Phase- and workload-dependent changes in corticospinal excitability to the biceps and triceps brachii during arm cycling

by

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ABSTRACT

Transcranial magnetic stimulation of the motor cortex and transmastoid electrical stimulation of the corticospinal tract can be used to assess changes in supraspinal and spinal excitability, respectively. These techniques have been used previously to determine differences in the neural control of isometric contractions compared to locomotor outputs. It has been shown that corticospinal excitability to the biceps brachii is not only different between isometric contractions and locomotor outputs, but also different during multiple cadences of arm cycling. This suggests that changes in workload, another method of changing intensity during arm cycling, may also result in differences in corticospinal excitability. The purpose of this study was to examine changes in corticospinal excitability between the biceps and triceps brachii during different relative workloads of arm cycling.

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LIST OF ABBREVIATIONS

μs – microseconds
μV - microvolts
CSE – corticospinal excitability
CMEP – cervicomedullary evoked potential
CPG – central pattern generator
EMG – electromyography
FCR – flexor carpi radialis
I-wave – indirect wave
kg – kilograms
mA – milliamps
MEP – motor evoked potential
MSO – maximum stimulator output
M_{max} – maximum m-wave
ms- milliseconds
mV – millivolts
M-wave – compound muscle action potential
PPO – peak power output
RMS – root mean square
RPM – revolutions per minute
s - seconds
SD – standard deviation
SE – standard error
TMES – transmastoid electrical stimulation
TMS – transcranial magnetic stimulation

CHAPTER 1 REVIEW OF LITERATURE

INTRODUCTION

Locomotion, and other rhythmic and alternating movements such as cycling, tend to be thought of as simple and not requiring conscious thought; however, through the use of techniques such as transcranial magnetic stimulation (TMS), transmastoid electrical stimulation (TMES), and peripheral nerve stimulation research has begun to illustrate how complex the mechanisms behind these motor outputs are. Both TMS and TMES activate neurones in the corticospinal tract, but at different locations. The corticospinal tract is the main descending pathway involved in voluntary movement in humans, it runs from the motor cortex to the spinal cord where it synapses with motoneurones. These synapses are largely monosynaptic to upper limb muscles such as biceps and triceps brachii, though triceps brachii also has many polysynaptic connections (Palmer & Ashby, 1992).

TMS activates cortical interneurones while TMES activates corticospinal axons at the level of the pyramidal decussation, both cause action potentials to travel from the site of stimulation along the corticospinal pathway and then motor evoked potentials (MEPs) or cervicomedullary evoked potentials (CMEPs) respectively, can be recorded from the muscle (Taylor et al., 2002). Peripheral nerve stimulation can be performed at any point along the nerve and evokes a similar response in a muscle, called a muscle action potential wave (M-wave). These techniques can be combined to determine where changes are occurring along the corticospinal pathway; changes in the evoked potentials are used

to infer changes in the central nervous system, which is referred to as changes in corticospinal excitability (CSE) (Taylor & Gandevia, 2004). Investigating CSE during rhythmic and alternating motor outputs, often referred to as locomotor outputs (e.g. locomotion, leg cycling), provides insight into the neural control of these movements which may be useful in guiding rehabilitation strategies for individuals with motor impairments.

CENTRAL PATTERN GENERATOR

Rhythmic and alternating movements, such as walking and cycling, are produced largely by a collection of neurones in the spinal cord called central pattern generators (CPG) (Grillner, 1981; Jordan, 1998; Zehr, 2005). In humans, these locomotion-type motor outputs are also modulated by supraspinal inputs and sensory feedback from muscle and skin receptors (Zehr, 2005; Sidhu et al., 2012). There is evidence to support that both upper and lower limb locomotor outputs have similar neural control mechanisms, primarily the CPG (Zehr, 2005). It has also been shown that motor outputs involving both upper and lower limbs, such as walking, have similar neural control mechanisms to those involving the upper or lower limbs alone, such as cycling (Zehr, 2005). These findings suggest that investigation into the neural control of arm cycling would be beneficial not only for rehabilitation purposes but also beneficial for understanding locomotion as a whole.

TECHNIQUES

Transcranial Magnetic Stimulation

TMS uses electromagnetic induction to cause a suprathreshold current in the brain (Rossini, 2015). This suprathreshold current directly activates corticospinal neurones deep in the fifth layer of the motor cortex if the stimulation is strong enough, and indirectly activates interneurones in the second and third layers of the motor cortex that synapse on to corticospinal neurones (Di Lazzaro et al., 2012; Taylor, 2006). Indirect, or transsynaptic, activation of corticospinal tract neurones elicits multiple descending volleys called indirect waves (I-waves) (Di Lazzaro et al., 1998). TMS responses, MEPs, can be recorded from the muscle of interest using electromyography (EMG) (Taylor et al., 2002). MEP's are electrical signals that are generated following TMS and recorded from the muscle. The peak-to-peak amplitude is the measurement typically used to quantify the MEP and is influenced by the number of recruited motoneurones, the number of motoneurones that discharge more than once, and/or by the synchronization of motoneurone discharge (Rossini et al., 2015). Generally, an increase or decrease in MEP amplitude represents an increase or decrease, respectively, in CSE. The corticospinal tract includes both supraspinal and spinal components, MEPs represent overall CSE, and so TMS is often paired with TMES in order to determine if changes in CSE are due to supraspinal mechanisms, spinal mechanisms, or both.

Transmastoid Electrical Stimulation

TMES activates corticospinal axons at the level of the cervicomedullary junction (Taylor, 2006). The corticospinal axons bend, or decussate, at this level, which makes them more susceptible to stimulation (Amassian et al. 1992; Maccabee et al. 1993). TMES elicits a single descending volley that travels from upper motoneurones to spinal motoneurones (Ugawa et al., 1991). The resulting responses, CMEPs, can be recorded from the muscle of interest using EMG. Taylor et al. (2002) investigated the interaction of TMS and TMES in humans by eliciting both stimulations at different interstimulus intervals. Short interstimulus intervals resulted in decreased MEP amplitudes in the biceps brachii, indicating that the antidromic volley from the TMES occluded many of the othodromic volleys elicited by the TMS. The MEP occlusion is evidence that both TMS and TMES activate the same axons, and supports the use of TMES (in combination with TMS) to determine changes in spinal excitability (Taylor, 2006). Together, TMS and TMES can be used to determine if changes in CSE are due to changes at the supraspinal level, spinal level, or both. For this purpose these techniques are useful when examining the neural control of motor outputs in humans.

CORTICOSPINAL EXCITABILITY TO UPPER LIMB MUSCLES DURING ISOMETRIC CONTRACTIONS

There has been considerable research to determine how increasing the intensity of isometric contractions alters CSE. Taylor et al. (1997) and Martin et al. (2006) investigated the effect of contraction strength on CSE in several arm muscles. Each of the

muscles tested showed an increase in CSE (increased MEP size) from rest to a slight contraction; however, the strength of contraction where MEP size peaked was different between muscles. Responses in biceps brachii and brachioradialis increased above 50% maximum voluntary contraction (MVC), whereas responses in distal muscles such as adductor pollicis and the first dorsal interosseous started to decline before 50% MVC. These intermuscle differences are thought to be due to the variation in how muscles increase force production; responses from muscles that primarily increase force through increased rate coding show plateaus at lesser contraction intensities than those that favor increased motor unit recruitment (Taylor et al. 1997; Martin et al. 2006; Gelli et al. 2007). Gelli et al. (2007) studied the relationship between force, surface electromyography (EMG), and CSE in biceps brachii and abductor digiti minimi. They found that the root mean square of EMG increased as force increased up to MVC, but that the median frequency of EMG and MEP size peaked at similar force levels prior to MVC (~40% MVC in abductor digiti minimi; ~70% MVC in biceps brachii). These findings support the premise that increased firing frequency, more than increased recruitment, limits MEP amplitude during strong contractions. While CSE is different during cycling and intensity matched isometric motor outputs (Carroll et al., 2006; Forman et al., 2014), it is important to understand the neural control of both in order to differentiate between changes that are due to the type of motor output versus those that are due to changes in intensity.

CORTICOSPINAL EXCITABILITY DURING ISOMETRIC CONTRACTIONS COMPARED TO CYCLING

Lower limb

Spinal mechanisms are largely responsible for the modulation of CSE during rhythmic and alternating movements; this has been demonstrated in lower limb studies (Weavil et al., 2015; Sidhu et al., 2012). Weavil et al. (2015) investigated CSE during multiple leg cycling workloads and intensity matched isometric knee extensions. They found that as intensity increased MEPs and CMEPs increased similarly for cycling and isometric contractions. Both MEPs and CMEPs plateaued before maximal intensity was reached in vastus lateralis; however, MEPs and CMEPS increased through all intensities in rectus femoris. These findings indicate that the increased CSE with increased workload is primarily due to spinal mechanisms, and also that there are intermuscular or task dependent differences in the modulation of CSE. Sidhu et al. (2012) also found that modulation of CSE during leg cycling was mainly driven by spinal mechanisms. They showed this via similar patterns of changes in MEPs and CMEPS in upper leg muscles throughout cycling. Notwithstanding these findings, it is clear that the motor cortex is also necessary for leg cycling. Sidhu et al. (2012) also compared subthreshold TMS responses in vastus lateralis during leg cycling and intensity matched isometric knee extension. Sub-threshold TMS activates inhibitory intracortical interneurones that synapse to corticospinal neurones; if the cells activated are involved in a motor output, a suppression in EMG may be seen (Davey et al., 1994). Sidhu et al. (2012) found a similar amount of EMG suppression during leg cycling and intensity matched isometric

contractions, which indicates that the motor cortex is directly involved in the activation of major muscles during leg cycling.

Upper limb

Isometric contractions are controlled through different neural mechanisms than rhythmic and alternating motor outputs such as arm cycling (Carroll et al. 2006; Forman et al., 2014). These differences are not evident immediately prior to movement (Copithorne et al., 2015), or at the onset of movement (Forman et al., 2016); however, they are apparent once arm cycling is steady state. Both Carroll et al. (2006) and Forman et al. (2014) compared CSE during arm cycling and intensity matched isometric contractions. Interestingly, Carroll et al. (2006) found that CSE was lower during arm cycling than during isometric contractions, while Forman et al. (2014) found the exact opposite: that CSE was higher during arm cycling. These contradicting findings indicate that changes in CSE are not only different between isometric contractions and cycling, but also that CSE is task dependent. Carroll et al. (2006) investigated flexor carpi radialis (FCR), which is active throughout cycling to stabilize the wrist; Conversely, biceps brachii is phasic, it is very active during the flexion phase of cycling and relatively inactive during the extension phase of cycling when triceps becomes the prime mover (Forman et al. 2014). Differences in CSE are not only seen between isometric contractions and cycling, they are also seen within each of these activities. Forman et al. (2016) compared the influence of neutral and pronated handgrip positions on CSE to the biceps brachii during isometric contractions and arm cycling. Neutral handgrip resulted in

increased CSE for both tasks during elbow flexion; however, the increase in CSE was primarily due to supraspinal mechanisms during isometric contractions, and both supraspinal and spinal mechanisms during arm cycling. Once steady-state arm cycling has been reached the neural control of this motor output has been repeatedly shown to be different than that of isometric contractions. The neural control during arm cycling is also different, depending on the muscle of interest, the handgrip position, and the phase of cycling being tested. These findings indicate that more research is needed to determine what other factors influence the neural control of arm cycling.

CORTICOSPINAL EXCITABILITY DURING ARM CYCLING

While much research has been done to investigate the neural control of isometric contractions, much less has investigated the neural control of arm cycling. As discussed above, differences in CSE have been shown within arm cycling using neutral and pronated handgrip positions. This suggests that other modifications, such as changes in intensity, and the method used to change intensity, may also result in variations in CSE. Forman et al. (2015) began this work by investigating how changes in cycling cadence affect CSE. At the 6:00 position, elbow flexion, they found that MEPs, CMEPs, and biceps background EMG all increased as cadence increased from 30, 60, and 90 rpm. At the 12:00 position, elbow extension, they found that MEPs increased at 90 rpm, while CMEPs decreased from 30, 60, and 90 rpm, and that biceps background EMG stayed consistent across all cadences. These findings indicate that during the elbow flexion phase of cycling biceps is mediated by spinal mechanisms, whereas during the elbow extension

phase of cycling biceps is mediated by supraspinal mechanisms. Together, these findings illustrate phase dependent modulation of CSE in the biceps brachii during arm cycling. Cycling intensity can be changed via cadence and/or workload, Forman et al. (2015) investigated cadence alone; however, workload and combinations of cadence and workload have yet to be studied during arm cycling.

INTERMUSCLE DIFFERENCES AND THEIR RELATIONSHIP TO CORTICOSPINAL EXCITABILITY

While the broad concept that there are neural control differences between isometric contractions and locomotor outputs is generalizable to multiple muscles, there are more specific differences that may vary depending on the muscle of interest. The findings of Carroll et al. (2006) and Forman et al. (2014) described above demonstrate task/muscle dependent differences in CSE during arm cycling. Sidhu et al. (2012) and Weavil et al. (2015) also showed intermuscle differences between rectus femoris and vastus lateralis during leg cycling; however, differences in CSE between antagonist muscles during arm cycling have yet to be investigated.

Biceps brachii and triceps brachii are antagonist muscles and are likely the prime movers during the flexion and extension phase, respectively, of arm cycling. Despite both being brachial muscles with complementary roles in arm cycling, these muscles have several anatomical and physiological differences that could influence neural control as seen by changes in CSE. Both muscles have monosynaptic connections to the corticospinal tract, but triceps brachii has a larger proportion of polysynaptic connections (Palmer & Ashby, 1992). As their names suggest triceps brachii has 3 heads, and biceps

brachii has two. While both heads of biceps are biarticular, crossing the elbow and shoulder, only the long head of triceps is biarticular. The lateral head of triceps is superficial and commonly used to record EMG, but unlike biceps and the long head of triceps, it is monoarticular, crossing only the elbow joint. The findings of Sidhu et al. (2012) and Weavil et al. (2015) both suggest that there are differences in CSE projecting to biarticular and monoarticular muscles; therefore, it is likely that there would be differences between biceps brachii and the lateral head of triceps brachii. Further research is needed to identify muscles that exhibit differences in neural control, and also determine the source(s) of these differences.

CONCLUSION

Previous research has shown that rhythmic and alternating motor outputs are controlled differently than isometric contractions (Carroll et al. 2006; Forman et al., 2014). It has also been shown that CSE during cycling is task dependent (Forman et al., 2014; Carroll et al., 2006; Weavil et al., 2015), phase dependent (Forman et al, 2015), and that there are intermuscle differences in task- and phase-dependent changes in CSE (Sidhu, 2012; Weavil 2015). The effect of intensity (altered through changes in cadence) on CSE during arm cycling has been investigated; however, in order to fully understand how changes in intensity alter CSE during arm cycling it is necessary to investigate changes in workload, as well as changes in workload combined with changes in cadence. It is also necessary to investigate more muscles that are involved in arm cycling, in order to determine where differences in neural control occur and what may be causing them.

Increasing the number of muscles studied, as well as the conditions of arm cycling will further develop the knowledge of the neural control locomotor movements. As the methods of control become more clear we will be better equipped to determine the source of interruption as caused by injury, and possibly be able to facilitate recovery.

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CHAPTER 2 PHASE- AND WORKLOAD-DEPENDENT CHANGES IN CORTICOSPINAL EXCITABILITY TO THE BICEPS AND TRICEPS BRACHII DURING ARM CYCLING

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Running Head: Phase- and workload-dependent modulation of corticospinal excitability

Keywords: motoneurone; transmastoid; transcranial; motor evoked potential, MEP; cervicomedullary evoked potentials, CMEP; cortical; muscle; central pattern generator

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Abstract

This is the first study to examine corticospinal excitability (CSE) to antagonistic muscle groups during arm cycling. Transcranial magnetic stimulation (TMS) of the motor cortex and transmastoid electrical stimulation (TMES) of the corticospinal tract were used to assess changes in supraspinal and spinal excitability, respectively. TMS induced motor evoked potentials (MEPs) and TMES induced cervicomedullary evoked potentials (CMEPs) were recorded from the biceps and triceps brachii at two positions, mid-elbow flexion and extension, while cycling at 5 and 15% of peak power output. While phase-dependent modulation of MEP and CMEP amplitudes occurred in the biceps brachii, there was no difference between flexion and extension for MEP amplitudes in the triceps brachii and CMEP amplitudes were higher during flexion than extension. Furthermore, MEP amplitudes in both biceps and triceps brachii increased with increased workload. CMEP amplitudes increased with higher workloads in the triceps brachii, but not biceps brachii, though the pattern of change in CMEPs was similar to MEPs. Differences between changes in CSE between the biceps and triceps brachii suggest that these antagonistic muscles may be under different neural control during arm cycling. Putative mechanisms are discussed.

INTRODUCTION

The basic pattern of rhythmic and alternating locomotor outputs in humans, such as arm cycling, are partially mediated via a spinally located network of neurones referred to as a central pattern generator (CPG) [1-5], though supraspinal input is required [6-8]. Studies have typically assessed spinal reflex modulation during locomotor outputs as a means to understand the neural control mechanisms underlying their production [9,10]. The results from these studies show that spinal reflexes, and thus the processing of sensory information, are both muscle- and phase- (e.g. flexion vs extension) dependent [9-11]. For example, Zehr and Chua (2000) demonstrated that cutaneous reflexes in some arm muscles were related to the amplitude of the ongoing background EMG (i.e. contraction intensity) while other muscles (e.g. biceps brachii) showed phase-dependent modulation.

Considerably less information is currently available regarding corticospinal excitability (CSE) modulation during locomotor outputs, though we are beginning to understand CSE modulation during leg [7,12,13] and arm cycling [14-17]. CSE can be assessed by measuring the amplitude of motor evoked potentials (MEPs) elicited via transcranial magnetic stimulation (TMS) of the motor cortex and transmastoid electrical stimulation (TMES) of corticospinal axons. Together, these measures give an indication of supraspinal and spinal excitability [16-21]. Using these techniques, Sidhu and colleagues have recently shown that CSE is phase-, muscle- and intensity-dependent to the leg muscles during cycling [7,12,13]. Work from our lab has shown phase-, task- and cadence-dependent modulation of CSE to the biceps brachii during arm cycling [14-17].

More specifically, while CSE to the biceps brachii increased throughout arm cycling as cadence increased, spinal excitability increased during elbow flexion and decreased during elbow extension [16]. Whether this occurs in different muscles during arm cycling is currently unknown.

The task-dependent neural control of arm muscles has been previously examined by assessing CSE during arm cycling compared to an intensity matched tonic contraction. These two motor outputs are compared because the generation of arm cycling is driven, in part, by spinal interneuronal networks [22]. A tonic contraction of similar muscle groups is chosen to represent a similar level of motoneurone output, but with reduced or absent activation of spinal interneuronal groups contributing to the production of arm cycling. Using this methodology, we recently showed that CSE to the biceps brachii was higher during the elbow flexion phase of arm cycling compared to an intensity-matched tonic contraction. This finding was in direct opposition to the findings of Carroll and colleagues (2006), who demonstrated that CSE to the FCR was higher during a matched tonic contraction compared to arm cycling. We suggested that the differences between CSE in the arm muscles may be related to the different functions of the arm muscles during arm cycling [17]. The FCR is used primarily to stabilize the wrist to allow for constant gripping of the hand pedals and is thus continuously active during arm cycling with little phase-dependence. The biceps brachii, however, demonstrates strong phase-dependence with high activation during elbow flexion to assist in propulsion and minimal activity during elbow extension occurring in the recovery phase. In addition to intermuscle differences in CSE during arm cycling, it was noted that the differences in CSE between arm cycling and tonic contraction were phase-dependent in both the biceps brachii [17]

and FCR [5]. CSE, likely supraspinal in origin, to the biceps brachii was higher during the mid-elbow flexion phase of arm cycling while spinal excitability was higher at the initiation of elbow flexion [17]. Conversely, CSE and spinal excitability (assessed via the H-reflex) projecting to the FCR during arm cycling were lower at mid-elbow flexion, as was spinal excitability at the initiation of elbow flexion [5]. No studies have simultaneously assessed CSE projecting to functional antagonists during arm cycling

The primary purpose of the present study was to determine whether CSE projecting to functional antagonists, the biceps and triceps brachii, was differentially modulated during arm cycling. A secondary objective was to assess load-dependent changes in CSE to the same muscles, also during arm cycling. We hypothesized that 1) both supraspinal and spinal excitability to the biceps brachii and triceps brachii would be phase-dependent (i.e. higher during flexion and extension, for the biceps and triceps brachii, respectively) and 2) supraspinal and spinal excitability would increase throughout arm cycling in both muscles as load increased.

MATERIALS AND METHODS

ETHICAL APPROVAL

The procedures of the experiment were verbally explained to each volunteer prior to the start of the session. Once all questions were answered, written consent was obtained. This study was conducted in accordance with the Helsinki declaration and approved by the Interdisciplinary Committee on Ethics in Human Research at Memorial University of

Newfoundland (ICEHR#: 20151928-HK). Procedures were in accordance with the Tri-Council guideline in Canada and potential risks were fully disclosed to participants.

PARTICIPANTS

Twelve male volunteers (26.3 ± 5.3 years of age, 182.7 ± 6.6 cm, 92.4 ± 17.8kg, ten right hand dominant, two left hand dominant) partook in this study [23]. All 12 participants received TMS while 8 of those 12 received TMES (see protocols below). Four participants did not receive TMES because the stimulation intensity required either activated nerve roots or was intolerable. Arm dominance was determined using the Edinburg handedness inventory: short form [24], to ensure that evoked potentials (described below) were recorded from the dominant arm. Given that the motor output assessed was bilateral, it was important to identify the dominant arm because of potential differences in their neural control [25,26]. Participants had no known neurological impairments. Prior to the experiment, all volunteers completed a magnetic stimulation safety-checklist in order to screen for contraindications to magnetic stimulation. Additionally, participants were required to complete a Physical Activity Readiness Questionnaire (PAR-Q+) to screen for any contraindications to exercise or physical activity.

EXPERIMENTAL SET-UP

This study was carried out on an arm cycle ergometer (SCIFIT ergometer, model PRO2 Total Body). Participants were seated upright at a comfortable distance from the hand pedals, so that during cycling, there was no reaching or variation in trunk posture. To further ensure that posture was maintained throughout all trials, each participant was

strapped securely to the ergometer seat with straps placed over the shoulders and crossed over the chest. Movement of the shoulders and arms was not impeded. The hand pedals of the ergometer were fixed 180 degrees out of phase and the seat height was adjusted so that the shoulders of each individual were approximately the same height as the centre of arm crank shaft. Participants lightly gripped the ergometer handles with the forearms pronated and wore wrist braces in order to limit the movement of the wrists during cycling as heteronymous reflex connections exist between the wrist flexors and biceps brachii [27].

Measurements were taken from two different locations; 6 and 12 o'clock relative to a clock face, whereby 12 o'clock was defined as the "top dead centre" of the arm crank and 6 o'clock was defined as the "bottom dead centre." These sites were relative to the hand dominance of each individual. For example, 12 o'clock for a right handed participant would have been when their *right* hand was positioned at "top dead centre" of the arm crank (see Fig. 1A; right handed participant at 6 o'clock position). For a left handed individual, 12 o'clock would have been set when their *left* hand was at "top dead centre." These two positions were chosen as they represent periods of high (6 o'clock) and low (12 o'clock) levels of biceps brachii activation during arm cycling, with the inverse true for the triceps brachii (see Fig. 1B). Movement between 3 o'clock (when the elbow reaches full extension) and 9 o'clock (when the elbow reaches maximal flexion) occurs when the elbow is flexing and the biceps and triceps brachii are most and least active, respectively. Movement between 9 o'clock and 3 o'clock occurs when the elbow is extending and the biceps and triceps brachii are less and more active, respectively. Measurements at each position were taken separately.

The study required participants to cycle at two different cycling power outputs; 5 and 15% of peak power output (PPO) as determined during a maximal sprint test (see below). Measurements were taken at 6 and 12 o'clock for a total of four separate trials. The order of the trials was randomized and responses (described below) were triggered automatically when the arm crank passed by one of the two pre-determined positions.

ELECTROMYOGRAPHY RECORDINGS

EMG activity of the biceps and triceps brachii of the dominant arm were recorded using pairs of surface electrodes (Medi-Trace 130 ECG conductive adhesive electrodes) positioned over the midline of the biceps brachii and the lateral head of the triceps brachii. A ground electrode was placed on the lateral epicondyle. Prior to electrode placement the skin was thoroughly prepared by removal of dead epithelial cells (using abrasive paper) followed by sanitization with an isopropyl alcohol swab. EMG was collected on-line at 5 KHz using CED 1401 interface and Signal 5.11 [Cambridge Electronic Design (CED) Ltd., Cambridge, United Kingdom] software program. Signals were amplified (gain of 300) and filtered using a 3-pole Butterworth with cutoff frequencies of 10-1000 Hz.

STIMULATION CONDITIONS

Motor responses from the biceps and triceps brachii were elicited via 1) electrical stimulation at Erb's point, 2) transcranial magnetic stimulation (TMS) and 3) transmastoid electrical stimulation (TMES). All volunteers had prior experience with TMS, TMES and Erb's point stimulation procedures. To determine the appropriate stimulation intensities (see below), participants were instructed to engage in the cycling

movement, but with the cycle ergometer cranks locked in place (the dominant hand pulling toward the body and non-dominate hand pushing away from the body) until the biceps brachii EMG matched a horizontal cursor set to 5% of their peak EMG recorded during the 10 second maximal sprint. Their dominant hand was placed at the 6 o'clock position and their non-dominant hand at the 12 o'clock position. The stimulation intensities for both TMS and TMES were made relative to the biceps brachii, though we also recorded from the triceps brachii as has been previously done [28].

BRACHIAL PLEXUS STIMULATION

The M_{max} of the biceps brachii was first determined by eliciting M-waves through electrical stimulation of the brachial plexus at Erb's point (DS7AH, Digitimer Ltd., Welwyn Garden City, Hertfordshire, United Kingdom). A pulse duration of 200 μs was used and intensities ranged from 100-300 mA. The cathode was placed in the supraclavicular fossa and the anode on the acromion process. The initial stimulation intensity was set at 25 mA and gradually increased until the elicited M-waves of the biceps brachii reached a plateau. Stimulation intensity was then increased by 10% to ensure maximal M-waves (i.e. M_{max}) were elicited throughout the study. Following analysis, MEP and CMEP amplitudes were normalized to the M_{max} during each trial in order to account for changes in peripheral neuromuscular propagation [21].

TRANSCRANIAL MAGNETIC STIMULATION

MEPs were elicited via TMS with the use of a Magstim 200 (Magstim, Dyfed, United Kingdom). Stimulations were delivered over the vertex via a circular coil (13.5cm outside diameter). Vertex was determined by measuring the mid-point between the

participant's nasion and inion, and the mid-point between the participant's tragi. The intersection of these two points was measured, marked and defined as vertex [8,20,29-31]. The coil was held tangentially to the participant's skull, approximately parallel to the floor, with the direction of the current flow preferentially activating either the left or right motor cortex (depending on hand dominance). The coil was held firmly against the participant's head by one of the investigators to ensure careful and consistent alignment over vertex for each trial. Stimulation intensity was started at approximately 25% of magnetic stimulator output (MSO) and gradually increased until a MEP amplitude equivalent to 15-20% of Mmax was found. This %MSO was used throughout the remainder of the experiment.

TRANSMASTOID ELECTRICAL STIMULATION

TMES was delivered using Ag-AgCl surface electrodes applied just inferior to the mastoid processes. The pulse duration was fixed at 100 μs and stimulations intensities of 125-275 mA were used (DS7AH, Digitimer Ltd., Welwyn Garden City, Hertfordshire, United Kingdom). Stimulation intensity began at 25 mA and gradually increased until the average of 8 CMEP amplitudes matched the average of the 8 MEP amplitudes previously determined [17,32]. This stimulation intensity was used throughout the remainder of the experiment.

EXPERIMENTAL PROTOCOL

Once the intensities for Erb's point stimulation, TMS, and TMES were determined, the four different workload trials (5 and 15% PPO at 6 and 12 o'clock) were performed. A cadence of 60 rpm was maintained for each trial, with 8 MEPs, 8 CMEPs and 2 $M_{max's}$

recorded at each workload and position. The order of these stimulations was randomized throughout the trial and stimulations were separated by approximately 7-8 s. To account for possible changes in the compound muscle action potential, a second trial consisting of 2 M-waves was performed immediately thereafter given that M_{max} may change over the course of an experiment [33]. These stimulations were elicited at the same workload, cadence and position as the previous MEPs and CMEPs. They were also separated by 7-8 s. These steps were then repeated for the remaining seven trials.

MEASUREMENTS

Data was analyzed off-line using Signal 5.11 software (CED, UK). The peak-to-peak amplitudes of MEPs, CMEPs and M_{max} of the biceps brachii were measured. The peak-to-peak amplitudes for all evoked potentials were measured from the initial deflection of the voltage trace from the baseline EMG to the return of the trace to baseline levels. Because changes in MEP and CMEP amplitudes can be the result of changes to M_{max}, both MEPs and CMEPs were normalized to the M_{max} evoked during the same trial. Pre-stimulus EMG, defined as a window of the mean rectified EMG immediately prior to the stimulation artifact, was measured from the rectified traces [17]. Measurements were taken from the averaged files of all 8 CMEPs, 8 MEPs and 2 M_{max}.

STATISTICS

All statistical analysis was performed using IBM's SPSS Statistics Version 23. Separate two-way repeated-measures ANOVAs with factors 'workload' and 'phase' were used to assess whether statistically significant differences in MEP or CMEP amplitudes (normalized to M_{max}) and the average of the pre-stimulus EMG occurred between the two

cycling workloads at each phase of the cycle (i.e. elbow flexion and extension). All data were normally distributed as determined using the Kolmogorov-Smirnov test for normality. Assumptions of sphericity were tested using the Mauchley test, and if it was violated, the appropriate correction was applied (i.e., Greenhouse Geisser or Huynh-Feldt). A Bonferroni *post hoc* test was performed to test for significant differences between interactions. All statistics were run on group data and a significance level of $P < .05$ was used. All data are reported in text as means ± SD and illustrated in figures as mean ± SE.

In order to make inferences as to changes in supraspinal and spinal excitability during cycling it is important that the intensity of the motor output, as estimated via pre-stimulus EMG levels, in the biceps brachii and triceps brachii be similar when MEPs and CMEPs were evoked. Thus, we compared pre-stimulus EMG levels between MEPs and CMEPs, within phase (flexion vs extension) and workload (5 or 15% PPO) using paired t-tests.

RESULTS

BICEPS BRACHII

Corticospinal excitability to the biceps brachii during arm cycling

MEP amplitude. Figure 2 (left panel) shows the average of 8 MEPs expressed as a percentage of M_{max} at 5% and 15% of PPO at the 6 o'clock and 12 o'clock positions (data is from one participant). In this example, MEPs expressed as a percentage of M_{max} at the 6 o'clock position are 22.03 and 53.2% during the 5 and 15% PPO trials, respectively. At

the 12 o'clock position MEPs are 1.44 and 2.19% M_{max} during the 5 and 15% PPO trials. There were significant main effects for position (flexion > extension, $p < 0.001$), load (15% > 5%, $p = 0.001$), as well as interaction effects ($p = 0.006$). As a group, MEP amplitudes during flexion were 45.10 and 77.89% Mmax at 5 and 15% PPO, respectively; during extension, average MEP amplitudes were 3.46 and 6.20% Mmax at 5 and 15% PPO, respectively (Fig. 3A).

Pre-stimulus EMG for MEPs. Significant main effects for position (flexion > extension, $p = 0.001$), load (15% > 5%, $p < 0.001$), as well as interaction effects ($p = 0.001$) were observed. As a group, pre-stimulus EMG during flexion was 86.4 and 230μV at 5 and 15% PPO, respectively; during extension, pre-stimulus EMG was 30.5 and 41.1μV at 5 and 15% PPO, respectively (Fig. 3C).

Spinal excitability to the biceps brachii during arm cycling

CMEP amplitude. Figure 2 (right panel) shows an example of the differences in CMEP amplitude between 5% and 15% PPO cycling loads at 6 o'clock and 12 o'clock positions. In this example, CMEPs expressed as a percentage of Mmax were 18, and 1.16% during the 5% PPO trial and 31.5 and 2.42% during the 15% PPO trial. There was a significant main effect for position (flexion > extension, $p < 0.001$) with no effect of load ($p = 0.179$). As a group, CMEP amplitudes during flexion and extension were 39.2 and 3.0% Mmax, respectively (Fig. 3B).

Pre-stimulus EMG for MEPs. Significant main effects for position (flexion > extension, $p = 0.004$), load (15% > 5%, $p = 0.001$) as well as interaction effects ($p = 0.017$) were observed. As a group, pre-stimulus EMG during flexion was 68.5 and

169.9μV at 5 and 15% PPO, respectively; during extension, pre-stimulus EMG was 29.8 and 40.2μV at 5 and 15% PPO, respectively (Fig. 3D).

Background EMG of biceps brachii between stimulation types as function of workload arm cycling. Paired t-tests reveal no significant effect of stimulation type at any intensity or position ($p = 0.284 – p = 0.893$). Thus general comparisons between changes in MEP and CMEP amplitudes are warranted.

TRICEPS BRACHII

Corticospinal excitability to the triceps brachii during arm cycling

MEP Amplitude. Figure 4 (left panel) shows an example of MEPs elicited at 5% and 15% PPO cycling loads at the 6 o'clock and 12 o'clock positions. In this example, MEPs expressed as a percentage of Mmax were 17.8 and 40.7% during the 5% PPO trial and 22.6 and 76.9% during the 15% PPO trial. There was a significant main effect for load (15% > 5%, $p = 0.006$), but no significant effect of position ($p = 0.246$), or interaction effects ($p = 0.053$). As a group, MEP amplitudes during were 19.2 and 31.3% Mmax at 5 and 15% PPO, respectively (Fig. 5A).

Pre-stimulus EMG for MEPs. Significant main effects for position (extension > flexion, $p < 0.001$), load (15% > 5%, $p < 0.001$) as well as interaction effects ($p < 0.001$) were observed. As a group, pre-stimulus EMG during extension was 54.7 and 129.7μV at 5 and 15% PPO, respectively; during flexion, pre-stimulus EMG was 22.2 and 45.9μV at 5 and 15% PPO, respectively (Fig. 5C).

Spinal excitability to the triceps brachii during arm cycling

CMEP amplitude. Figure 4 (right panel) shows an example of the differences in CMEP amplitude between 5% and 15% PPO cycling loads at 6 o'clock and 12 o'clock positions. In this example, CMEPs expressed as a percentage of Mmax were 18.2, and 3.81% during the 5% PPO trial and 19.8 and 8.3% during the 15% PPO trial. There were significant main effects for position (flexion > extension, $p = 0.042$) and load (15 > 5%, $p = 0.003$), and no interaction effect ($p = 0.353$). As a group, CMEP amplitudes during flexion and extension were 15.2 and 23.6% Mmax,, respectively (Fig. 5B). Aaverage CMEP amplitudes were 16.4 and 22.5% Mmax at 5 and 15% PPO, respectively (Fig. 5D).

Pre-stimulus EMG for CMEPs. Significant main effects for position (extension > flexion, $p = 0.002$), load (15% > 5%, $p = 0.001$) as well as interaction effects ($p = 0.005$) were observed. As a group, pre-stimulus EMG during extension was 52.5 and 122.3μV at 5 and 15% PPO, respectively; during flexion, pre-stimulus EMG was 20.6 and 41.0μV at 5 and 15% PPO, respectively (Fig. 5E).

Background EMG of triceps brachii between stimulation types as function of workload during arm cycling. Paired t-tests reveal no significant effect of stimulation type at any intensity or position ($p = 0.69$ – $p = 0.988$). Thus general comparisons between changes in MEP and CMEP amplitudes are warranted.

DISCUSSION

This is the first study to report on corticospinal excitability of antagonistic muscle groups during arm cycling. As expected, corticospinal and spinal excitability projecting to the biceps brachii was higher during elbow flexion than extension and was increased with a higher relative workload. The triceps brachii, however, provided some unexpected results. First, there were no phase-dependent differences in CSE projecting to the lateral head of the triceps brachii, though CSE did increase with an increased intensity. Second, spinal excitability was *higher during elbow flexion than extension*. Thus, there are intermuscle differences in the phase- and workload-dependent changes to corticospinal excitability during arm cycling.

PHASE-DEPENDENT MODULATION OF CORTICOSPINAL AND SPINAL EXCITABILITY

Corticospinal and spinal excitability to the biceps brachii was significantly greater during elbow flexion than extension, a finding we have demonstrated previously [17]. The phase-dependent differences in CSE can be partially accounted for by changes in supraspinal and spinal excitability (given the same pattern of change as those in CSE, Figs. 3A and B), though the exact mechanisms are not yet known. Our previous work showed that supraspinal excitability was different between cycling and tonic contraction during elbow flexion and we suggested that increased supraspinal excitability during this phase of arm cycling was to enhance the descending drive to the spinal cord to increase the recruitment and firing rates of the spinal motoneurones, thus producing adequate torque generating capabilities [17]. However, the cortical mechanisms associated with this increase in excitability have yet to be determined. At the spinal level, changes in

synaptic input and/or intrinsic motoneurone properties that would act to increase spinal motoneurone excitability could also explain the larger CMEP amplitude during elbow flexion compared to extension when the motor pool is less active and likely receiving reciprocal inhibitory input from the triceps brachii motor pool [17,34].

We hypothesized that overall CSE and spinal excitability projecting to the triceps brachii would be greater during elbow extension than flexion and were thus surprised that there was no phase-dependent difference in CSE to the triceps brachii, despite the significant phase-dependent difference in the pre-stimulus EMG amplitude (i.e. EMG higher during elbow extension; see Fig. 5C). This apparent dissociation between CSE and EMG suggests that changes in overall CSE assessed via TMS-evoked MEPs relate to differences in central motor command as opposed to changes in central drive required to increase EMG levels. That is, changes in MEP amplitude do not necessary relate to changes in ongoing muscle activity. This may be the case in the present study (i.e. dissociation between EMG and changes in CSE), especially given that arm cycling likely involves the operation of a spinal CPG [35] and is under different neural control than tonic contractions [11,14,17,22,36]. This also suggests that the central command controlling the triceps and biceps brachii may be different, given the phase-dependent modulation of CSE in the biceps brachii. Intermuscle differences in CSE during locomotor outputs in the legs have been previously reported [7]. Sidhu and colleagues (2012) demonstrated differences in the CSE to the rectus femoris and biceps femoris compared to the vastus lateralis during leg cycling and suggested that intermuscle differences in the phase-dependent modulation of CSE was a function of biarticular versus monoarticular muscles. It is noted that arm cycling is a bilateral motor output and

we did not assess the activity of the non-dominant limb. It is possible that the participants relied on elbow flexion of the non-dominant limb to produce elbow extension in the dominant limb, resulting in a lack of phase-dependency in CSE to the triceps brachii. Though we cannot rule out this possibility we consider it unlikely given that the EMG of the triceps brachii was higher during elbow extension than flexion in the dominant limb.

Even more surprising was that spinal excitability to the triceps brachii was higher during elbow flexion than extension, despite the higher pre-stimulus EMG during elbow extension (Figs 5B and E). There are several factors to consider for explaining this finding. First, higher spinal excitability during flexion than extension combined with a lack of phase-dependent modulation of CSE suggests that supraspinal excitability may be reduced to the triceps brachii during elbow flexion phase. Second, it is noted that we recorded the activity of the lateral head of the triceps brachii, a monoarticular muscle, which although active in elbow extension does not necessarily represent the activity or excitability in the other three elbow extensors (i.e. long and medial head of triceps brachii and the anconeus). The motoneurones projecting to the lateral head have lower recruitment thresholds than the long head when shoulder and elbow joint angles are 0 and 90 degrees of flexion respectively, during isometric contractions [37]. Those joint angles are equivalent to the elbow flexion position in the present study. Thus, the larger CMEPs during elbow flexion could be muscle specific and due to increased recruitment of spinal motoneurones. It is presently unclear how corticospinal and/or spinal excitability to the other elbow extensors is modulated during arm cycling.

Third, during elbow flexion the triceps brachii are in a stretched position compared to elbow extension, which would presumably increase muscle spindle activity. Increased

input from Ia afferents is known to exert a strong excitatory influence on motoneurone excitability, which may lead to increased recruitment and/or firing rate by activating persistent inward currents (PICs), for example, which amplify synaptic inputs [38,39]. Wilson and colleagues (2015) recently demonstrated, via indirect measures, that the contribution of PICs to motoneurone excitability was higher in the lateral head of the triceps brachii than the biceps brachii during isometric contractions. It is also noted that 1) motoneurones with lower recruitment thresholds, such as those in the lateral head of the triceps brachii, also have a higher incidence of PICs and 2) there is a higher incidence of PICs in extensor compared to flexor motoneurones [40,41]. It is possible that the stretch activated facilitation of PICs to the triceps brachii during elbow flexion may have increased spinal motoneurone excitability, thus increasing CMEP amplitude. The contribution of PICs to motoneurone excitability may be reduced during elbow extension when the triceps brachii are no longer in a stretched position, thus reducing PIC related amplification of synaptic input [42].

Finally, though corticomotoneuronal excitation occurs monosynaptically for both the biceps and triceps brachii, the incidence of those connections are much less in the triceps brachii, which involves a larger portion of polysynaptic connections in the corticomotoneuronal pathway [43,44]. Thus, although TMES-evoked CMEPs are suggested to represent spinal motoneurone excitability [18], CMEPs represent the ability of motoneurones to respond to synaptic input, not changes in the intrinsic properties of spinal motoneurones that are modifiable during locomotor outputs, such as the voltage threshold for action potential initiation and afterhyperpolarization amplitude [45-47]. With more interneurones relaying the information to the triceps brachii, TMES-evoked

CMEPs in the triceps brachii are thus more heavily influenced by interneuronal excitability than the biceps brachii. Given that arm cycling has been shown to be generated, in part, via a spinally located CPG [22,48], it is likely that many last order interneurones (excitatory and inhibitory) are active [49], thus influencing motoneurone excitability as seen in the CMEP amplitudes. The relative contribution of the corticomotoneuronal pathway to various muscles during locomotor output may thus be different, with some populations of motoneurone pools receiving greater cortical input than others. It may be that the observed intermuscle differences presented in corticospinal control herein represent different, muscle-dependent neural control strategies.

One possibility that we consider unlikely to account for similar spinal excitability of the triceps brachii during elbow extension and flexion, but cannot rule out with certainty, is that the higher pre-stimulus EMG during elbow extension could have blunted the CMEP amplitude due to the fact that the motoneurone pool was already highly active (i.e. the stimulation was insufficient to activate additional motoneurones or to increase their firing rate). However, when pre-stimulus EMG is carefully considered, the pre-stimulus EMG levels during elbow flexion and 15% PPO are not significantly different from those during elbow extension and 5% PPO, yet the CMEP amplitude during flexion are much larger than those during extension and 5% PPO (see Figs. 5B and E).

LOAD-DEPENDENT MODULATION OF CORTICOSPINAL AND SPINAL EXCITABILITY

Load-dependent increases in CSE were expected and did occur in both the biceps and triceps brachii during both flexion and extension phases of arm cycling. The loads used in the present study were significantly different from each other in terms of motoneurone

output as seen in the pre-stimulus EMG (see Figs. 3C, D and 5C, E), which is a general measure of muscle contraction intensity (i.e. the higher the pre-stimulus EMG the more active the muscle). Previous work examining the CSE to the biceps brachii during isometric contractions have reported increases in both MEP and CMEP amplitudes as the contraction intensity increases, up to a limit of approximately 60% of maximal voluntary contraction force output [20,26]. This suggests that spinal excitability contributed to the overall increase in CSE seen during these experiments. In the present experiment, significantly larger MEPs were recorded from both the biceps (Fig. 3A) and triceps brachii (Fig. 5A) muscles during arm cycling at 15% as opposed to 5% of PPO. Significantly larger CMEPs were recorded for the triceps but not biceps brachii at 15% vs 5% PPO, though the changes in CMEPs in the biceps brachii followed a similar pattern changes in MEP, suggesting that spinal excitability contributed to the increase in MEP amplitude. Perhaps the most novel and interesting point to consider is that it appears as though the type of intensity may be important in determining CSE during arm cycling. As opposed to isometric contractions, one can alter the intensity of arm cycling by changing the load, cadence, or a combination of both. In the present study we show that by increasing the load, the CSE to the biceps brachii increases during flexion and extension. In our previous work, however, we used cadence to alter the intensity of cycling and demonstrated that although overall CSE was increased to the biceps brachii during both phases as cadence increased, spinal excitability actually *decreased*, suggesting an overall increase in supraspinal excitability (see Figs 4A and D, Forman et al. 2015). Triceps brachii data, unfortunately, was not assessed and there is currently no information available regarding CSE to the triceps brachii during different intensity tonic contractions.

CONCLUSION

The most novel finding in the present study was that the phase-dependent modulation of corticospinal and spinal excitability appears to be different for the biceps and triceps brachii. While corticospinal and spinal excitability to the biceps brachii were both higher during elbow flexion compared to extension, as expected, corticospinal excitability to the triceps brachii was not phase-dependent and spinal excitability was actually higher during elbow flexion than extension. These findings suggest that the neural control of these antagonistic muscle groups may be differentially controlled by supraspinal and spinal centres. These findings warrant further investigation to determine their underlying mechanisms.

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CONFLICTS OF INTEREST: The authors have no conflict of interest to report.

FIGURE LEGEND

Figure 1. Experimental set-up. (A) Participants were seated with their shoulders at approximately the same height as the axis of the crank shaft on the cycle ergometer while cycling at 60 rpm at two different workloads (5 and 15% of PPO). Measurements were taken at the 6 o'clock (shown here) and 12 o'clock positions from the dominant arm. (B) Raw electromyography (EMG) trace for the biceps and triceps brachii from one participant. Note the monophasic and biphasic activation patterns of the biceps and triceps brachii, respectively. Black arrows labelled 12 and 6 represent the positions according to the face of a clock where stimulations were elicited (no stimulations in the traces shown).

Figure 2. Biceps brachii representative example (n=1). Average motor evoked potentials (MEPs; left panel) and cervicomedullary evoked potential (CMEPs; right panel) traces following 8 stimulations during arm cycling at 5% PPO (dashed gray line), and 15% PPO (solid black line) at the 6 o'clock (top panels) and 12 o'clock (bottom panels) positions. Amplitudes are expressed as a percentage of maximal M-wave (Mmax).

Figure 3. Group data (means ± SE, n = 12) for biceps brachii MEP amplitudes (A), and pre-stimulus EMG prior to transcranial magnetic stimulation (TMS; C). Group data (means ± SE, n = 8) for CMEP amplitudes (B) and pre-stimulus of the biceps brachii

prior to TMES (D). MEP and CMEP amplitudes are expressed relative to the Mmax taken during cycling at the same cadence and workload. *Significant difference ($P < 0.05$).

Figure 4. Triceps brachii representative example (n=1). Average motor evoked potentials (MEPs; left panel) and cervicomedullary evoked potentials (CMEPs; right panel) traces following 8 stimulations during arm cycling at 5% PPO (dashed gray line), and 15% PPO (solid black line) at the 6 o'clock (top panel) and 12 o'clock (bottom panel) positions. Amplitudes are expressed as a percentage of maximal M-wave (Mmax).

Figure 5. Group data (means ± SE, n = 12) for triceps brachii MEP amplitudes (A), and pre-stimulus EMG prior to transcranial magnetic stimulation (TMS; C). Group data (means ± SE, n = 8) for CMEP amplitudes based on position (B) and % PPO (D), as well as pre-stimulus of the triceps brachii prior to TMES (E). MEP and CMEP amplitudes are expressed relative to the Mmax taken during cycling at the same cadence and workload. *Significant difference ($P < 0.05$).

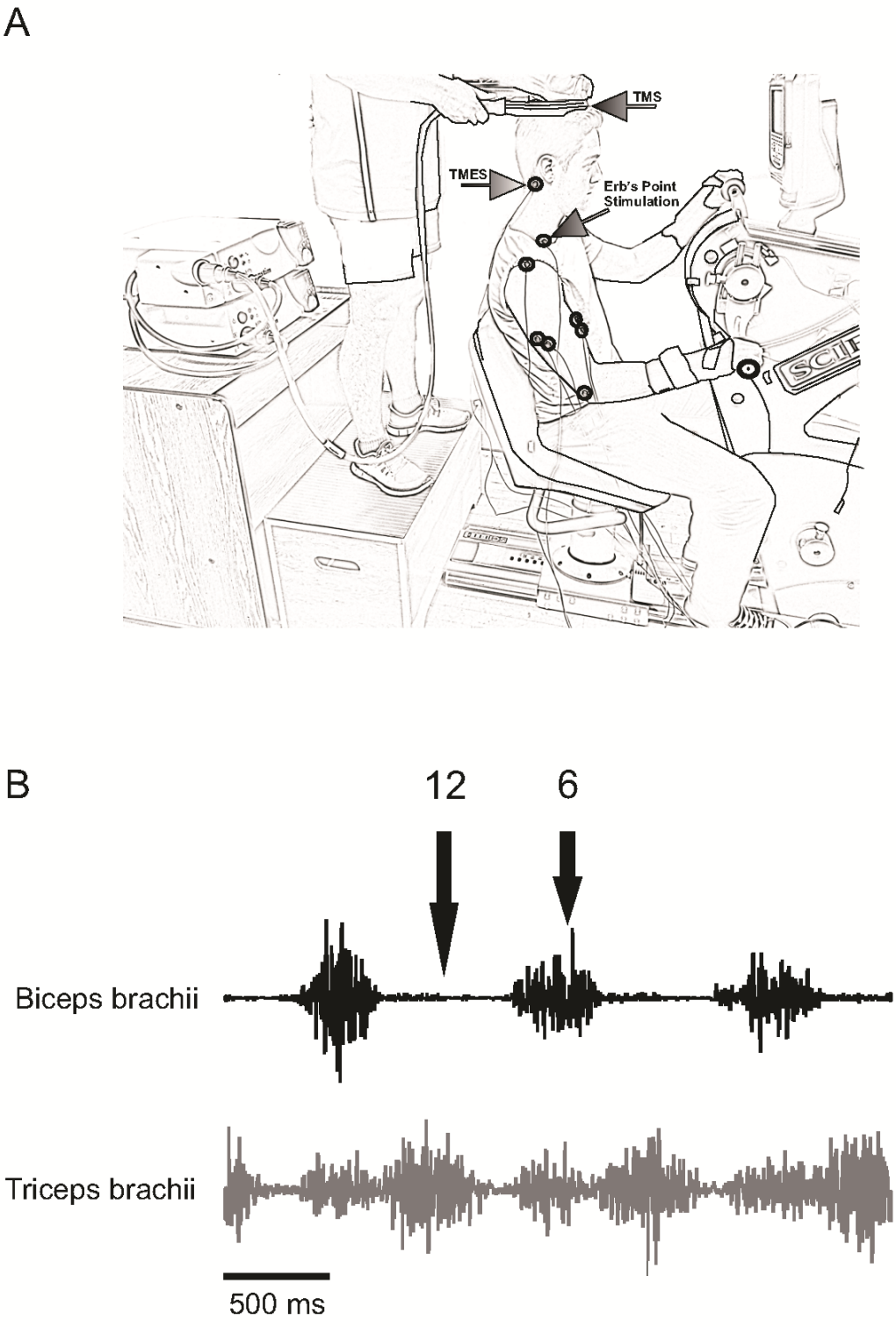

Figure 1 Experimental Set-up

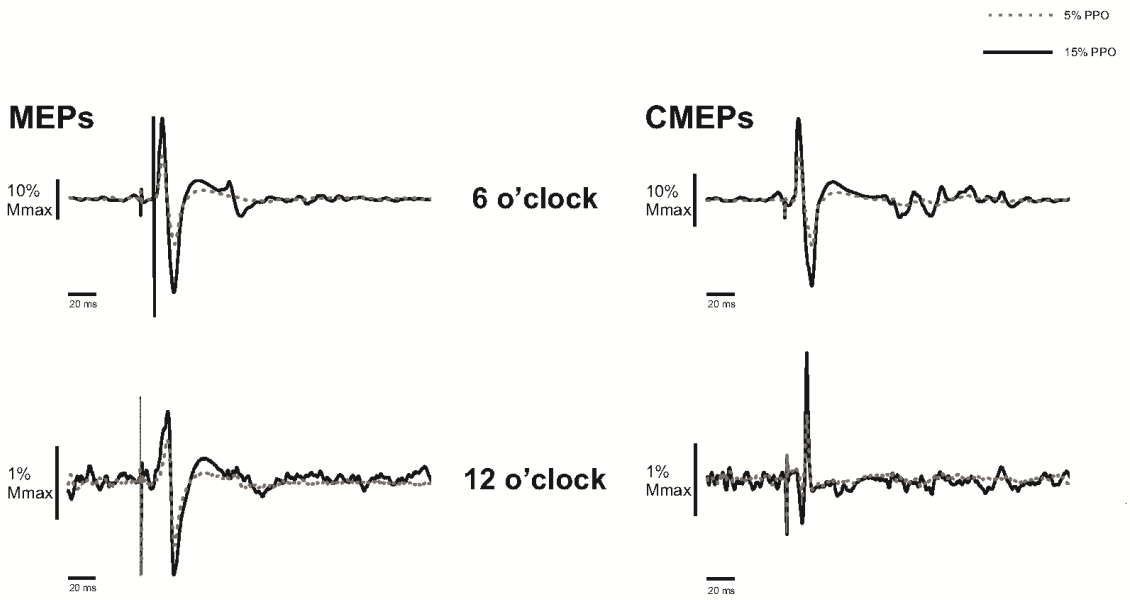

Figure 2 Biceps Brachii Representative Example

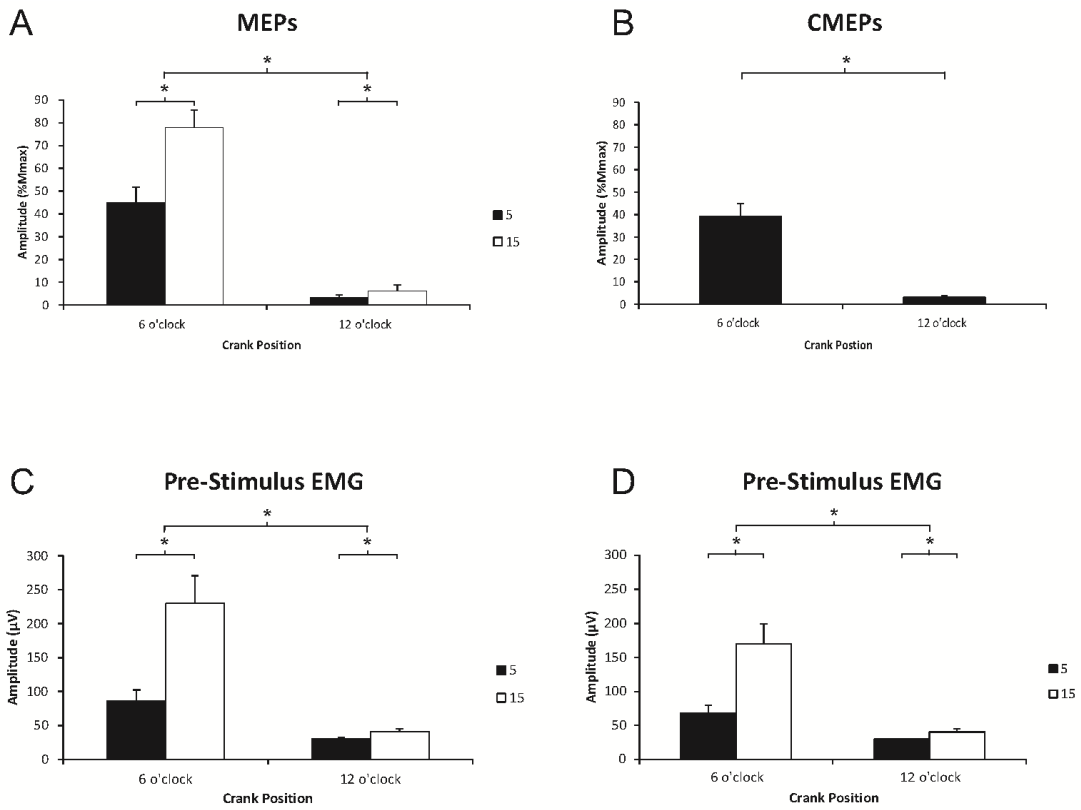

Figure 3 Biceps Brachii Group Data

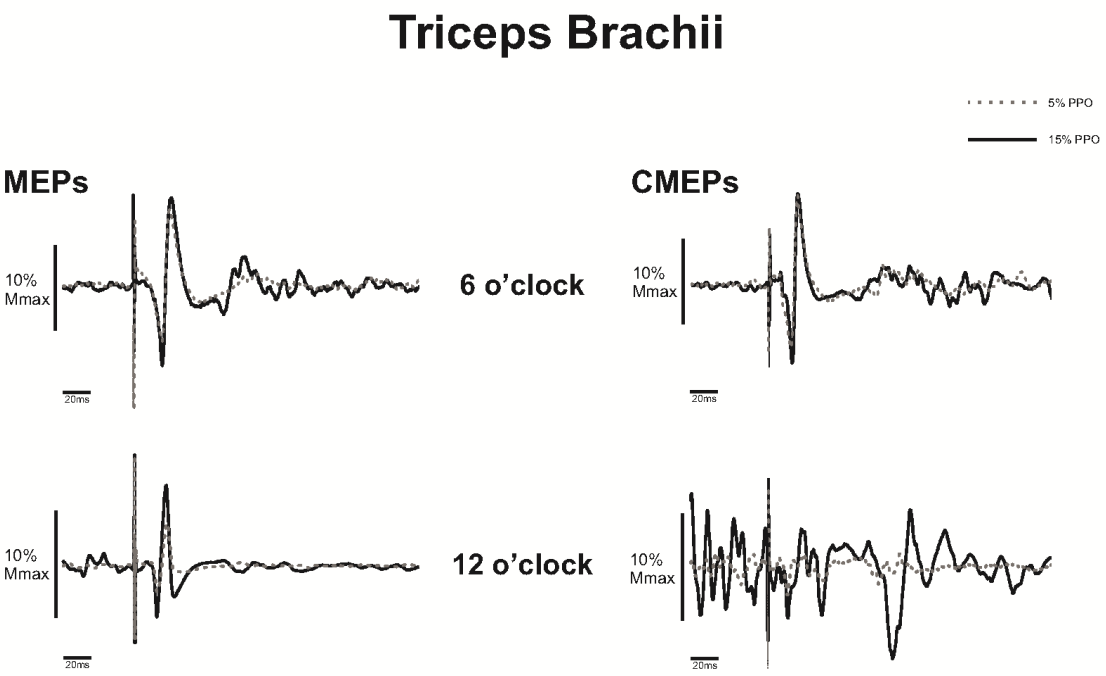

Figure 4 Triceps Brachii Representative Example

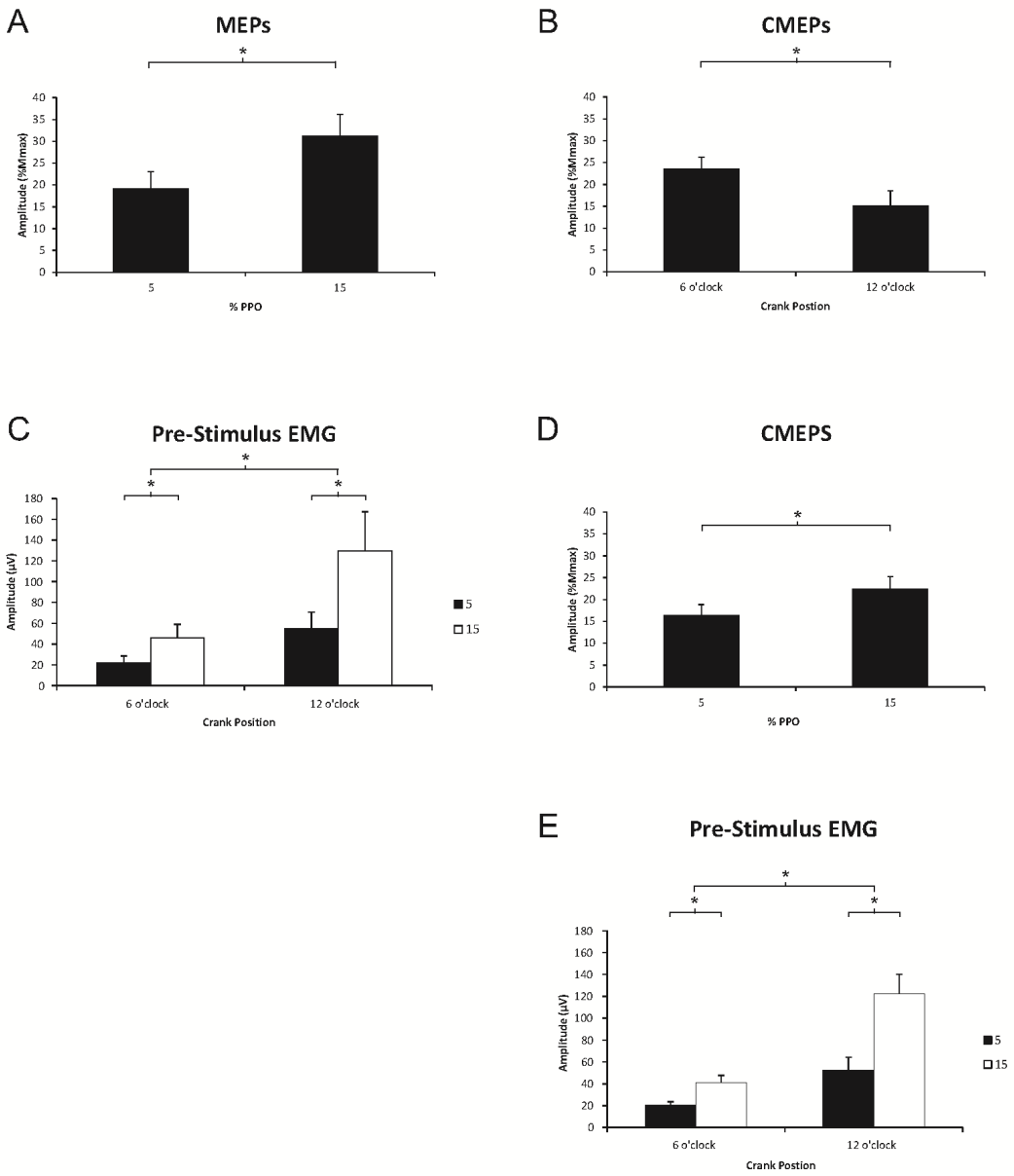

Figure 5 Triceps Brachii Group Data

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GENERAL SUMMARY AND REFLECTION

This project was initially designed to determine the differences in corticospinal excitability between intensity-matched isometric contractions and arm cycling using relative workloads; however, throughout the process some unexpected occurrences caused the purpose to shift. The first issue to arise was that participants were unable to match their isometric contraction intensity to the arm cycling intensity, which has proven difficult to do in multiple studies, though it is possible. Participants watched a computer screen with two horizontal lines placed at ± 10% of their mean rms EMG (mean rms EMG was calculated from the 50ms prior to stimulation in the cycling trial) and were asked to contract to a level where their EMG fell between the two lines. While the participants appeared to be keeping their EMG within the lines during the sessions, the subsequent statistical analysis showed a significant difference between the isometric contraction and arm cycling intensities. When we discovered this we had to decide whether to re-collect the entire project, or to remove the isometric piece and continue. We decided to continue without isometric contractions because multiple studies had already shown that corticospinal excitability is different during arm cycling than intensity matched isometric contractions.

The other surprise came when we were interpreting the results. The biceps brachii results followed the pattern of previous findings: corticospinal and spinal excitability were both higher during elbow flexion compared to extension and increased with increased relative workload; however, the triceps brachii results did not follow the same pattern. In the triceps brachii corticospinal excitability was not phase-dependent and

spinal excitability was higher during elbow flexion than extension. We then changed focus to the novel intermuscle differences findings as opposed to the original focus on relative workload.

Reflecting on the project and its outcome, I think it would be beneficial to do a similar study and include the comparison to isometric contractions. A more reliable method would be necessary to ensure that the isometric contraction intensity could be matched to the arm cycling intensity, but assuming that was possible it would be ideal to be able to directly compare corticospinal excitability between the two motor outputs. I also think the findings of this study advocate for including both the long head and the lateral head of triceps brachii when looking at arm cycling. Including the long and lateral head of triceps brachii will enable comparison between a monoarticular and biarticular head of the same muscle. That, in combination with the addition of another monoarticular muscle, such as brachialis, would provide insight into whether this dissociation between EMG and corticospinal excitability is a function of muscle articulation or muscle action (flexion vs extension). Brachialis, similar to the lateral head of triceps brachii, only crosses the elbow joint, and would therefore be an ideal choice to compare a monoarticular flexor to a monoarticular extensor. The inclusion of these muscles would allow a comparison between monoarticular and biarticular muscles that produce flexion and that produce extension, thus providing valuable insight into intermuscle differences in phase-dependent changes to corticospinal excitability.

Collecting from the long head of triceps brachii and brachialis in addition to the lateral head of triceps brachii and biceps brachii would help to clarify which, if any, of our suggested factors influenced the higher spinal excitability found during flexion

compared to extension, and lack of phase-dependent modulation of corticospinal excitability. We postulated that the lateral head of triceps brachii may not be representative of the other elbow extensors. It has a lower recruitment threshold than the long head when the shoulder and elbow joint angles are at 0 and 90 degrees, respectively. Including both the long and lateral heads of the triceps brachii would facilitate comparisons between both extensors. We also suggested these findings could be a result of the lateral head of triceps brachii being in a stretched position during elbow flexion compared to during elbow extension. The long head of triceps brachii would not experience the same degree of stretch at this position because it crosses both the shoulder and elbow joint. To further explore the effect of stretch on our findings, it would be beneficial to collect from more joint angles. In the current study we collected during mid-elbow flexion and extension, it would also be worthwhile to collect during the end range of elbow flexion and extension.

It is necessary to understand how the neural control of antagonistic muscle groups may be differentially controlled, particularly when both muscles are arguably of equal importance to arm cycling. It is also important to determine the source of such differences. Looking forward, we should include both the long head and the lateral head of triceps brachii, as well as biceps brachii and brachialis as muscles of interest during arm cycling.