in SICI appears to be dependent on resistance training background. Specifically, during the more moderate strength force outputs (25 and 40% MVC), the amount of SICI in the chronic-RT group was significantly lower than the non-RT group. In fact, no SICI was even observed in chronic-RT group during 40% MVC force. The current results provide evidence for a neural adaptation in intracortical interactions following chronic resistance training.

4.7.1 SICI as a function of contraction intensity

The observed decrease in the amount of SICI with increasing force output, is somewhat similar to that shown elsewhere. For example, going from rest to a weak muscle contraction (10% of maximal rectified and integrated EMG), Fisher et al. (2002) observed that SICI was significantly reduced during the contraction compared to when the muscle was at rest (Fisher et al. 2002). Moreover, Zoghi, Pearce and Nordstrom (2007) found that SICI of the abductor pollicis brevis (APB) and the FDI muscles was progressively reduced as force output increased from rest to 25% MVC (Zoghi and Nordstrom 2007). Also, Ortu et al. (2008) examined SICI of the FDI muscle during a range of force outputs from 10 to 50% MVC. They found SICI was present at 10% but not at 25 – 50% MVC of the FDI (Ortu et al. 2008). In the current study, SICI of the biceps brachii

was observed at 15% MVC and still occurred at 40% MVC of elbow flexion in the non-RT group. Thus, it appears that the overall decrease in SICI as a function of increased force output may be muscle-dependent. There are several reasons why the findings in the current study (i.e. SICI of the biceps brachii was present at higher contraction intensites) may have differed from those aforementioned in the ABP and FDI muscles. First, the paired-pulse TMS stimulation protocol we used, applied a CS intensity equal to 80% AMT at an ISI of 2.5 ms to produce the maximum SICI. However, in the previous studies, a wide range of CS intensities from 70 to 90% of AMT as well as various ISIs from 1 to 5 ms were utilized, potentially leading to a different quantification of SICI. For example, by applying a low CS intensity (70% of AMT), no inhibition was reported during weak contraction (20% MVC) of the FDI (Ortu et al. 2008). Secondly, the aforementioned studies examined small hand muscle groups, while we investigated SICI projecting the biceps brachii muscle. It has been shown that the organization of the intracortical circuits projecting to the intrinsic hand muscles, due to the motor control function, are different from those of proximal arm muscles. Distal hand muscles which are involved in fine movements, should have stronger inhibitory control compared to proximal muscles which are normally engaged in tonic postural motor tasks (Abbruzzese et al. 1999). Lastly, as shown here, resistance training background alters SICI as a function of increased force output. The previous studies did not report any details regarding the resistance training background of their participants.

4.7.2 SICI is reduced more in non-RT individuals as force output increases

Chronic resistance training has been shown to alter corticospinal excitability. Using single-pulse TMS, Pearcey, Power, and Button (2014) evaluated changes in biceps brachii MEPs from the dominant arm of chronic-RT and non-RT participants over a range of force outputs from 10% to 100% MVC. They found that MEP amplitudes increased progressively from weak to stronger

elbow flexor contractions (up to 60% MVC) in both groups, however, at the highest contraction intensities (> 60% MVC), chronic-RT participants had lower MEP amplitudes than non-RT participants. The rationale for the discrepancy in MEPs at these high muscle contraction intensities between groups is not known, though the chronic-RT group may have had lower MEPs due to enhanced spinal mechanisms underlying the force output at those higher percentages of MVC (Pearcey et al. 2014). It was suggested that perhaps this reduced a potential neural adaptation to chronic resistance training, in that at high contraction forces, less descending input is required by the motor cortex to produce the appropriate force. However, the influence of inhibitory or facilitatory circuits on the development of MEP amplitudes in chronic-RT and non-RT individuals have not been compared until now. Latella, Kidgell, and Peace (2012) found reduced corticospinal silent periods (indicating decreased inhibition) following 4-8 weeks of resistance training and suggested that the change in the corticospinal silent period may have been due to increased intracortical inhibition (Latella et al. 2012). Additionally, using similar paired-pulse TMS protocols, Weier, Pearce, and Kidgell (2012) and Goodwill, Pearce, and Kidgell (2012), found that SICI was reduced following acute periods of either bilateral or unilateral strength training of the quadriceps. However, in these studies, SICI was only measured during a single force output (10% MVC) and thus may not be indicative how SICI is modulated at various force outputs following training (Goodwill et al. 2012; Weier et al. 2012).

While both groups in the present study showed a reduction in SICI with increased force output from 15-40% MVC, SICI was observed in the chronic-RT group during elbow flexor force outputs equal to or stronger than 25% MVC. If SICI reduction was the only mechanism responsible for MEP modulation, the same amount of intracortical inhibition should have been observed in the two groups as they showed the same change in MEP amplitude from 15-40% MVC. Since SICI

was reduced significantly in the chronic-RT group, the cortical circuitry underlying the development of a MEP is chronic resistance training-dependent at weak to moderate elbow flexors contractions. Chronic resistance training-induced adaptations to the intracortical inhibitory circuitry may be an effective mechanism to reduce the descending input required to produce a MEP and subsequently to generate force output. Additional research should be performed to determine if other potential cortical circuitry that underlies the development of a MEP is altered by chronic resistance training.

Since the intensity of the CS in the SICI protocol (in the current study) was below AMT, it could not evoke a descending volley by itself (Di Lazzaro et al. 2012). Therefore, the alteration of the MEP response with increased force output is of cortical origin and is due to the modulation of intracortical circuits (Kujirai et al. 1993; Ridding et al. 1995). There are two main intracortical circuits: inhibitory and facilitatory. Although these intracortical networks are two separate phenomena, they both project to the corticospinal neurons indirectly by changing the interneurons' activation responsible for the various population of the I waves (Ziemann et al. 1996). It has been suggested that intracortical inhibitory circuit at short ISI (between 1 to 5 ms) can suppress late I waves, yet, facilitatory circuit affects early I waves (Di Lazzaro et al. 2012). During all various target forces, these intracortical circuits interact with each other to affect the overall cortical output. Accordingly, a reduction in the activation of the inhibitory interneurons responsible for the late I waves could be a potential mechanism to reduce SICI as the force output increases. Because SICI was absent during stronger force outputs (> 25% MVC) in chronic-RT group, chronic resistance training may inhibit the interneurons activating late I waves and therefore less inhibitory output were produced in chronic-RT group.

Activation of facilitatory interneurons producing early I waves could be another potential mechanism to reduce the amount of SICI during higher force outputs. Zoghi, Pearce, and Nordstrom (2003) applied TMS with different coil orientation (antero-posterior vs. postero-anterior) to selectively activate various types of I wave population during rest as well as weak isometric contraction of the intrinsic hand muscles. They observed increased facilitation of both early and late I waves during muscle contraction, compared to rest. The early I waves, however, had more contribution to the MEP response than the late I waves (Zoghi et al. 2003). SICF is one of the most important of these facilitatory networks through the M1 area and is also known as I-wave facilitation (Hanajima et al. 2002; Ilic et al. 2002). To activate SICF network, a supra-threshold CS intensity should be applied at the ISIs equal to those of SICI activation. Therefore, the utilized TMS protocol to elicit SICI, was not able to activate SICF network. However, if any training-induced adaptive changes in this intracortical facilitatory neurons occurred, the threshold intensity required to activate these interneurons could be decreased. Therefore, it would be possible for the SICF network to be activated with lower CS intensity, probably close to sub-threshold intensity required for SICI. Our data strongly supported this hypothesis. During weak contraction, 15% MVC, the applied sub-threshold CS was not able to activate SICF network. However, during higher force outputs (25 and 40% MVC) the chronic-RT group may have had neural adaptations that allowed for activation of SICF circuitry at the same time as the SICI circuitry. A concomitant activation of both circuits would allow for, in part, a cancelling out of inhibition exerted by the SICI circuitry. Ortu et al. (2008) examined the interaction between SICI and SICF circuits during rest and muscle contraction in non-RT individuals (Ortu et al. 2008). Accordingly, during rest, increased inhibitory postsynaptic potentials (IPSPs), caused by the SICI protocol, could inhibit the MEP responses. However, during muscle contraction, reduced activation threshold of the SICF

circuit, produced excitatory postsynaptic potentials (EPSPs) with the same intensity used for SICI protocol. Thus, during a muscle contraction, SICI does not present a pure inhibition but rather a balance between inhibition and facilitation (Ni and Chen 2008). Therefore, during stronger force outputs, the activation of facilitatory circuits could have also led to the reduction in SICI and the activation of these circuits may have been more pronounced in chronic-RT participants. However, the effect of chronic resistance training on SICF remains unknown.

Increased feedback from the periphery also reduces SICI. Since increasing force output is accompanied by an increase in afferent feedback, SICI could be gradually reduced as force output increases. Ridding and Rothwell (1999) observed that there was a decrease in SICI during peripheral nerve stimulation and voluntary contraction. However, motor imagery activity, where the afferent feedback was absent, did not reduce SICI (Ridding and Rothwell 1999). Increased neural activity generated by afferent feedback and voluntary command has been shown to be an important mechanism affecting intracortical inhibition during and following a repetitive task with hand muscles (Nordstrom and Butler 2002). Since Chronic-RT individuals produce more force at a given percentage of MVC compared to non-RT individuals (Pearcey et al. 2014; Philpott et al. 2015), it is plausible that chronic-RT individuals have higher level of afferent feedback and subsequently reduced SICI. However, since we did not directly measure the afferent feedback in the current study, we cannot be certain that this is the case.

4.7.3 Chronic resistance training has been shown to alter corticospinal excitability

Another important finding of our study was that chronic-RT individuals had lower AMT for MEPs of the biceps brachii during elbow flexor contractions at 15, 25 and 40% MVC. However, very little is reported about the effect of resistance training on cortical motor threshold (CMT) including; resting motor threshold (RMT) and active motor threshold (AMT). According

to the report of an International Federation of Clinical Neurophysiology, CMT including RMT and AMT is subject to intra-subject and inter-subject variations when repeatedly measured and consequently, it is of limited value to test corticospinal excitability (Groppa et al. 2012). However, most neurophysiological studies, report CMT. A combination of various mechanisms at the supraspinal and spinal levels along with peripheral nervous system may alter CMT. Decreased amount of CMT was reported in patients suffering from various neurological disorders involving CNS such as ALS (Desiato et al. 2002) and epilepsy (Groppa et al. 2008). Ziemann, Lönnecker, Steinhoff, and Paulus (1996) observed that AMT and RMT did not alter following the use of GABA enhancing medications such as Lorazepam. Therefore, it was concluded that excitability of the intracortical circuits projecting to corticospinal neurons could not affect CMT. However, the result of another study by Pennisi et al. (2002) who studied motor cortex excitability in Alzheimer disease supported the idea that intracortical facilitation and/or inhibition can affect the CMT and cortically originated MEP response (Pennisi et al. 2002). Here, we found that AMT is decreased following chronic resistance training. As discussed above, a reduction in intracortical inhibition observed in chronic-RT compared to non-RT individuals could be explained by a lower threshold for intracortical facilitatory circuit activation. Perhaps chronic resistance training affected the AMT in the same way; a reduced activation threshold for the interneurons responsible for facilitation of the MEP. A lower TMS intensity in the chronic-RT group might be able to activate the intracortical facilitatory circuit and facilitate the corticospinal volley to evoke the MEP response, while, in the non-RT group a higher stimulation intensity was required to activate a similar proportion of cortical neurons to produce the target force. We tested this hypothesis by investigating the correlation between AMT and SICI in all participants, independent of the resistance training background. The result showed a strong negative correlation between AMT and

SICI during 25 and 40% MVC. Accordingly, the threshold of intracortical circuits activation could be correlated to the threshold needed to produce AMT. If this is the case, it is likely for these two effects to be controlled by, at least in part, a common population of cortical neurons. Therefore, it is likely for the intracortical interactions to modulate the AMT when the target muscle is performing a strong contraction. Also, due to adaptive changes in this common intracortical networks, lower AMT and lower activation threshold of the intracortical facilitatory circuit can be achieved following chronic resistance training.

4.8 Conclusion

In summary, regardless of resistance training background, SICI of the biceps brachii is reduced as elbow flexor force output is increased. However, chronically-RT individuals show further reductions in SICI, with it being completely abolished by 40% MVC. This abolishment of SICI in the chronic-RT group may occur due to an adaptive neural process associated with training through which complex interactions between intracortical inhibitory and/or facilitatory circuits play a role. Furthermore, chronic-RT individuals also had reduced AMT at all contraction intensities compared to non-RT individuals. Reduced SICI and AMT of the biceps brachii during weak to moderate elbow flexor force outputs in chronic-RT individuals may, in part along with other mechanisms, underlie the greater absolute force production at these relative contraction intensities. We suggest that chronic resistance training leads to an adaptive neural process through the intracortical inhibitory and facilitatory circuits which can cancel out intracortical inhibition to some extent and maybe increase activation of the facilitatory circuits in the cortex during the generation of force. Future studies should determine the effect of chronic resistance training on SICF or ICF circuits.

Acknowledgments

This study was supported by the Natural Sciences and Engineering Research Council of Canada.

Compliance with ethical standards

Conflict of interest

The authors declare they have no conflict of interest.

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4.10 Figure legends:

Fig 1 Schematic diagram of the experimental set-up **(A)** and protocol **(B)**. Participants were asked to complete 10, 7s duration, elbow flexor contractions at 15, 25 and 40% MVC (total of 30 contractions, 10 at each %MVC). Participants received two (top panel B, a test stimulus to measure MEP) or four (bottom panel B, a condition and test stimulus to measure SICI) transcranial magnetic stimulations of the motor cortex during each contraction at 1.5 and 5.5s. For each %MVC participants performed 5 contractions to measure MEP and 5 contractions to measure SICI.

Fig 2 Individual raw data from two participants. Corticospinal responses during 15, 25 and 40 % MVC recorded from a non-RT (top) and chronic-RT (bottom) biceps brachii. MEPs recorded from the single pulse stimulation protocol are shown with dash line and conditioned MEPs (recorded from paired-pulse protocol) are illustrated by the solid line. For the test pulse TMS protocol, stimulation intensity of 120% AMT was used. Conditioned stimulation intensity of 80% AMT was applied 2.5 ms prior to test stimulus to inhibit the test MEP during paired pulse TMS protocol. Notice that SICI was not present in chronic-RT participants during stronger force outputs (25 and 40% MVC) while it was present at all force output levels in the non-RT participants.

Fig 3 Chronic resistance training increases MVC. The chronic RT (471.5 ± 57.5 N) group produced significantly more force than the non-RT (298.6 ± 48.7 N) group. Bars represent means ± SD and asterisk represents statistical significance of $p < 0.001$.

Fig 4 Chronic resistance training alters AMT (% MSO) during 15, 25 and 40% MVC. The chronic RT had lower AMTs compared to the non-RT group (43 ± 1.8% vs. 50 ± 2.2%, 41 ± 1.3% vs. 49 ± 2.2%, 41 ± 1.9% vs. 47 ± 2.2%) at 15, 25 and 40% MVC, respectively. Data points represent means ± SD and asterisks represents statistical significance of $p < 0.001$.

Fig 5 Chronic resistance training alters SICI during 15, 25 and 40% MVC. SICI was expressed as the ratio between conditioned MEPs and test MEPs. During 15% MVC both chronic-RT and non-RT groups exhibited SICI. However, during 25 and 40% MVC SICI was observed only in the non-RT participants. Data points represent means ± SD and asterisks represents statistical significance of $P < 0.05$.

Fig 6 Corticospinal excitability of the biceps brachii increases with increased contraction intensity. Data is reported as normalized test MEP responses to M_{max}. As force increased, corticospinal excitability increased. MEP responses recorded during 15, 25, and 40 %MVC were all significantly different from one another. Data points represent means ± SD and asterisks represents statistical significance of $P < 0.05$.

4.11 Figures:

Fig 1

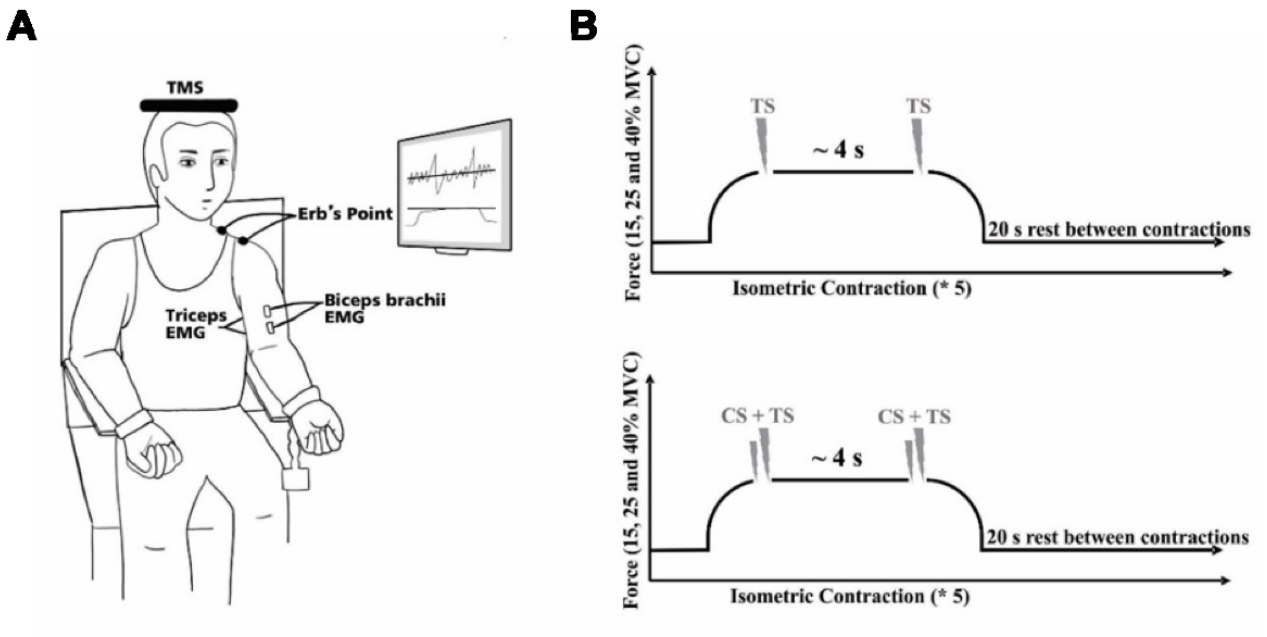
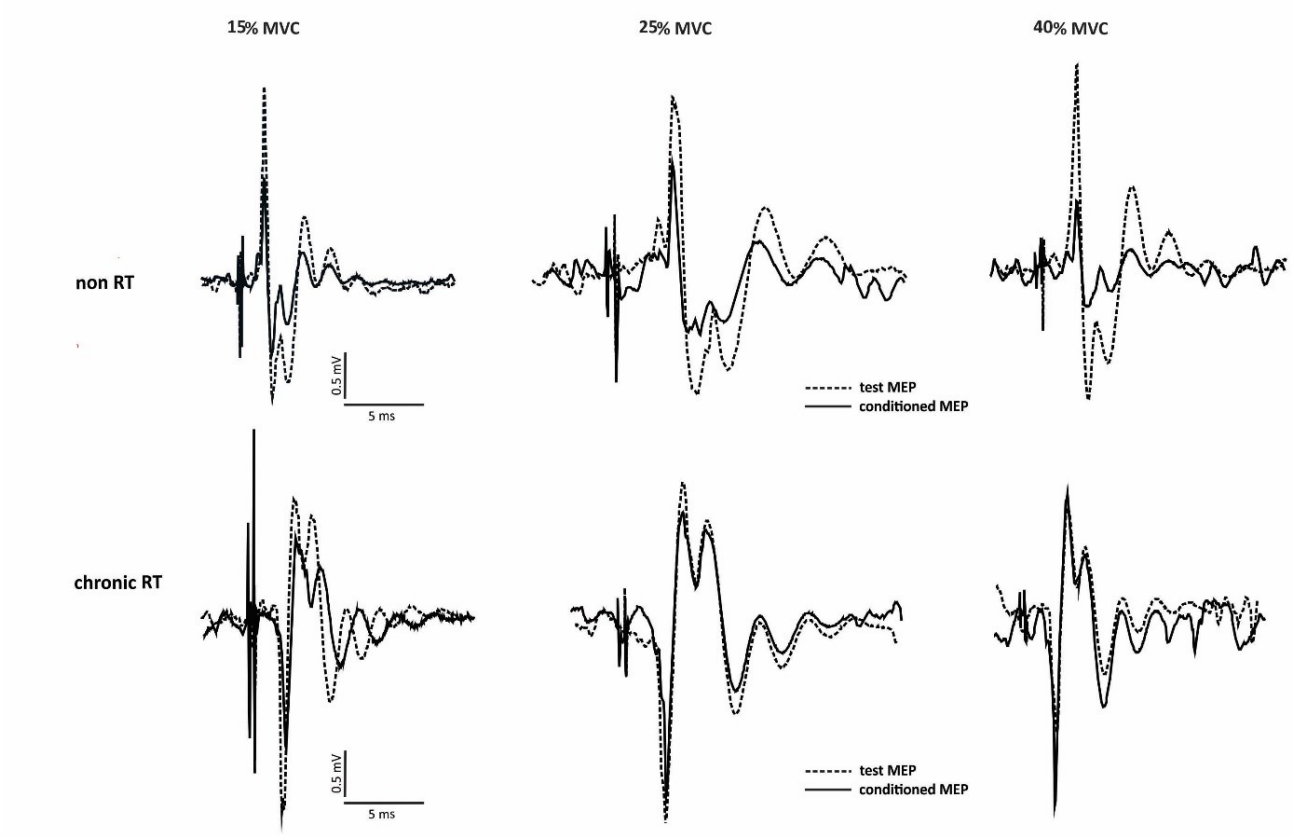


Fig 2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25% MVC
40% MVC
non RT
chronic RT
0.5 mV
5 ms
test MEP
conditioned MEP

Fig 3

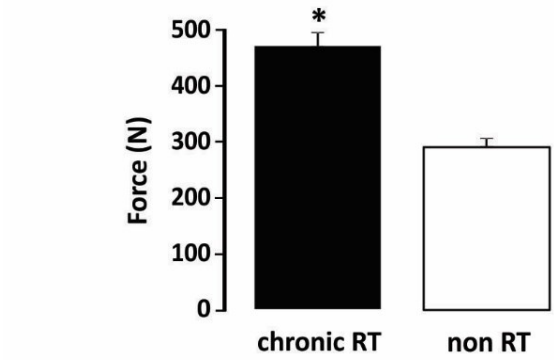

*
500
400
300
200
100
0
Force (N)
chronic RT
non RT

Fig 4

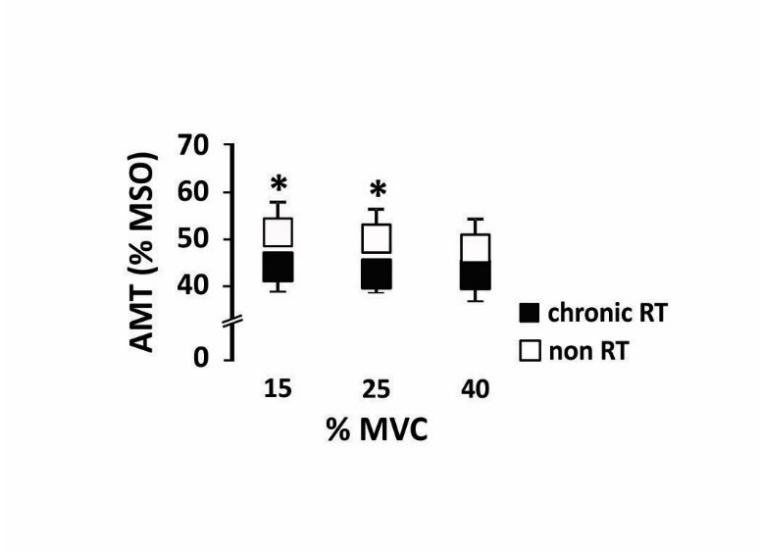

70
60
50
40
0
AMT (% MSO)
*
*
chronic RT
non RT
15
25
40
% MVC

Figure 5

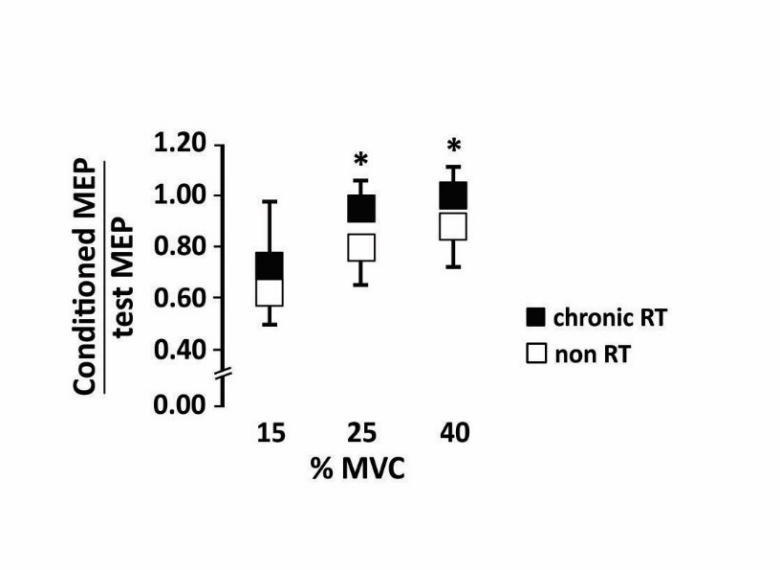
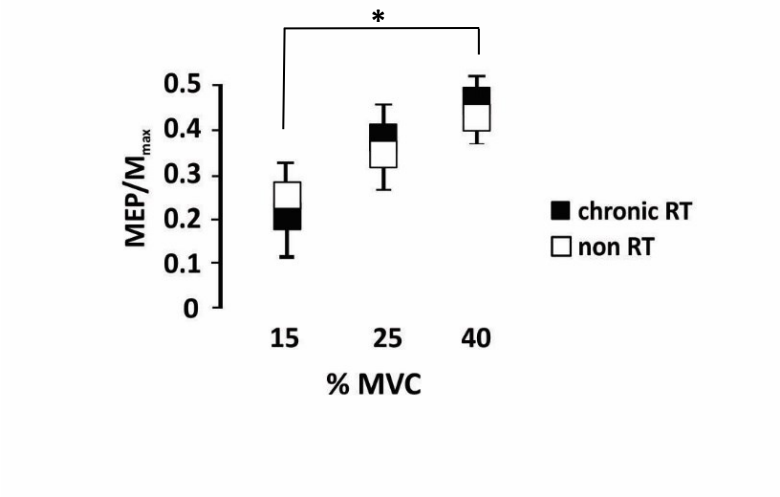


Fig 6



Appendix 1: Magnetic Stimulation Safety Checklist

Please read the checklist below. If the answer to any of the questions is yes please indicate that you are ineligible to participate in the study.

You are NOT required to circle a response nor are you required to provide any further information. This checklist is for safety screening only.

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**

If you are using any of the medications listed in the table below you are ineligible to participate in this study.

1) Tricyclic Antidepressants
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)

- Trifluprom azine (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 2: Informed Consent Form

Title: Modulation of corticospinal excitability and short intracortical inhibition during different levels of voluntary contraction in untrained and chronic resistance trained subjects.

Researcher(s):

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You are invited to take part in a research project entitled "Modulation of corticospinal excitability and short intracortical inhibition during different levels of voluntary contraction in untrained and chronic resistance trained subjects." This project is the M Sc thesis of Mr. Behzad Lahouti which is supervised by Dr. Duane Button.

This form is part of the process of informed consent. It should give you the basic idea of what the research is about and what your participation will involve. **It also describes your right to withdraw from the study at any time**. In order to decide whether you wish to participate in this research study, you should understand enough about its risks and benefits to be able to make an informed decision. This is the informed consent process. Take time to read this carefully and to understand the information given to you. Please contact the lead researcher, Mr. Behzad Lahouti, 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 study. If you choose not to take part in this research or if you decide to withdraw from the research once it has started, there will be no negative consequences for you, now or in the future.

Introduction:

This research is being conducted by Mr. Behzad Lahouti, a Master's Student in the School of Human Kinetics and Recreation at Memorial University of Newfoundland, to investigate the contribution of cortical and spinal mechanisms in the neural control of various isometric contraction intensities in biceps brachii muscle. In the other words, we are examining how central nervous system will change as a function of contraction intensity, and whether the probable changes are the same between untrained and chronic resistance trained subjects. It has been shown

that corticospinal volley is the balance between excitatory and inhibitory components. As such, by using different stimulation protocols, we will examine some of the most important excitatory and inhibitory mechanisms responsible for contractions' control.

Purpose of study:

The purpose of this study is to examine corticospinal and intracortical excitability modulation during different levels of isometric contractions in untrained subjects compared to chronic resistance trained subjects.

What you will do in this study:

We will use a combination of Transcranial Magnetic Stimulation (TMS) protocols to assess corticospinal excitability and intracortical inhibition during different levels of isometric voluntary contractions of elbow flexors. Prior to the test, we will shave and place Electromyography (EMG) and stimulator electrodes on your Biceps and Triceps Brachii muscle, lateral epicondyle of the humerus, supra clavicular fossa, and acromion process. Then you will be positioned on a chair. After positioning, you will perform some submaximal isometric contractions to become accustomed to the testing procedure and to warm up your muscle. Thereafter, you will perform three maximal isometric contractions to determine Maximal Voluntary Contraction (MVC). Then, we will find M max by evoking M-waves during weak contraction (5% of MVC). The value of the M-max will be used to normalize MEP responses. Then, you will be asked to perform 3 sets of isometric contractions at different intensities, 15%, 25% and 40%, respectively. During these contractions, we will deliver TMS to determine your Active Motor Threshold in each of those intensities. Then the main experiment will be commenced. During the main test, you will be required to perform total of 30 isometric contractions, 10 contraction at each intensity. The

duration of contractions is 7s during which two TMS will be delivered. These contractions will be divided in three identical blocks. You will have 20s rest interwall between contractions as well as 10 min break in between the three blocks.

GENERAL DESCRIPTION OF PROCEDURES

As mentioned above, during the experiment, you will be asked to complete isometric voluntary contractions at different intensities with your elbow flexors accompanied by TMS. These contractions will be divided up into three blocks, each with 10 unique contractions. You will perform this protocol with ≥20s rest between contractions, and ≥10 min rest between trials.

While you are performing the contractions, single-pulse TMS protocol and paired-pulse TMS protocol will be delivered to your nervous system and the responses, unconditioned MEP and conditioned MEP, will be recorded via surface electromyography electrodes over the biceps brachii muscle. After your participation, we will analyze the amplitude of the evoked responses to investigate whether your central nervous system is more excitable during higher levels of muscle contraction and in chronic resistance trained subject. Also, we would be able to examine changes in one of the most important inhibitory networks in the cortex.

SPECIFIC DESCRIPTION OF STIMULATION CONDITIONS

The brain stimulation technique that we will use is referred to as TMS and will occur over the brain. The stimulation will be delivered via a circular coil to the brain tissue and responses will be recorded from muscle. This method is widely used to test 'motor cortical' excitability. By the comparison of the size of the Motor Evoked Potentials recorded from the muscle during different levels of contractions, useful information about the differences of motoneuron excitability will be obtained. Also, the electrical stimulation will be delivered via electrode located on supraclavicular

fossa and acromion process to record M-Wave. The values of M-wave, M-max, will be used to normalize the Motor Evoked Potentials recorded from the muscle.

These stimulations are designed for human research. They are completely safe and have been used extensively by Drs. Power and Button. Skin preparation will be undertaken for all electrodes, including shaving hair off the desired area followed by cleansing with an isopropyl alcohol swab. The electrodes do contain an adhesive that allows them to stick to the skin.

I will gladly answer any questions or concerns you may have regarding any portion of the study if the procedures are not completely clear.

Length of time:

Participation in this study will require you to come to a lab located in the School of Human Kinetics and Recreation at Memorial for one session of about two hours, accompanied by two breaks of 10 min each, between three trials of the test.

Withdrawal from the study:

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

Possible benefits:

The benefit of participating in my study is that you will learn about the functioning of your nervous system during different levels of isometric contraction. Also, your participation will definitely help us to understand mechanisms of impaired muscle function or performance and potential mechanisms to improve motor control, which may have positive impact in rehabilitation after injury and athletic training. The findings of this research may be used for guiding rehabilitation strategies and exercise interventions for clinical and non-clinical populations.

Possible risks:

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

1) You will have electrodes placed on the front and back of your arm. These electrodes have an adhesive that has a tendency to cause redness and minor irritation of the skin. This mark is temporary (usually fades within 1-2 days) and is not generally associated with any discomfort or itching.
2) As mentioned above, electrical stimulation will be delivered to the brachial plexus before the main experiment to record M-wave. This will be used to analyze the response and adjust the stimulus intensity. As mentioned, this stimulation protocol will be performed prior to start the test, and will not repeat during the test procedure. The electrical stimulations will cause twitching of the the muscles. The sensation will give you a sharp pain and discomfort, yet, will be very brief (less than a second) and will in no way result in any harm to either muscles or skin in a long-term period.

3) TMS is used to assess brain excitability and is applied at the surface of the top of the skull. This will cause activation of the brain resulting in small muscle contraction. The stimulation is not painfull and most individuals do not experience any discomfort.
4) Post experiment muscle soreness, simlilar to that following an acute bout of exercise will be experienced by some participants.
5) Psychological risks such as nervousness or anxiety may be experienced due to the various stimulation techniques used (top of head and transmastoid). You will be given the opportunity to ask any questions you have.

Each investigator is first aid certified and has access to emergency services in the unlikely event that you require medical assistance. The following address is for the University Counselling Centre should you feel the need to avail of their services.

University Counselling Centre

5th Floor University Centre, UC-5000

Memorial University of Newfoundland

St. John's NL A1C 5S7

Tel: (709) 864-8874

Fax: (709) 864-3011

Director/Associate Professor: Peter Cornish, Ph.D.

NOTE: The stimulators used for the experiment are designed for human research, are completely safe and have been used extensively by Dr. Button.

Confidentiality:

The ethical duty of confidentiality includes safeguarding participants' identities, personal information, and data from unauthorized access, use, or disclosure.

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

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

Anonymity:

Anonymity refers to protecting participants' identifying characteristics, such as name or description of physical appearance. Only the researchers will be aware of your participation. In addition to Drs. Duane Button and Kevin Power, the other researchers, all masters students, required to assist with data collection are:

1. Behzad Lahouti
2. Shawn Wiseman

Every reasonable effort will be made to ensure anonymity; and you will not be identified in publications without explicit permission.

Recording of Data:

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

Storage of Data:

The only individuals who will access to this data are the researchers involved in this study. Data will be retained for a minimum of five years, as per Memorial University policy on Integrity in Scholarly Research after which time it will be destroyed. All data will be kept in a secured location: paper-based records will be kept in a locked cabinet in the office of Dr. Button while computer based records will be stored on a password protected computer in the office of Dr. Button. The data collected as a result of your participation can be withdrawn from the study at your request up until the point at which the results of the study have been accepted for publication (~1 year post study). During this period, participants' data will be removed from the study by using participant codes.

Reporting of Results:

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

Sharing of Results with Participants:

Following completion of this study please feel free to ask any specific questions you may have about the activities you were just asked to partake in. Also, if you wish to receive a brief summary of the results then please indicate this when asked at the end of the form and provide us with your contact information, including name and Email address.

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: Mr. Behzad Lahouti (blahouti@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 destroyed.
- You understand that if you choose to withdraw **after** data collection has ended, your data can be removed from the study up to one year after the conclusion of data collection.

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.

☐ I would like to receive a summary of the results of the study. (If you check this box, please provide us with your Email address and/or Mail address)

_____ _____

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