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Investigating premotor corticospinal excitability in fast and slow voluntary contractions of the elbow flexors

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Abstract

Corticospinal excitability (CSE) increases prior to a voluntary contraction; however the relative contributions of cortical and spinal mechanisms are not well understood. It is also unknown whether the intended voluntary contractile rate affects the change in CSE. Therefore, the purpose was to assess cortical and spinal contributions to premotor CSE prior to fast (ballistic) and slow (ramp) contractions.

Eighteen young, healthy participants (9F) completed isometric elbow flexion contractions targeting 50% maximal voluntary contraction (MVC) force, at fast (as fast as possible) and slow (25% MVC/s) contractile rates. Participants were cued to contract with warning (red) and "GO" (green) visual signals. Magnetic and electric stimulations were applied to elicit motor evoked potentials (MEPs), cervicomedullary evoked potentials (CMEPs), and M-waves, respectively, in the surface electromyogram (EMG) recorded over the biceps brachii. MEPs and CMEPs were collected at 0, 25, 50 and 75% of premotor reaction time (RT) following the "GO" signal and compared to a resting baseline.

MEP amplitude demonstrated a 45% increase from baseline at 75% RT ($p = 0.009$). CMEP amplitude was already 49% larger than baseline at 0% RT ($p<0.001$) and increased progressively closer to EMG onset of the contraction. However, there were no differences in MEP and CMEP amplitudes when compared between contractile conditions (all $p>0.05$). Normalized to the CMEP, there was no difference in MEP amplitude from baseline in either contractile condition (all $p>0.05$).

These results indicate that increases in premotor CSE are predominantly spinally mediated. Furthermore, the increase in premotor CSE is not influenced by the intended voluntary contractile rate.

Keywords

Corticospinal tract, magnetic stimulation, electric stimulation, contractile rate, isometric contractions, reaction time.

Summary for Lay Audience

In preparation for a voluntary muscular contraction, there is an increase of excitability in connections made between the brain, spinal cord, and active muscles. However, it is not well understood whether this increase of excitability arises at the level of the brain or spinal cord. Additionally, it is unknown whether the rate of contraction to be performed (fast or slow) influences preparatory activity in the brain and spinal cord. This study investigated the factors influencing increased excitability before voluntary muscle contractions, and whether brain or spinal cord mechanisms play a more significant role when compared between fast and slow contractions.

Eighteen healthy (9 female) young adults participated in the study, completing elbow flexion contractions at both fast (as fast as possible) and slow ramp (2s) rates. Participants received visual cues indicating when to begin their contractions. Magnetic and electrical stimulation were used to elicit responses recorded from surface electrodes placed on the skin over the biceps brachii muscle. Magnetic stimulation was used to excite the motor cortex of the brain resulting in motor evoked potentials (MEP), whereas electrical stimulation was used to excite the spinal cord and brachial plexus, resulting in cervicomedullary evoked potentials (CMEP) and M-waves in the biceps muscle.

Results showed that the brain evoked response (MEP) increased from baseline before voluntary contractions, however this excitatory response was not different when compared between fast and slow contractions. The CMEP also increased significantly before the onset of contraction, indicating greater excitability at the spinal level, but this response was not different between fast and slow contractions either. When comparing MEP and CMEP responses, these findings revealed that spinal mechanisms play a more significant role to increase excitability in preparation for voluntary contractions. Although, this increase of excitability is not dependent on the rate of voluntary contraction to be performed.

Co-Authorship Statement

Daniel C. Basile, Michael T. Paris, and Charles L. Rice conceptualized and designed the study. Daniel C. Basile and Michael T. Paris participated in data collection and analysis. Daniel C. Basile, Michael T. Paris, Chris J. McNeil, and Charles L. Rice participated in interpretation of experimental data.

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List of Abbreviations

CMEP – Cervicomedullary evoked potential

CNS – Central nervous system

CSE – Corticospinal excitability

CST – Corticospinal tract

EMG –Surface electromyography

ITT – Interpolated twitch technique

LMN – Lower motor neuron

MEP – Motor evoked potential

MVC – Maximal voluntary contraction

MU – Motor unit

MUDR – Motor unit discharge rate

RFD – Rate of force development

RT – Reaction time

TMS – Transcranial magnetic stimulation

TMES – Transmastoid electrical stimulation

UMN – Upper motor neuron

VA – Voluntary activation

Chapter 1

1 Literature Review

1.1 General Introduction

Voluntary movement requires coordinated activity within the motor system, as descending signals from the motor cortex must be transmitted to the spinal cord and skeletal muscle to ultimately initiate a contraction. Synaptic connections made along the corticospinal tract are required to transmit motor signals from the cortex, and the efficacy in relaying these descending neural signals to generate action potentials within the motoneuron pool is termed corticospinal excitability (CSE). The influence of CSE on voluntary movement has been investigated previously using numerous paradigms and demonstrated to increase from a resting level prior to movement (Chen et al., 1998; Leocani et al., 2000; Cirillo et al., 2021; Baudry & Duchateau, 2021), indicating that premovement motor preparatory activity is occurring along the corticospinal pathway and resulting in an increase of premotor CSE. Therefore, just prior to initiating a voluntary contraction there is an increase of CSE to relay descending synaptic input to the motoneuron pool, however the relative influence of cortical and spinal contributions to premotor CSE is poorly understood. Developing a better understanding of this relationship would highlight the role of motor preparation on subsequent motor output, as cortical and spinal mechanisms concurrently influence premotor CSE and provide descending neural drive to the motoneuron pool to facilitate effective voluntary contractions.

1.2 Rate of Force Development

During voluntary contractions, rate of force development (RFD) is fundamentally dependent on two neural determinants related to motor unit (MU) structure and function: MU recruitment and MU discharge rate (MUDR) (Enoka & Duchateau. 2017). The first is the degree or speed of MU recruitment and the second is the frequency of action potential discharges reaching the active muscle. A motor unit consists of an individual motor neuron, and all the muscle fibres that the motor neuron innervates. Traditionally,

MU recruitment across most contractile modalities is governed by the “Henneman size principle” (Ducheateau & Enoka., 2011). This principle states that the order of recruitment is dependent on the size of motor neurons, as small, low-threshold motor neurons are recruited first for actions requiring small increases in force, whereas large increases in force would be achieved by the additional recruitment of larger, high-threshold motor neurons (Henneman. 1957). MUDR refers to the rate at which motor neurons discharge action potentials, and is proportional to the synaptic input (descending neural drive) that they receive in relation to other excitatory and inhibitory inputs (Enoka & Duchateau., 2017). Greater relative excitatory synaptic input to the motor neurons should increase the frequency (often measured in Hertz or Hz) of action potentials discharged by the individual motor units and facilitate an increase of contractile RFD.

1.3 Ballistic and Ramp Contraction

MUDR is also quite task-dependent, relevant to the contractile modality and how quickly a muscle may be required to increase force output. A gradual increase in muscle force during slow (ramp) contractions requires small, lower threshold MUs to be recruited first and then larger, higher threshold MUs to be additionally recruited as force increases to an upper limit reaching 80-90% MVC in many muscles (Kukulka & Clamann. 1981; De Luca et al., 1982), with a maximal MUDR of about 30-60Hz in a variety of limb muscles (Duchateau & Enoka., 2011). In contrast, MUs are recruited at much lower recruitment thresholds at the onset of rapid (ballistic) contractions, resulting in a much higher initial MUDR at the onset of muscle activation and subsequent decline during successive discharges to achieve a much faster increase in force production. Although this contractile modality has been tested in fewer muscles, MUDRs in rapid contractions can reach 60-120Hz in healthy individuals (Desmedt and Godaux., 1977) theoretically indicating greater excitatory synaptic input to the motoneuron pool. Therefore, although the discharge rate increases progressively to recruit larger, higher-threshold MUs during a slow-ramp contraction, ballistic contractions are characterized by a high initial discharge rate at the onset of rapid muscle activation, in order to recruit MUs as fast as possible and result in a much higher instantaneous MUDR.

1.4 Motor Preparation

To facilitate voluntary contractions, specific neural adjustments should precede the onset of movement stemming from an increase of excitability along the corticospinal tract and providing excitatory synaptic input to the motoneuron pool within a premovement, or motor preparatory phase, of voluntary contraction (i.e., <500ms prior to contraction). Prior studies in humans using TMS of the motor cortex (Chen et al., 1998; Leocani et al., 2000; Cirillo et al., 2021; Baudry & Duchateau, 2021), reported an increased amplitude of motor evoked potential (MEP) prior to a voluntary contraction, reflecting an increase of CSE before the onset of agonist muscle activation. CSE has also been shown to increase from a resting level after a warning signal is provided during a simple reaction time (RT) task, facilitating a motor preparatory phase in anticipation of the upcoming "GO" cue (Kennefick et al., 2014) and suggesting that the motor system is preparing to execute a movement (Deeke, 1996). Contrarily, if an individual is at rest with no intention to initiate a movement, then an increase of corticospinal excitability relative to a resting baseline should not be expected (Kalmar. 2018). Greater excitatory synaptic input to the pool of motoneurons should increase the frequency of action potentials discharged by the individual motor units, and subsequently facilitate the initiation of a voluntary contraction.

1.5 Corticospinal Tract

The corticospinal tract (CST) is a major neuronal pathway of the CNS, responsible for facilitating voluntary movement. The CST connects the cortex with the spinal cord, sending descending motor signals through bundles of motor fibers from cortical regions to the spinal cord, in order to initiate and modulate movement of the distal extremities. 75-90% of the fibers will decussate to the contralateral side through the pyramidal decussation (border between the medulla oblongata and the spinal cord), crossing the midline and continuing to the spinal cord through the lateral corticospinal tract (Welniarz et al., 2017). The lateral corticospinal tract sends fibers primarily to the extremity muscles and the innervation is contralateral, meaning that the left motor cortex controls extremities on the right side of the body, and vice versa. 5 to 15% of fibers that do not decussate within the pyramidal decussation make up the anterior corticospinal tract (Jang.

2014). This tract extends into the spinal cord, but only travels down to the level of the lower thoracic cord to control trunk or axial muscles.

The corticospinal pathway also contains both upper motor neurons (UMN) and lower motor neurons (LMN). UMN have nerve fibers responsible for communication between the brain and spinal cord, whereas LMN have nerve fibers that communicate between the spinal cord and muscle. When a motor impulse is generated from the primary motor cortex, UMN in the cortex transmit the impulse along the corticospinal tract to the anterior horn of the spinal cord, at which point the UMN will synapse with LMN in the spinal cord. The impulse from the LMN will then leave the spinal cord and reach the neuromuscular junction, transmitting the motor impulse to the active muscle fibers and resulting in a contraction.

1.6 Corticospinal Excitability

Voluntary movement requires coordinated activity from many parts of the motor system, as descending motor signals from cortical regions will be transmitted by the corticocortical and corticospinal (upper) motor neurons, which ultimately synapse with the spinal (lower) motor neurons at the spinal cord to activate the skeletal muscle (Kalmar. 2018). The efficacy of the corticospinal pathway to relay motor signals from the cortex, generate action potentials within the motoneuron pool at the spinal cord and transmit them down to the muscle reflects the overall “excitability” of the corticospinal pathway (Weavil & Amann., 2018).

At a cortical level, the response to a depolarizing stimulus is dependent upon synaptic efficacy of the corticocortical neurons which input to the corticospinal neurons, the intrinsic excitability of the corticospinal motor neurons and the net excitatory/inhibitory input to the corticospinal motor neurons. The efficacy of these connections collectively determines the strength of the descending volley transmitted to the spinal motor neurons (Kalmar. 2018). At a spinal level, the descending volley will activate the spinal motor neurons; with activation depending on corticospinal synaptic efficacy, intrinsic excitability of the spinal motor neurons and net excitatory/inhibitory input that the spinal motor neurons receive. Cortical and spinal transmission of a descending volley represent

more “central” mechanisms contributing to CSE. Transmission of the descending volley can also be impacted by various mechanisms at the “peripheral” level. Strength of the volley may be altered at the neuromuscular junction, along the sarcolemma, or even between a muscle and recording electrode (used for measuring CSE). Therefore, in this context CSE represents activity along the entire corticospinal pathway: encompassing the motor cortex, upper and lower motor neurons at the cortical and spinal level, neuromuscular junction and all the muscle fibers innervated by the motor neurons. Cortical, spinal, and peripheral mechanisms all provide an overall contribution to CSE, making it difficult to attribute changes in CSE to one particular level on the corticospinal pathway. Rather, to comprehensively assess CSE one can perform measurements which attempt to reflect the excitability of cortical, spinal, and peripheral mechanisms individually, and evaluate modulations at each level in concert with any changes in overall CSE.

1.7 Surface Electromyography

The electrical activity associated with the contraction of muscle fibers in motor units can be recorded non-invasively using surface electromyography (EMG). Surface electrodes can be positioned on the skin overlying a muscle to obtain a recording of generally non-selective electric activity across multiple active motor units during a contraction. However, given that surface EMG is percutaneous (no direct contact with the active muscles), the surface-detected signal can be attenuated by skin and subcutaneous adipose thickness, resulting in poor spatial selectivity with an inability to track small changes in individual motor unit activity (Duchateau et al., 2006). Additionally surface EMG often provides worse signal-to-noise ratios compared to intramuscular EMG, limiting the ability to interpret muscular activity.

1.8 Transcranial Magnetic Stimulation

Transcranial magnetic stimulation (TMS) was introduced by Barker et al. (1985), used to non-invasively stimulate the human brain by depolarizing neurons in the cortex to generate action potentials through electromagnetic induction. A simple TMS device consists of a few circular turns of copper wire connected to the terminals of a large

electrical capacitor through a switch. The device produces pulses of current which generate brief magnetic fields to penetrate into the brain without attenuation by the scalp or skull. When a circular TMS coil is placed on the scalp, the site of stimulation will cover a large area of the brain but the depth of penetration into the brain is small.

Changes in CSE are often evaluated with TMS of the motor cortex, measured through the size of motor evoked potentials (MEPs) recorded at the muscle with surface electromyography (EMG). A single high-intensity TMS pulse of the motor cortex will evoke a series of descending excitatory corticospinal volleys which travel along the corticospinal tract and descend to the motoneuron pool. The peak-to-peak MEP amplitude represents descending excitatory drive to the entire motoneuron pool, which can be subject to change based on both extrinsic factors (i.e., TMS intensity) as well as intrinsic factors (i.e., mental activity, level of muscle activation) and collectively used as a measure of CSE. Although, because the corticospinal pathway involves synaptic activation at both a cortical and spinal level, the size of the MEP depends on excitability of both cortical neurons and spinal motoneurons (Taylor. 2006). Therefore, MEPs provide a general representation of CSE along the corticospinal pathway, but they cannot effectively distinguish between cortical and spinal contributions to CSE, making it difficult to interpret changes in CSE using MEPs alone.

1.9 Transmastoid Electrical Stimulation

Transmastoid electrical stimulation (TMES) is another non-invasive method employed to assess spinal excitability, which involves passing a brief (100 - 500us) high-voltage electric pulse between adhesive electrodes fixed near the mastoid processes. Given the positioning of the electrodes, TMES evokes a short-latency excitatory response in descending axons located at the level of the cervicomedullary junction near the pyramidal decussation; producing recordings of electrical activity known as cervicomedullary motor evoked potentials (CMEPs) which can be recorded from the muscle with EMG (Taylor. 2006). TMES activates many of the same descending axons as TMS through the corticospinal pathway, however the CMEP is a result of motor neuron activation by a single descending volley, elicited through excitation of the corticospinal axons at a sub-cortical level (McNeil et al., 2013).

Whereas MEPs (elicited by TMS) represent synaptic activation at both a cortical and spinal level, the large monosynaptic component of CMEPs are evoked without synaptic activation in the cortex (Petersen et al., 2002) and hence often are used to interpret changes in MEPs by isolating for spinal motor neuron excitability. Using this method, it is generally easier to elicit CMEPs in the proximal upper limb muscles (i.e., biceps brachii) compared to distal upper limb muscles (i.e., FDI). Additionally, responses in the lower limb muscles (i.e., tibialis anterior) to TMES usually require higher intensities of stimulation and may not even evoke CMEPs in all individuals. The CMEP currently represents the most direct assessment of motoneuron excitability at the spinal level (McNeil et al., 2013), as the single excitatory descending volley and monosynaptic component underlying the CMEP helps discern between cortical and spinal contributions to overall CSE.

1.10 Compound Muscle Action Potential

Electrical stimulation of the peripheral nerve is an effective technique to elicit muscular contractions without voluntarily generated neural drive, enabling direct control over stimulation strength and frequency to the muscle. Electric stimulation can also be used to determine the maximal compound muscle action potential (maximal CMAP) recorded by EMG over the active muscle belly, and achieved through percutaneous stimulation of the nerve innervating a muscle of interest. The maximal CMAP represents a summation of all MU action potentials within the muscle, typically measured by peak-to-peak amplitude of the M-wave elicited by electric stimulation (*M*max). For a more generalized representation of neural activation, recordings are often made with a monopolar electrode configuration: active electrode positioned over the muscle belly and reference electrode positioned over the nearest tendon. To determine the maximal CMAP, percutaneous electrical stimulation over the designated muscle-innervating nerve is gradually increased in intensity until the size of the EMG recorded M-wave reaches a maximal peak-to-peak amplitude (*M*max).

1.11 Biceps Brachii

The biceps brachii is a large, bi-articular muscle on the ventral portion of the arm, composed of a short head and a long head. The short head originates from the coracoid process, whereas the long head originates from the supraglenoid tubercle. Both the long and short heads of the biceps insert on the posterior aspect of the radial tuberosity. The main arterial blood supply for biceps brachii is via the muscular branches of the brachial artery, and the nerve supply to the biceps is provided by the musculocutaneous nerve (root C5, C6) (Pacha Vicente et al., 2005). The primary function of the biceps brachii is to supinate the forearm, flex the elbow, and play a small role in flexion of the shoulder (Landin et al., 2017). Biceps brachii is a fusiform muscle, meaning the muscle belly fibers are arranged in parallel to each other, and composed of similar proportions of type 1 and type 2 muscle fibers (Bellemare et al., 1983).

1.12 Isometric Contractions

During isometric contractions, an individual contracts against a force transducer which is locked in a set position. Force is produced by the active muscles but although there is some internal shortening, the joint angle does not change. Isometric contractions have utility for electromyographical studies, as there is generally a greater signal-to-noise ratio and the recording area over the active muscle is more constant (minimal electrode displacement with no changes in muscle length) compared to dynamic contractions, which facilitate large changes in muscle length.

1.13 Purpose and Hypothesis

Limited work has investigated the potential difference in cortical and spinal contributions to premotor CSE prior to voluntary contractions, and the integration of these mechanisms functioning to increase premotor CSE is still poorly understood. Additionally, very few studies have attempted to investigate changes in CSE in concert with anticipated contractile rate, specifically to highlight differences in premotor CSE that may occur prior to voluntary contractions performed at fast (ballistic) and slow (ramp) contractile rates. Developing a better understanding of this relationship will provide unique and novel insights into the role of corticospinal mechanisms on subsequent voluntary

contractile activity, by assessing the effects of potentially different cortical and spinal contributions influencing CSE in preparation for both fast and slow voluntary contractions. Therefore, the purpose of this thesis was to investigate differences in cortical and spinal contributions to premotor CSE measured in the premotor reaction time (RT) period prior to initiating isometric fast (ballistic) and slow (ramp) contractions voluntarily. Preparing to initiate a voluntary contraction should facilitate an increase of premotor CSE within the premotor RT period, producing greater excitatory synaptic input through both cortical and spinal mechanisms prior to the onset of contraction compared to resting baseline values. Additionally, a greater increase in premotor CSE relative to baseline should be expected prior to initiating ballistic contractions compared to ramp contractions.

Chapter 2

2 Premotor corticospinal excitability in fast and slow voluntary contractions of the elbow flexors

2.1 Introduction

Rate of force development (RFD) is the ability to rapidly increase force output from a resting level at the onset of a contraction, and represents an important factor in determining the explosive strength of individuals as they perform successful movements (Maffiuletti et al., 2016). RFD is principally determined by two neural mechanisms related to motor unit (MU) structure and function: MU recruitment and MU discharge rate (MUDR). The first is the degree or speed of MU recruitment and the second is the frequency of action potential discharges reaching the active muscle. To produce slow graded (ramp) contractions in many skeletal muscles, MU recruitment proceeds until approximately 80% MVC (Kukulka & Clamann. 1981; De Luca et al., 1982), with MUDR achieving 30-60Hz at MVC (Duchateau & Enoka., 2011). In contrast, MUs are recruited at much lower recruitment thresholds at the onset of rapid (ballistic) contractions with much higher initial MUDRs reaching approximately 60-120Hz in healthy individuals (Desmedt and Godaux., 1977), functioning to achieve much faster RFD. Therefore, modulations in MUDR are often task-dependent and relevant to the contractile rate required; fundamentally driven by corticospinal input strength to the spinal motor neurons prior to discharge (Del Vecchio et al., 2019).

Initiating any voluntary movement requires synaptic connections along the corticospinal tract to transmit motor signals from the cortex, and the efficacy in relaying these descending neural signals to generate action potentials within the motoneuron pool is termed corticospinal excitability (CSE). CSE is often assessed using transcranial magnetic stimulation (TMS), by stimulating the primary motor cortex (M1) and inducing an electromagnetic current throughout a pool of motoneurons. Single-pulse TMS is used to evoke a series of descending excitatory volleys in the motoneurons, which travel along the corticospinal pathway to the active muscle and can be recorded as a motor evoked potential (MEP) using surface electromyography (Reis et al., 2008). However, when

delivered in the preparation or premovement phase prior to a voluntary contraction (prior to muscle activation), TMS measures have demonstrated a premotor increase in MEP amplitude, likely reflecting an increase of CSE in preparation for the upcoming movement (Chen et al., 1998; Leocani et al., 2000; Cirillo et al., 2021; Baudry & Duchateau, 2021). For example, prior findings have demonstrated that CSE (evaluated with MEPs) can increase from a resting level after a warning signal is provided during a simple reaction time (RT) task, facilitating a motor preparatory phase in anticipation of the upcoming imperative cue (Kennefick et al., 2014) and suggesting that the motor system is preparing to execute a movement (Deecke. 1996). However, using TMS to assess CSE through MEPs only represents excitability along the entire corticospinal pathway, making it difficult to correctly attribute changes in CSE to one particular level (cortical, spinal, peripheral) of the corticospinal pathway.

Changes at the spinal level have also been demonstrated in preparation for voluntary contractions when tested in the dorsiflexors (Kagamihara et al., 1992; Baudry and Duchateau., 2021), as these prior studies utilized the H-reflex and F-wave test, respectively, to evaluate spinal excitability. However, the H-reflex test is subject to presynaptic inhibition of the 1a afferents (McNeil et al., 2013) and axonal hyperpolarization in sensory and motor axons with fatigue (Vagg et al., 1998), which can alter the size of the H-reflex response and potentially misrepresent spinal motoneuron excitability. Additionally, the F-wave test can be relatively insensitive to changes in motoneuron excitability, as the motoneurons which generate F-waves are often limited to large motoneurons (Espiritu et al., 2003). Using transmastoid electrical stimulation (TMES) to elicit a cervicomedullary evoked potential (CMEP) currently represents the most direct method to assess spinal excitability (McNeil et al., 2013), as the CMEP is primarily the result of motoneuron activation by a single descending volley elicited by excitation of corticospinal axons (Ugawa et al., 1991; Gandevia et al., 1999). Hence, they can be effectively used to interpret changes in MEPs by isolating for spinal motoneuron excitability. Kennefick et al. (2019) previously assessed cortical and spinal changes to the MEP with a movement complexity task, however very limited work has investigated the potential difference in cortical and spinal contributions to premotor CSE with voluntary

contractions performed at different contractile rates, and the integration of these mechanisms functioning to increase premotor CSE is still poorly understood.

Therefore, using a simple RT task, this study will investigate differences in cortical and spinal contributions to premotor CSE measured in the premotor RT period prior to initiating isometric ballistic and ramp contractions voluntarily. Facilitating a motor preparatory phase with a warning signal should initiate an increase of premotor CSE, producing greater excitatory synaptic input through both cortical and spinal mechanisms prior to the onset of contraction compared to resting baseline. Additionally, a greater increase in premotor CSE relative to baseline should be expected prior to initiating ballistic contractions compared to ramp contractions.

2.2 Methods

Participants

Young (18-30 years), healthy males (n=9) and females (n=9) visited the lab on two occasions (1st: familiarization with TMS and various electrical stimulations, as well as practicing ballistic and ramp contractions) and 2nd: the experimental protocol). Completion of both sessions took approximately 2.5 hours total for each participant. Exclusion criteria included the presence of any neuromuscular disorders, discomfort lying down supine for extended periods of time, and TMS exclusion criteria. All participants gave informed consent, and the experimental protocol was approved by the institutional ethics review board.

Experimental Set-Up

Participants were situated in a supine position on a custom-built experimental table, with their left arm secured in an isometric force transducer at an angle of ~90 deg. flexion at the elbow joint, while restricting any motion of the shoulder joint to isolate the action of the elbow flexors. The forearm was in a neutral grip position and a strap at the wrist firmly secured the forearm to the force transducer. Elbow flexor force was measured with a linear strain gauge. Surface EMG of the biceps brachii was recorded via adhesive Ag–AgCl electrodes (20 mm diameter) arranged in a monopolar configuration. The active

recording electrode was positioned over the belly of the biceps muscle, and the reference electrode was placed over the distal tendon for the biceps.

In all experiments, force and EMG data were recorded to the computer using a 12-bit A/D converter (CED 1401 Plus; Cambridge Electronic Design Ltd, Cambridge, UK) in conjunction with Spike2 software (v. 6.06; Cambridge Electronic Design). The force and EMG signals were sampled at 2000 and 5000 Hz, respectively. EMG data were amplified (×100) and bandpass filtered (16–1000 Hz) using a NeuroLog system (Digitimer Ltd.). EMG of the biceps was also displayed on a computer monitor in real time for participants to receive visual feedback.

Brachial Plexus Stimulation

A constant current stimulator (DS7A, Digitimer) was used to deliver single electrical stimuli (200μs pulse width; 400 V) to the brachial plexus at Erb's point to record the maximal compound muscle action potential in the biceps. The cathode and anode (Cardinal Health™ adhesive Ag–AgCl electrodes, 20mm diameter) were placed in the supraclavicular fossa and over the acromion, respectively. Experimental stimulus intensity (i.e., 60-125mA) was gradually increased until the M-wave amplitude reached a plateau indicating a maximal M-wave response (*M*max) obtained at the active muscle in a rested state.

Transcranial Magnetic Stimulation (TMS)

To elicit a MEP from the biceps brachii, stimulation of the motor cortex was delivered over the vertex using a circular coil (13.5 cm outside diameter) attached via a BiStim unit to two Magstim 200 stimulators (Magstim, Dyfed, UK). The experimental stimulus intensity (i.e., 50-100%) was set to evoke a MEP in the biceps of ∼10% *M*max in a rested state.

Transmastoid Electric Stimulation (TMES)

To elicit a CMEP from the biceps brachii, a brief high-voltage electrical current (200μs duration, Digitimer DS7A) was passed between adhesive electrodes (Cleartrace) fixed to the skin approximately ~1cm superior and medial to the mastoid processes (Gandevia et al., 1999). The cathode was placed above the mastoid on the right side, and the anode was placed above the mastoid on the left side. The stimulation intensity (i.e., 125-325mA) was set to evoke a CMEP in the biceps of ~10% *M*max in a rested state.

Experimental Protocol

Session 1 - Familiarization

The familiarization session began by determining each participant's MVC, using the interpolated twitch technique (ITT) to determine voluntary activation (VA, Herbert & Gandevia. 1999). The required ITT current was achieved with percutaneous electric stimulation, with the cathode and anode (Cardinal Health™ adhesive Ag–AgCl electrodes, 20mm diameter) placed approximately ~5cm apart upon the muscle belly of the biceps brachii. Participants were familiarized with making isometric elbow flexion contractions and performed approximately 10 ballistic and 10 ramp practice trials to get accustomed with completing these contractions to ~50% MVC. Ballistic practice contractions were used to establish the mean premotor RT for each participant, defined as the time between presentation of the imperative "GO" cue and the onset of EMG activity. Given that RTs differ between participants, assessing CSE at time points relative to premotor RT ensures that the same preparatory processes are being measured across all participants when the stimulation is delivered (Kennefick et al., 2019). The *M*max was then determined in the relaxed biceps brachii, by increasing the electrical stimulation current incrementally until the amplitude of the M-wave reached a plateau. Participants were familiarized with TMS and TMES that elicited a MEP and CMEP amplitude equivalent to ~10% of the *M*max, to ensure participants were responsive to these measures in a rested state and comfortable with the associated physical sensations.

Session 2 – Experimental

The experimental session began by completing the same measures as the familiarization session, except for the premotor RT test. This was performed to account for any changes in MVC and peripheral excitability that may occur between sessions. Participants also performed approximately 10 practice trials of ballistic and ramp contractions to ~50% MVC, prior to beginning the protocol. The subsequent experimental protocol consisted of a simple RT task, meaning participants completed a pre-determined contraction as fast as possible after a visual imperative "GO" cue (green light) was provided. All experimental trials were separated by a 15s time interval, and included a visual warning cue (red light) provided 1000ms prior to the imperative "GO" cue to facilitate a motor preparatory phase. TMS and TMES were delivered at 4 separate time intervals relative to the imperative "GO" cue: 0%, 25%, 50%, and 75% RT (participant RTs determined in session 1) to assess cortical and spinal contributions to premotor CSE, respectively. Each time interval represented 1 individual trial, and 12 trials collectively represented 1 block of trials (Figure 1). When prompted with the "GO" cue, each participant performed either a ballistic or ramp, isometric elbow flexion of their biceps brachii muscle to ~50% MVC (contraction to be performed determined before beginning the block). With no prompt from visual cues, 2 TMS and 1 TMES were delivered in each block at random to determine a resting baseline for CSE, indicated by an absence of background EMG activity in the biceps muscle. Thus, changes in MEP and CMEP amplitudes at each time interval within the block were referenced to resting baseline. The order of experimental trials was randomized and counterbalanced, ensuring each participant ultimately completed an equal number of ballistic and ramp contractions throughout the protocol. Participants were instructed to react "as fast as possible" after the imperative "GO" cue appeared and initiate the ballistic or ramp contraction. Finally, all participants received visual feedback of their force output in real-time from a monitor placed ~1 m away.

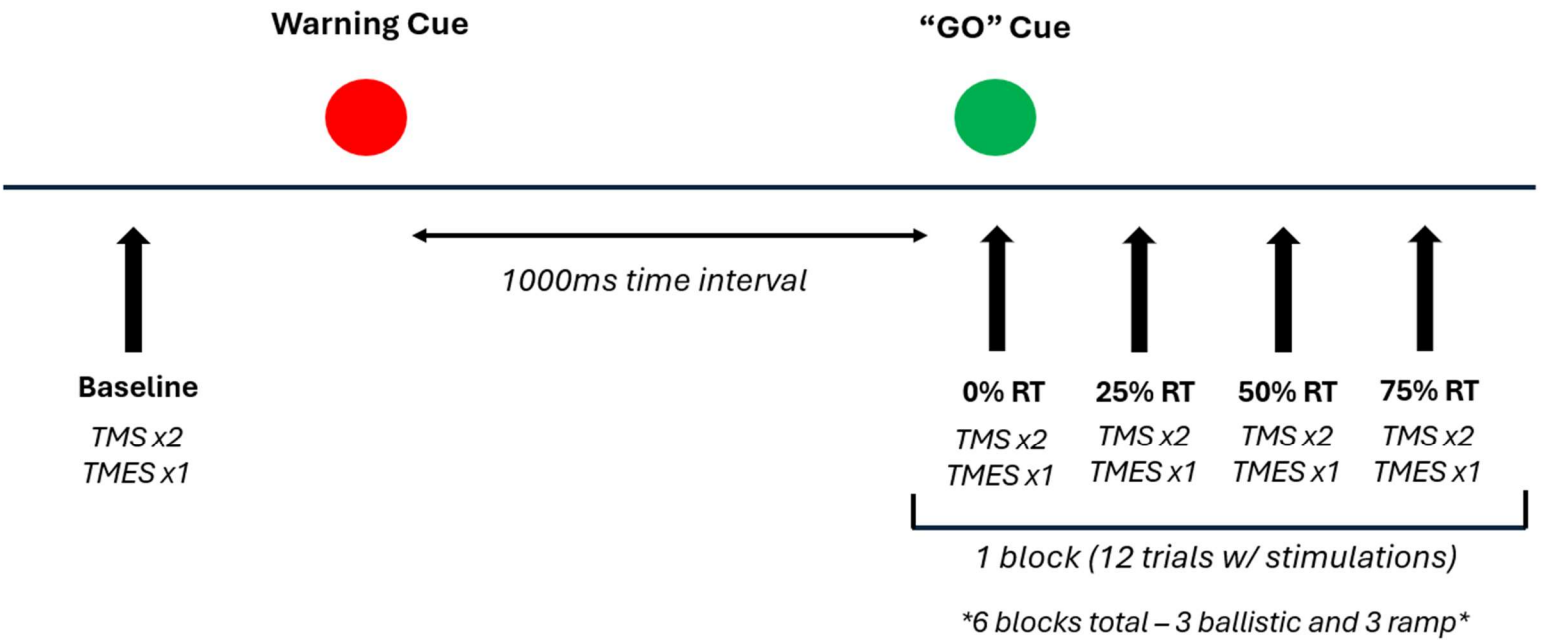

Figure 1 Experimental protocol schematic with visual representation of TMS and TMES stimulation points relative to the "GO" cue. Arrows pointing upwards indicate the timing of TMS and TMES stimulations.

Data Analyses

All analyses were performed off-line using Spike2. The amplitude of each evoked potential (*M*max, MEP, and CMEP) was measured between cursors marking the initial deflection from baseline EMG activity to the second crossing of the horizontal axis (Martin et al., 2006). MEP and CMEP amplitudes at baseline were normalized to 10% of each participant's *M*max amplitude, and changes in MEP and CMEP amplitudes over time were expressed relative to baseline values. MEP amplitude was used to assess CSE in each condition and CMEP amplitude was used to evaluate the spinal contribution to any changes in CSE. To assess the cortical contribution to any changes in CSE, MEP amplitude was normalized to CMEP amplitude obtained under the same conditions and time points (i.e., MEP at 0% RT/CMEP at 0% RT). Evoked potentials with an amplitude greater than 2 SDs from the overall mean of each participant were discarded from analysis (Kennefick et al., 2019). To assess voluntary EMG onset, the root mean square (RMS) of the background EMG activity was measured in the 100ms period before each TMS or TMES pulse was delivered. If background RMS-EMG activity was 2 SDs or more above baseline levels prior to stimulation (indicating agonist muscle pre-activation), the entire trial was removed from the analysis (Kennefick et al., 2019). Approximately 5% of all experimental trials were removed from analysis due to pre-activation of the agonist muscle.

Statistical Analysis

All statistical analysis was performed in R (version 4.1.2; R Foundation for Statistical Computing, Vienna, Austria). The main dependent variables were MEP amplitude (corticospinal excitability), CMEP amplitude (spinal excitability), and MEP/CMEP ratio values (cortical excitability). The independent variables included: Type (ballistic or ramp conditions) as a within-subjects factor, time (baseline, 0%, 25%, 50%, 75% premotor RT) as a within-subjects factor, and sex (male and female) as a between-subjects factor. Evoked potentials were assessed for normality using the Shapiro-Wilk's test. Both MEP and CMEP amplitudes were significantly non-normal ($p < 0.05$), so MEP and CMEP data were subjected to a logarithmic transformation (Manikandan, 2010). Log-transformed

MEP and CMEP data were analyzed with a three-way repeated measures analysis of variance (RM-ANOVAs), using TYPE (2 levels - ballistic and ramp) x TIME (5 levels - baseline, 0%, 25%, 50%, 75% RT) x SEX (2 levels - male and female) variables. If main effects were significant, post hoc Dunnett's test and Bonferroni-corrected unpaired samples Student's t-tests were performed, where appropriate. MVC (in Newtons) and VA (%) pre-and-post experimental protocol was analyzed using paired sample Student's t-tests, to compare the difference in values pre-post protocol. Significant differences in the data were defined as $p < 0.05$. All data are presented as mean ± SD.

2.3 Results

Control Measures

Mmax was measured pre- and post-protocol at rest, with the participant's arm secured in the force transducer as described in the "Methods" section. Across all participants, mean peak-to-peak amplitude of Mmax in the biceps brachii was 22.0 ± 6.4 mV pre-protocol, and 21.9 ± 6.2 mV post-protocol. Mean baseline absolute MEP and CMEP amplitudes were 2.44 ± 1.32 mV (11.0% Mmax) and 2.29 ± 1.28 mV (10.4% Mmax), respectively. The mean premotor RT was 252.8 ± 27 milliseconds. Mean pre-protocol MVC pooled across all participants was 72.6 ± 29.1 Newtons, and post-protocol MVC was 71.2 ± 28.7 Newtons, indicating that the protocol did not induce any substantial neuromuscular fatigue which was corroborated with no significant change in VA (93.6% ± 1.7% pre-protocol and 93.1% ± 1.8% post-protocol). Mean values for all data in absolute units are summarized in Table 1.

Table 1 Participant demographics, control measures, MEP and CMEP data. Control measures, MEP and CMEP data are presented in absolute units as mean ± SD.

Participant Demographics	**n = 18**	
Age (yrs)	23.5 ± 3.3	
Height (cm)	172.3 ± 11.2	
Body mass (kg)	69.2 ± 12.4	
Reaction time (ms)	252 ± 27	
ITT current (mA)	204 ± 70	
*M*max current (mA)	90 ± 26	
TMES intensity (mA)	198 ± 55	
TMS intensity (%)	82 ± 19	
Control Measures	**Pre-Protocol**	**Post-Protocol**
MVC (N)	72.61 ± 29.06	71.22 ± 28.69
VA (%)	93.56 ± 1.72	93.50 ± 1.82
*M*max (mV)	22.0 ± 6.48	21.90 ± 6.24
10% *M*max (mV)	2.20 ± 0.65	2.19 ± 0.62
MEP (mV)	**Ballistic**	**Ramp**
Baseline	2.44 ± 1.32	2.78 ± 1.39
0% RT	2.88 ± 1.62	2.87 ± 1.48
25 % RT	2.97 ± 1.66	3.09 ± 1.64
50% RT	3.11 ± 1.67	3.34 ± 1.80
75% RT	3.42 ± 1.60	3.47 ± 1.56
CMEP (mV)	**Ballistic**	**Ramp**
Baseline	2.24 ± 1.30	2.32 ± 1.27
0% RT	3.35 ± 2.33	3.20 ± 1.68
25% RT	3.32 ± 2.08	3.26 ± 1.90
50% RT	3.55 ± 2.28	3.43 ± 2.06
75% RT	3.72 ± 2.17	3.53 ± 1.62

MEP

The three-way RM-ANOVA revealed a significant main effect of time ($p = 0.0009$), with no effect of type ($p = 0.097$), sex ($p = 0.105$) or interactions for MEP amplitude. This indicates that MEP amplitude significantly increased from baseline across RT points, but there was no difference in MEP amplitude between the ballistic and ramp conditions, as well as no difference between male and female participants. Post hoc Dunnett's test was used to inform the effect of time, revealing a significant difference in MEP amplitude (pooled ballistic and ramp data) at 75% RT ($p = 0.009$) compared to baseline (Figure 2).

CMEP

There was a significant main effect of time ($p = <0.0001$) and sex ($p= 0.038$), with a significant interaction between time:sex ($p = 0.011$), but no effect of type ($p = 0.379$). This indicates that CMEP amplitude increased significantly from baseline across RT points irrespective of contraction type, and there was a significant difference in amplitude between male and female participants. However, CMEP amplitude was not different between the ballistic and ramp conditions, and therefore post hoc Dunnett's test was used to inform the effect of time, revealing a significant difference in CMEP amplitude (pooled ballistic and ramp data) at all RT points compared to baseline (all $p = <0.0001$) (Figure 3). Bonferroni-corrected post-hoc unpaired t-tests were used to inform the interaction effect of time:sex, revealing a significant difference in CMEP amplitude between male and female participants at 25% RT ($p = 0.009$) and 75% RT ($p = 0.002$). (Figure 4).

MEP/CMEP

For the ratio of MEP/CMEP, the analysis revealed no significant main effect of type ($p = 0.220$), time ($p = 0.264$), or sex ($p = 0.873$). This indicates that there were no significant differences in cortical excitability between the ballistic and ramp conditions, cortical excitability did not change significantly from baseline across RT points, and there was no difference in cortical excitability responses between male and female participants (Figure 5).

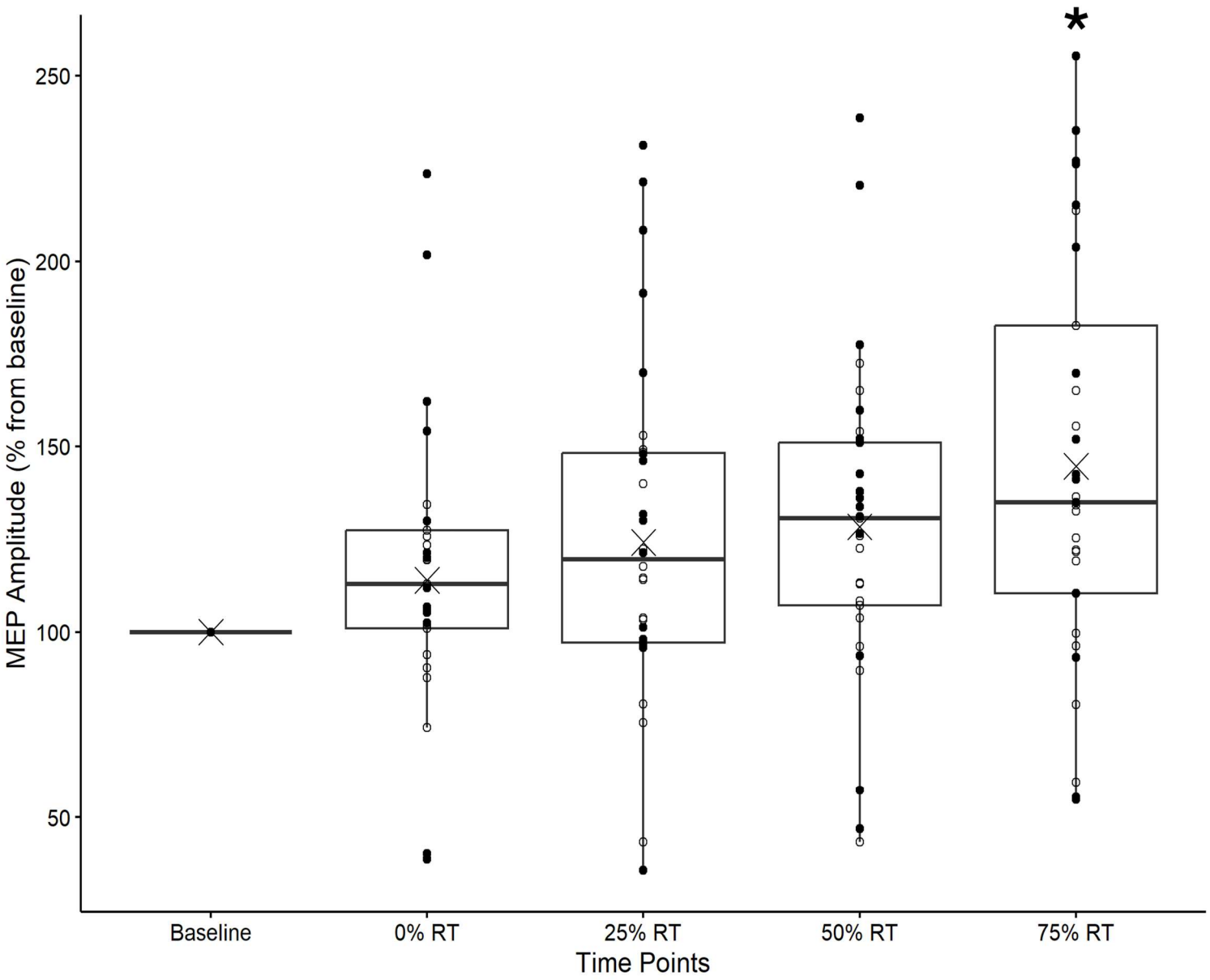

Figure 2 Individual participant ballistic (filled circles) and ramp (open circles) changes in MEP amplitude (%) relative to baseline. Solid horizontal lines in each box represent the median value, and the box outlines represent the upper (top, Q3) and lower (bottom, Q1) quartile range of the data. "X" represents the mean value for pooled data between ballistic and ramp conditions at each RT point. *****Significant differences in MEP amplitude from baseline ($p < 0.05$).

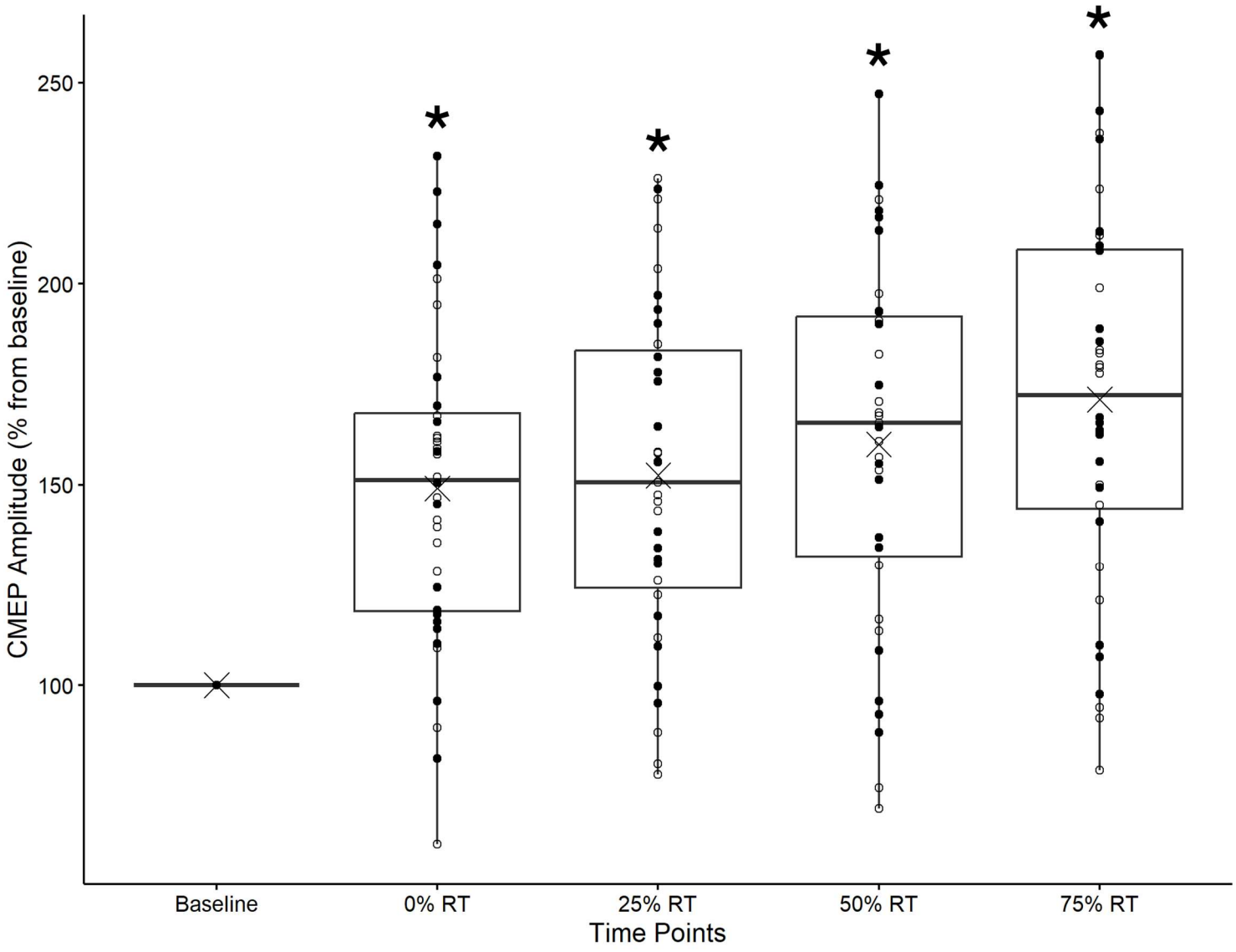

Figure 3 Individual participant ballistic (filled circles) and ramp (open circles) changes in CMEP amplitude (%) relative to baseline. Solid horizontal lines in each box represent the median value, and the box outlines represent the upper (top, Q3) and lower (bottom, Q1) quartile range of the data. "X" represents the mean value for pooled data between ballistic and ramp conditions at each RT point. *Significant differences in CMEP amplitude from baseline ($p < 0.05$).

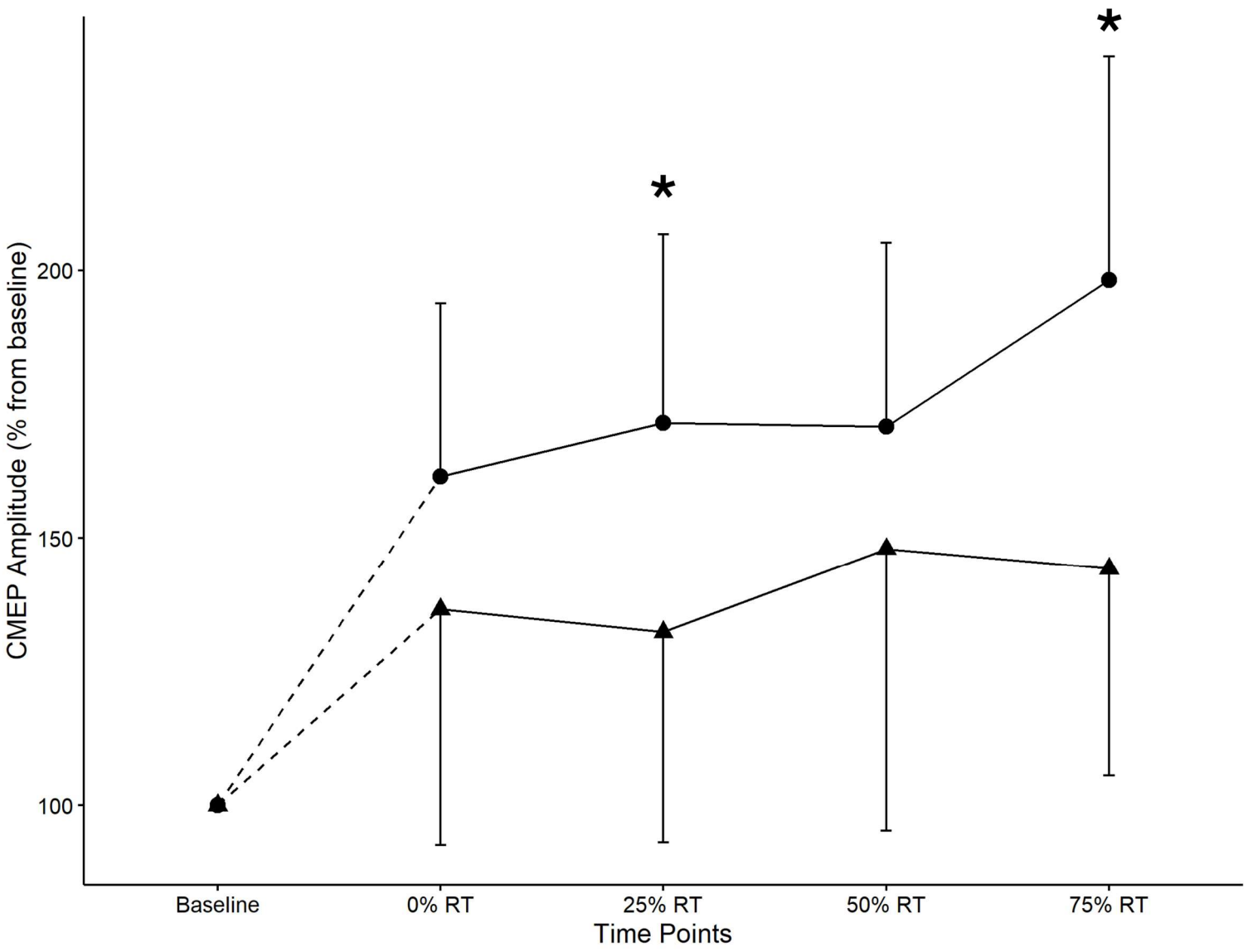

Figure 4 Pooled male (filled triangles) and female (filled circles) changes in CMEP amplitude (%) relative to baseline. Data points displayed represent the mean value at each RT point and error bars represent SD. *Significant differences between males and females in CMEP amplitude ($p < 0.05$).

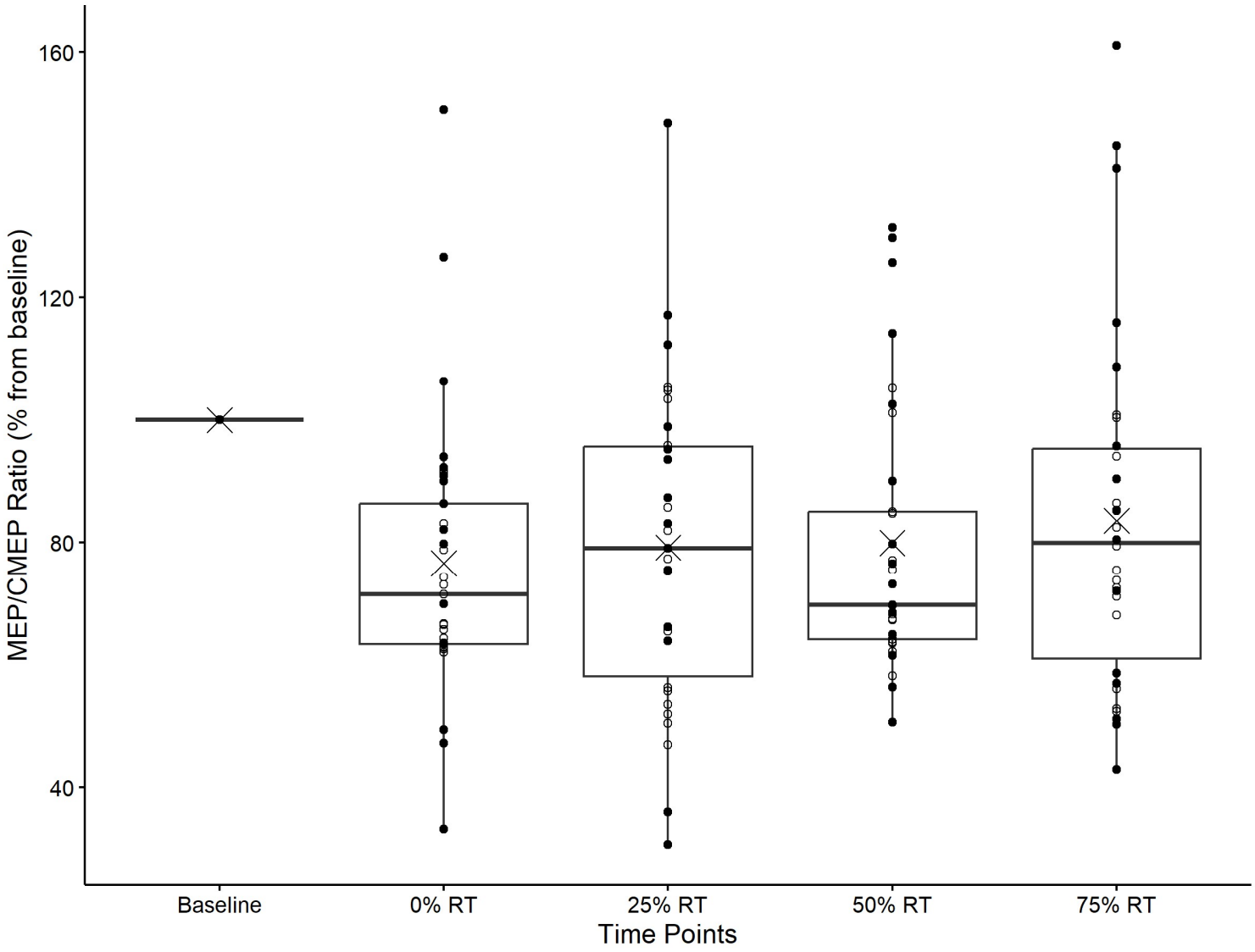

Figure 5 Individual participant ballistic (filled circles) and ramp (open circles) changes in MEP/CMEP ratio values (%) relative to baseline. Solid horizontal lines in each box represent the median value, and the box outlines represent the upper (top, Q3) and lower (bottom, Q1) quartile range of the data. "X" represents the mean value for pooled data between ballistic and ramp conditions at each RT point. No significant differences in MEP/CMEP ratio from baseline ($p > 0.05$).

3 Discussion and Summary

3.1 Discussion

Premotor CSE

The goal of this study was to investigate the modulation of CSE in the premotor RT period prior to movement (EMG) onset, and to delineate between cortical and spinal contributions to any changes in CSE. Additionally, these premotor CSE responses (MEP, CMEP, MEP/CMEP) were compared between ballistic and ramp contractile conditions. The first major finding of this study was an increase in MEP (Figure 2) and CMEP (Figure 3) amplitudes during the premotor RT period, which occurred before the onset of voluntary EMG. This change in excitability (relative to resting baseline levels) occurred with minimal background EMG activity prior to stimulation, indicating that the muscle was effectively relaxed before receiving TMS and TMES. There was also a lack of differences in premotor MEP amplitude, CMEP amplitude and MEP/CMEP ratio responses with ballistic compared to ramp contractions, indicating that premotor CSE was not specific to the intended RFD of the ensuing contraction.

An increase of MEP amplitude prior to movement (EMG onset) has been demonstrated with simple RT task paradigms in prior studies to assess premotor CSE in the abductor pollicis brevis (Chen et al., 1998; Cirillo et al., 2021), extensor pollicis brevis (Leocani et al., 2000), and flexor digitorum superficialis (Kennefick et al., 2014). A few studies have also reported an inhibitory effect on MEPs with a simple RT task (Touge et al., 1998; Ibanez et al., 2018), although this finding could be due to the timing of stimulation. That is, Touge et al. (1998) and Ibanez et al. (2018) delivered TMS prior to the presentation of an imperative ”GO” signal, while the participants were still waiting to receive their cue to contract. It has been suggested that inhibition on CSE may serve as a “braking” mechanism to counter premature motor output (Duque & Ivry., 2009), and prevent participants from initiating a contraction too early. However, in the present study all measures were taken in the premotor RT period occurring after the presentation of an imperative “GO” cue to contract, therefore it is unlikely that our participants would have demonstrated a similar inhibitory effect. Although these earlier findings reported a

change in CSE prior to movement (occurring approximately 50-1500ms before EMG onset) the use of TMS alone to assess CSE did not allow the researchers to distinguish between cortical and spinal mechanisms contributing to the overall MEP.

The present study assessed spinal excitability contributions to the changes in MEP amplitude, and demonstrated an increase in CMEP amplitude prior to initiating both ballistic and ramp contractions, which occurred at all time points within the premotor RT period relative to resting baseline (Figure 3). Whereas MEP amplitude represents excitability along the entire corticospinal pathway, CMEP amplitude is influenced by descending neural drive from the cortex (Martin et al., 2006), and represents motoneuron activation from a single descending volley elicited by excitation of corticospinal axons (Ugawa et al., 1991). Thus a change in spinal excitability, as assessed by an increase in CMEP amplitude, should be facilitated from excitatory drive in higher cortical centers. However, the CMEP test is delivered below the level of the cortex and does not reflect cortical excitability modulations contributing to the MEP. Normalizing the MEP to the CMEP (MEP/CMEP ratio, matched at the same time point/conditions) facilitates the assessment of cortical excitability by accounting for any changes in the MEP which may have occurred in the cortex, although the present data reported no significant differences from baseline in MEP/CMEP responses over time (Figure 5). The increase in MEP (corticospinal excitability) and CMEP (spinal excitability) amplitude occurred without a concomitant increase in MEP/CMEP (cortical excitability), suggesting that cortical excitability was not strongly affected by movement preparation for the simple RT task when measured within the premotor RT period. Therefore, the increase in premotor CSE from resting baseline during the premotor RT period was mediated at the spinal level.

Few studies have attempted to assess spinal excitability in concert with premotor CSE. Notably, Kennefick et al. (2019) assessed premotor CSE with a movement complexity task and demonstrated an increase in MEP and CMEP amplitude without a concomitant increase in cortical excitability (MEP/CMEP), concluding that the increase in CSE associated with greater movement complexity was attributable to spinal excitability. However, that study made no attempt to compare premotor CSE responses between different voluntary contractile rates (i.e., ballistic versus ramp). Using a reaction time task

Mackinnon and Rothwell (2000) found an increase in premotor CSE without an increase in spinal excitability, attributing the increase in CSE to cortical excitability mechanisms. Although, Mackinnon and Rothwell (2000) used the H-reflex to investigate spinal excitability, which can be influenced by presynaptic inhibition (McNeil et al., 2013). Therefore, any changes in spinal excitability could have been diminished by presynaptic inhibition in the premotor period and contributed to the discrepant results.

Ballistic versus Ramp Contractions

The present study also demonstrated a lack of differences in MEP, CMEP and MEP/CMEP responses when compared between contractile conditions (i.e., MEP at 0% RT in ballistic vs. MEP at 0% RT in ramp), indicating that the increase in premotor CSE from baseline is not specific to the RFD of the upcoming contraction (Figures 2, 3, and 5). To our knowledge, only one study previously has investigated the differences in premotor CSE between ballistic and ramp contractions (Duchateau and Baudry, 2021). Relative to EMG onset, they reported an increase of premotor CSE that occurred approximately 100ms earlier in the ramp compared to ballistic condition, with larger increases in MEP amplitude prior to the ramp compared to ballistic condition. They also reported an increase in spinal excitability, as measured by the F-wave test, prior to the ramp contraction but not ballistic, indicating differences in cortical and spinal-mediated contributions to premotor CSE between ballistic and ramp contractions. These findings conflict with those of the present study, which may be explained by methodological differences in the experimental protocol. Duchateau and Baudry (2021) required participants to self-initiate their ballistic and ramp contractions, whereas the present study used a visual imperative ”GO” cue to initiate the intended contraction. Thus, assessing an individual in preparation for a self-initiated contraction may facilitate differences in cognitive and motor preparatory processes compared to preparation for a simple RT task (Chen et al., 1998), which could explain the temporal difference of increased CSE reported between ballistic and ramp conditions. The present study also demonstrated a significant increase in spinal excitability from baseline prior to completing both ballistic and ramp contractions, whereas Duchateau and Baudry (2021) only reported an increase in spinal excitability prior to ramp contractions in a small subset of participants (n=4).

However, Duchateau and Baudry (2021) used the F-wave test to measure spinal excitability, which reflects the excitability of motoneurons reactivated by antidromic impulses following supramaximal stimulation of a peripheral nerve (McNeil et al., 2013). The F-wave measure has been challenged as a flawed assessment of spinal excitability given that F-waves generated are often limited to large motoneurons, thereby making the test relatively insensitive to changes in spinal excitability (Espiritu et al., 2003). The present study used the CMEP test which is considered a very direct method to assess spinal excitability (McNeil et al., 2013), as it has demonstrated a large monosynaptic component in the upper limb (Petersen et al., 2002) without being affected by presynaptic inhibition like the H-reflex (Nielsen and Petersen, 1994). The CMEP is more sensitive to any changes in motoneuronal excitability than the F-wave measure, which could have contributed to the discrepancy in spinal excitability results between studies.

Sex-Based Differences

Lastly, the findings here demonstrated a sex-based difference in CMEP responses at 25% and 75% premotor RT with pooled ballistic and ramp data. Specifically, female participants had a larger CMEP amplitude than male participants at 25% and 75% RT, (23% and 27% larger, respectively) (Figure 4). To our knowledge, this is the first study to report sex-based differences in premotor spinal excitability, and the findings might be explained by differences in neuromodulatory input to the motoneuron pool. The release of neuromodulators such as serotonin and norepinephrine do not directly activate motoneurons within the pool, but rather modify the properties of voltage-sensitive ion channels and increases the intrinsic electrical excitation of the target neuron, thereby making the motoneurons more sensitive to excitatory ionotropic input (Heckman et al., 2009). A recent study by Jenz et al. (2023) demonstrated a larger PIC estimate in females compared to males when investigating motoneurons of the lower limb, suggesting the differences were partly attributable to differences in neuromodulatory drive between the sexes. Females generally have greater circulating levels of sex hormones such as progesterone and estradiol than males, which pose effects on the release of brainstem-derived monoamines; most notably serotonin (Jenz et al., 2023). Taken together, an increased circulating concentration of progesterone and estradiol may have contributed to

greater neuromodulatory drive in female participants primarily through the release of serotonin, facilitating an enhanced intrinsic excitation of the motoneuron pool and resulting in a sex-based difference of spinal excitability noted at a few time points within the premotor RT period.

3.2 Considerations and Limitations

Investigating the modulation of premotor CSE using single-pulse TMS and TMES with a simple RT paradigm presents some limitations. Specifically, we did not investigate any inhibitory mechanisms (i.e., using paired-pulse TMS) which could have influenced the time course of change in CSE (Duque & Ivry. 2009), however our methodology and results support the role of excitatory mechanisms facilitating an increase in CSE during the premotor RT period. Our findings with a simple RT task demonstrate enhanced CSE predominantly mediated at the spinal level, although these findings may not be generalizable to movement preparation for daily functional tasks which are more often self-paced (not initiated by an imperative ”GO” cue). However, previous work investigating premotor CSE with self-paced movements (Chen et al., 1998; Duchateau and Baudry. 2021) also demonstrated an increase of CSE prior to EMG onset (evaluated through MEPs), but the previous studies did not delineate between cortical and spinal contributions to premotor CSE (Chen et al., 1998) and used a different method to assess spinal excitability (Duchateau and Baudry. 2021). Lastly, we evaluated sex-based differences in premotor CSE between participants although we did not control for specific potential affects within the menstrual cycle among the female participants. Indeed, the limited prior work on sex-based differences in CSE have primarily focused on evaluating neural responses to fatigue (Yacyshyn & McNeil, 2020), or evaluating CSE within clinical populations such as concussion (Pauhl et al., 2022); demonstrating no evidence to suggest that CSE would vary among female participants depending on phase within the menstrual cycle.

3.3 Conclusions

The current findings support the role of excitatory mechanisms facilitating an increase of CSE from baseline in the premotor RT period, which is predominantly a spinally mediated response. Additionally, there was no difference in premotor CSE prior to initiating ballistic and ramp contractions, suggesting that the increase in premotor CSE is not specific to the intended RFD of the ensuing contraction. Spinal excitability response level may be influenced by sex, with differences in CMEP amplitude between males and females demonstrated within the premotor RT period.

3.4 Future Directions

CSE is often assessed during simple isometric tasks (no joint movement), with the assumption that results can be extrapolated to functional movements outside a laboratory setting (Kalmar. 2018). However, limited work has been conducted to investigate CSE mechanisms in dynamic contractions (Clos et al., 2022), or further attempt to discern between cortical and spinal mechanisms functioning to control CSE during concentric (muscle shortening) and eccentric (muscle lengthening) contractions. Additionally, motor unit properties fundamentally are driven by corticospinal input strength (neural drive) to the spinal motor neurons prior to MU discharge (Del Vecchio et al., 2019), although MU firing rates are also investigated far more often during isometric contractions. The isometric modality provides a simpler model to record single motor units with minimal electrode displacement, and without the influence of ongoing changes in joint position or velocity. However, dynamic contractions represent a more functional modality relevant to daily movements, as concentric and eccentric contractions are necessary to facilitate wider degrees of purposeful movement. There are few studies describing MU firing rate changes during dynamic contractions in human models, (Del Valle & Thomas., 2005; Harwood et al. 2011; Kirk et al., 2021) although given the differences in MU firing rates between concentric and eccentric movements (Del Valle & Thomas., 2005), integrating CSE responses with MUDR in both concentric and eccentric contractions may elucidate different neural control mechanisms originating from cortical and spinal contributions to neural drive.

Further work could also determine whether the relationship between CSE and MU firing rates is altered in older individuals who, for example, have slowed movements contributing to trips and falls, which may be related to impairments in CSE (Oliviero et al., 2006) and/or intrinsically lower MU firing rates (Klass et al., 2008). Thus, creating a foundation of understanding and methodology for investigating corticospinal input with MU activity, particularly during dynamic contractions, would provide greater insight on integrated neuromuscular control during dynamic functional movements.

References

Amann, M., Sidhu, S. K., McNeil, C. J., & Gandevia, S. C. (2022). Critical considerations of the contribution of the corticomotoneuronal pathway to central fatigue. *The Journal of Physiology*, *600*(24), 5203–5214. https://doi.org/10.1113/JP282564

Barker, A. T., Jalinous, R., & Freeston, I. L. (1985). Non-invasive magnetic stimulation of human motor cortex. *Lancet (London, England)*, *1*(8437), 1106–1107. https://doi.org/10.1016/s0140-6736(85)92413-4

Baudry, S., & Duchateau, J. (2021). Changes in corticospinal excitability during the preparation phase of ballistic and ramp contractions. *The Journal of Physiology*, *599*(5), 1551–1566. https://doi.org/10.1113/JP281093

Bellemare, F., Woods, J. J., Johansson, R., & Bigland-Ritchie, B. (1983). Motor-unit discharge rates in maximal voluntary contractions of three human muscles. *Journal of Neurophysiology*, *50*(6), 1380–1392. https://doi.org/10.1152/jn.1983.50.6.1380

Chen, R., Yaseen, Z., Cohen, L. G., & Hallett, M. (1998). Time course of corticospinal excitability in reaction time and self-paced movements. *Annals of Neurology*, *44*(3), 317–325. https://doi.org/10.1002/ana.410440306

Cirillo, G., Di Vico, I. A., Emadi Andani, M., Morgante, F., Sepe, G., Tessitore, A., Bologna, M., & Tinazzi, M. (2021). Changes in Corticospinal Circuits During Premovement Facilitation in Physiological Conditions. *Frontiers in Human Neuroscience*, *15*, 684013. https://doi.org/10.3389/fnhum.2021.684013

Clos, P., Mater, A., Legrand, H., Poirier, G., Ballay, Y., Martin, A., & Lepers, R. (2022). Corticospinal Excitability Is Lower During Eccentric Than Concentric Cycling in Men. *Frontiers in Physiology*, *13*, 854824. https://doi.org/10.3389/fphys.2022.854824

De Luca, C. J., LeFever, R. S., McCue, M. P., & Xenakis, A. P. (1982). Behaviour of human motor units in different muscles during linearly varying contractions. *The Journal of Physiology*, *329*, 113–128. https://doi.org/10.1113/jphysiol.1982.sp014293

Deecke, L. (1996). Planning, preparation, execution, and imagery of volitional action. *Brain Research. Cognitive Brain Research*, *3*(2), 59–64. https://doi.org/10.1016/0926-6410(95)00046-1

Del Valle, A., & Thomas, C. K. (2005). Firing rates of motor units during strong dynamic contractions. *Muscle & Nerve*, *32*(3), 316–325. https://doi.org/10.1002/mus.20371

Del Vecchio, A., Negro, F., Holobar, A., Casolo, A., Folland, J. P., Felici, F., & Farina, D. (2019). You are as fast as your motor neurons: Speed of recruitment and maximal discharge of motor neurons determine the maximal rate of force development in humans. *The Journal of Physiology*, *597*(9), 2445–2456. https://doi.org/10.1113/JP277396

Desmedt, J. E., & Godaux, E. (1979). Voluntary motor commands in human ballistic movements. *Annals of Neurology*, *5*(5), 415–421. https://doi.org/10.1002/ana.410050503

Duchateau, J., & Enoka, R. M. (2011). Human motor unit recordings: Origins and insight into the integrated motor system. *Brain Research*, *1409*, 42–61. https://doi.org/10.1016/j.brainres.2011.06.011

Duchateau, J., Semmler, J. G., & Enoka, R. M. (2006). Training adaptations in the behavior of human motor units. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, *101*(6), 1766–1775. https://doi.org/10.1152/japplphysiol.00543.2006

Duque, J., & Ivry, R. B. (2009). Role of Corticospinal Suppression during Motor Preparation. *Cerebral Cortex*, *19*(9), 2013–2024. https://doi.org/10.1093/cercor/bhn230

Enoka, R. M., & Duchateau, J. (2017). Rate Coding and the Control of Muscle Force. *Cold Spring Harbor Perspectives in Medicine*, *7*(10), a029702. https://doi.org/10.1101/cshperspect.a029702

Espiritu, M. G., Lin, C. S. -Y., & Burke, D. (2003). Motoneuron excitability and the F wave. *Muscle & Nerve*, *27*(6), 720–727. https://doi.org/10.1002/mus.10388

Gandevia, S. C., Petersen, N., Butler, J. E., & Taylor, J. L. (1999). Impaired response of human motoneurones to corticospinal stimulation after voluntary exercise. *The Journal of Physiology*, *521*(3), 749–759. https://doi.org/10.1111/j.1469-7793.1999.00749.x

Harwood, B., Davidson, A. W., & Rice, C. L. (2011). Motor unit discharge rates of the anconeus muscle during high-velocity elbow extensions. *Experimental Brain Research*, *208*(1), 103–113. https://doi.org/10.1007/s00221-010-2463-4

Heckman, C. J., Mottram, C., Quinlan, K., Theiss, R., & Schuster, J. (2009). Motoneuron excitability: The importance of neuromodulatory inputs. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, *120*(12), 2040–2054. https://doi.org/10.1016/j.clinph.2009.08.009

Henneman, E. (1957). Relation between size of neurons and their susceptibility to discharge. *Science (New York, N.Y.)*, *126*(3287), 1345–1347. https://doi.org/10.1126/science.126.3287.1345

Herbert, R. D., & Gandevia, S. C. (1999). Twitch Interpolation in Human Muscles: Mechanisms and Implications for Measurement of Voluntary Activation. *Journal of Neurophysiology*, *82*(5), 2271–2283. https://doi.org/10.1152/jn.1999.82.5.2271

Ibáñez, J., Hannah, R., Rocchi, L., & Rothwell, J. C. (2020). Premovement Suppression of Corticospinal Excitability may be a Necessary Part of Movement Preparation. *Cerebral Cortex*, *30*(5), 2910–2923. https://doi.org/10.1093/cercor/bhz283

Jang, S. H. (2014). The corticospinal tract from the viewpoint of brain rehabilitation. *Journal of Rehabilitation Medicine*, *46*(3), 193–199. https://doi.org/10.2340/16501977-1782

Jenz, S. T., Beauchamp, J. A., Gomes, M. M., Negro, F., Heckman, C. J., & Pearcey, G. E. P. (2023). Estimates of persistent inward currents in lower limb motoneurons are larger in females than in males. *Journal of Neurophysiology*, *129*(6), 1322–1333. https://doi.org/10.1152/jn.00043.2023

Kagamihara, Y., Komiyama, T., Ohi, K., & Tanaka, R. (1992). Facilitation of agonist motoneurons upon initiation of rapid and slow voluntary movements in man. *Neuroscience Research*, *14*(1), 1–11. https://doi.org/10.1016/S0168-0102(05)80002-1

Kalmar, J. M. (2018). On task: Considerations and future directions for studies of corticospinal excitability in exercise neuroscience and related disciplines. *Applied*

Physiology, Nutrition, and Metabolism, *43*(11), 1113–1121. https://doi.org/10.1139/apnm-2018-0123

Kennefick, M., Burma, J. S., Van Donkelaar, P., & McNeil, C. J. (2019). The Time Course of Motoneuronal Excitability during the Preparation of Complex Movements. *Journal of Cognitive Neuroscience*, *31*(6), 781–790. https://doi.org/10.1162/jocn_a_01394

Kennefick, M., Maslovat, D., & Carlsen, A. N. (2014). The time course of corticospinal excitability during a simple reaction time task. *PloS One*, *9*(11), e113563. https://doi.org/10.1371/journal.pone.0113563

Kirk, E. A., Gilmore, K. J., & Rice, C. L. (2021). Anconeus motor unit firing rates during isometric and muscle-shortening contractions comparing young and very old adults. *Journal of Neurophysiology*, *126*(4), 1122–1136. https://doi.org/10.1152/jn.00219.2021

Klass, M., Baudry, S., & Duchateau, J. (2008). Age-related decline in rate of torque development is accompanied by lower maximal motor unit discharge frequency during fast contractions. *Journal of Applied Physiology*, *104*(3), 739–746. https://doi.org/10.1152/japplphysiol.00550.2007

Kukulka, C. G., & Clamann, H. P. (1981). Comparison of the recruitment and discharge properties of motor units in human brachial biceps and adductor pollicis during isometric contractions. *Brain Research*, *219*(1), 45–55. https://doi.org/10.1016/0006-8993(81)90266-3

Landin, D., Thompson, M., & Jackson, M. R. (2017). Actions of the Biceps Brachii at the Shoulder: A Review. *Journal of Clinical Medicine Research*, *9*(8), 667–670. https://doi.org/10.14740/jocmr2901w

Leocani, L., Cohen, L. G., Wassermann, E. M., Ikoma, K., & Hallett, M. (2000). Human corticospinal excitability evaluated with transcranial magnetic stimulation during different reaction time paradigms. *Brain*, *123*(6), 1161–1173. https://doi.org/10.1093/brain/123.6.1161

MacKinnon, C. D., & Rothwell, J. C. (2000). Time-varying changes in corticospinal excitability accompanying the triphasic EMG pattern in humans. *The Journal of Physiology*, *528*(3), 633–645. https://doi.org/10.1111/j.1469-7793.2000.00633.x

Maffiuletti, N. A., Aagaard, P., Blazevich, A. J., Folland, J., Tillin, N., & Duchateau, J. (2016). Rate of force development: Physiological and methodological considerations. *European Journal of Applied Physiology*, *116*(6), 1091–1116. https://doi.org/10.1007/s00421-016-3346-6

Manikandan, S. (2010). Data transformation. *Journal of Pharmacology & Pharmacotherapeutics*, *1*(2), 126–127. https://doi.org/10.4103/0976-500X.72373

Martin, P. G., Gandevia, S. C., & Taylor, J. L. (2006). Output of human motoneuron pools to corticospinal inputs during voluntary contractions. *Journal of Neurophysiology*, *95*(6), 3512–3518. https://doi.org/10.1152/jn.01230.2005

McNeil, C. J., Butler, J. E., Taylor, J. L., & Gandevia, S. C. (2013). Testing the excitability of human motoneurons. *Frontiers in Human Neuroscience*, *7*, 152. https://doi.org/10.3389/fnhum.2013.00152

McNeil, C. J., Giesebrecht, S., Gandevia, S. C., & Taylor, J. L. (2011). Behaviour of the motoneurone pool in a fatiguing submaximal contraction. *The Journal of Physiology*, *589*(Pt 14), 3533–3544. https://doi.org/10.1113/jphysiol.2011.207191

Nielsen, J., & Petersen, N. (1994). Is presynaptic inhibition distributed to corticospinal fibres in man? *The Journal of Physiology*, *477*(1), 47–58. https://doi.org/10.1113/jphysiol.1994.sp020170

Oliviero, A., Profice, P., Tonali, P. A., Pilato, F., Saturno, E., Dileone, M., Ranieri, F., & Di Lazzaro, V. (2006). Effects of aging on motor cortex excitability. *Neuroscience Research*, *55*(1), 74–77. https://doi.org/10.1016/j.neures.2006.02.002

Pacha Vicente, D., Forcada Calvet, P., Carrera Burgaya, A., & Llusá Pérez, M. (2005). Innervation of biceps brachii and brachialis: Anatomical and surgical approach. *Clinical Anatomy (New York, N.Y.)*, *18*(3), 186–194. https://doi.org/10.1002/ca.20057

Pauhl, A., Yasen, A., & Christie, A. (2022). Corticospinal Excitability and Inhibition Are Not Different between Concussed Males and Females. *Brain Sciences*, *12*(7), 824. https://doi.org/10.3390/brainsci12070824

Petersen, N. T., Taylor, J. L., & Gandevia, S. C. (2002). The effect of electrical stimulation of the corticospinal tract on motor units of the human biceps brachii. *The Journal of Physiology*, *544*(Pt 1), 277–284. https://doi.org/10.1113/jphysiol.2002.024539

Reis, J., Swayne, O. B., Vandermeeren, Y., Camus, M., Dimyan, M. A., Harris-Love, M., Perez, M. A., Ragert, P., Rothwell, J. C., & Cohen, L. G. (2008). Contribution of transcranial magnetic stimulation to the understanding of cortical mechanisms involved in motor control. *The Journal of Physiology*, *586*(2), 325–351. https://doi.org/10.1113/jphysiol.2007.144824

Taylor, J. L. (2006). Stimulation at the cervicomedullary junction in human subjects. *Journal of Electromyography and Kinesiology*, *16*(3), 215–223. https://doi.org/10.1016/j.jelekin.2005.07.001

Taylor, J. L., Petersen, N. T., Butler, J. E., & Gandevia, S. C. (2002). Interaction of transcranial magnetic stimulation and electrical transmastoid stimulation in human subjects. *The Journal of Physiology*, *541*(3), 949–958. https://doi.org/10.1113/jphysiol.2002.016782

Touge, T., Taylor, J. L., & Rothwell, J. C. (1998). Reduced excitability of the cortico-spinal system during the warning period of a reaction time task. *Electroencephalography and Clinical Neurophysiology/Electromyography and Motor Control*, *109*(6), 489–495. https://doi.org/10.1016/S0924-980X(98)00050-2

Ugawa, Y., Rothwell, J. C., Day, B. L., Thompson, P. D., & Marsden, C. D. (1991). Percutaneous electrical stimulation of corticospinal pathways at the level of the pyramidal decussation in humans. *Annals of Neurology*, *29*(4), 418–427. https://doi.org/10.1002/ana.410290413

Vagg, R., Mogyoros, I., Kiernan, M. C., & Burke, D. (1998). Activity-dependent hyperpolarization of human motor axons produced by natural activity. *The Journal of Physiology*, *507*(3), 919–925. https://doi.org/10.1111/j.1469-7793.1998.919bs.x

Weavil, J. C., & Amann, M. (2018). Corticospinal excitability during fatiguing whole body exercise. *Progress in Brain Research*, *240*, 219–246. https://doi.org/10.1016/bs.pbr.2018.07.011

Welniarz, Q., Dusart, I., & Roze, E. (2017). The corticospinal tract: Evolution, development, and human disorders. *Developmental Neurobiology*, *77*(7), 810–829. https://doi.org/10.1002/dneu.22455

Yacyshyn, A. F., & Mcneil, C. J. (2020). The Sexes Do Not Differ for Neural Responses to Submaximal Elbow Extensor Fatigue. *Medicine & Science in Sports & Exercise*, *52*(9), 1992–2001. https://doi.org/10.1249/MSS.0000000000002342

Appendix

Western Research

Date: 4 March 2024

To: Charles Rice

Project ID: 107505

Review Reference: 2024-107505-90080

Study Title: Motor neuron and muscle fiber resilience in humans

Application Type: Continuing Ethics Review (CER) Form

Review Type: Delegated

Date Approval Issued: 04/Mar/2024 15:45

REB Approval Expiry Date: 07/Mar/2025

Dear Charles Rice,

The Western University Research Ethics Board has reviewed the application. This study, including all currently approved documents, has been re-approved until the expiry date noted above.

REB members involved in the research project do not participate in the review, discussion or decision.

Western University REB operates in compliance with, and is constituted in accordance with, the requirements of the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The REB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Please do not hesitate to contact us if you have any questions.

Electronically signed by:

Mr. Joshua Hatherley, Ethics Coordinator on behalf of Dr. N. Poonai, HSREB Chair 04/Mar/2024 15:45

Reason: I am approving this document

Note: This correspondence includes an electronic signature (validation and approval via an online system that is compliant with all regulations).

Curriculum Vitae for Daniel C. Basile

Post-secondary Education and Degrees	**Bachelor of Kinesiology** University of Toronto Toronto, Ontario, Canada 2017 – 2022
	Master of Science The University of Western Ontario London, Ontario, Canada 2022 – Present
Related Work Experience	**Graduate Teaching Assistant** **University of Western Ontario (2022 – Present)** **London, Ontario, Canada**
	Undergraduate Research Assistant **University of Toronto (2021 – 2022)** **Toronto, Ontario, Canada**

Peer-Reviewed Papers

Bentley RF, **Basile DC**, Di Salvo AN, Schwartz JL (2023). "Effect of a semi-upright body position on oxygen consumption kinetics during submaximal cycling". *PLOS One (Submitted).*

Submitted Abstracts

Basile DC, Paris MT, McNeil CJ, Rice CL (2024). "Investigating premotor corticospinal excitability in fast and slow voluntary contractions of the elbow flexors". American College of Sports Medicine, Boston, Massachusetts, May 2024. *(In Press).*

Conference Presentations

Basile DC, Paris MT, McNeil CJ, Rice CL (2024). "Investigating premotor corticospinal excitability in fast and slow voluntary contractions of the elbow flexors". Exercise Neuroscience Group. June 2023. Memorial University of Newfoundland. *(Oral Presentation).*

Bentley RF, **Basile DC**, Di Salvo AN, Schwartz JL (2023). "Effect of a semi-upright body position on oxygen consumption kinetics during submaximal cycling". Bertha Rosenstadt Undergraduate Research Conference. April 2022. University of Toronto. *(Oral Presentation).*