Corticospinal excitability of the biceps brachii is higher during arm cycling than an intensity-matched tonic contraction

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Running Head: Modulation of corticospinal excitability during arm cycling

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ABSTRACT

Human studies have not assessed corticospinal excitability of an upper-limb prime mover during arm cycling. The purpose of the present study was to determine whether supraspinal and/or spinal motoneurone excitability of the biceps brachii was different between arm cycling and an intensity-matched tonic contraction. We hypothesized that spinal motoneurone excitability would be higher during arm cycling than an intensity-matched tonic contraction. Supraspinal and spinal motoneurone excitability were assessed using transcranial magnetic stimulation (TMS) of the motor cortex and transmastoid electrical stimulation (TMES) of the corticospinal tract, respectively. TMS-induced motor evoked potentials (MEPs) and TMES-induced cervicomedullary evoked potentials (CMEPs) were assessed at three separate positions (3, 6 and 12 o’clock relative to a clock face) during arm cycling and an intensity-matched tonic contraction. MEP amplitudes were 7.2 and 8.8% \( M_{\text{max}} \) larger during arm cycling when compared to a tonic contraction at the 3 (\( P < 0.001 \)) and 6 o’clock (\( P < 0.001 \)) positions, respectively. There was no difference between tasks during elbow extension (12 o’clock). CMEP amplitudes were 5.2% \( M_{\text{max}} \) larger during arm cycling when compared to a tonic contraction at the 3 o’clock position (\( P < 0.001 \)) with no differences seen at mid-flexion (6 o’clock) or extension (12 o’clock). The data indicate an increase in the excitability of corticospinal neurones which ultimately project to biceps brachii during the elbow flexion portion of arm cycling and increased spinal motoneurone excitability at the onset of elbow flexion during arm cycling. We conclude that supraspinal and spinal motoneurone excitability are phase- and task-dependent.
Rhythmic and alternating motor outputs, such as locomotion, are initiated via descending commands (Jordan et al., 2008) that enhance the excitability of spinal interneurones, thereby causing the central pattern generator (CPG) (Grillner, 1981, Jordan, 1998) to oscillate and recruit spinal motoneurones. Occurring simultaneously with CPG operation is a drastic re-organization of intraspinal excitability, including modulation of spinal reflex pathways and changes in the presynaptic regulation of sensory transmission and interneuronal excitability. Essentially, a new functional locomotor “state” is created within the spinal cord when compared to rest. Ultimately, however, it is the spinal motoneurone that must transform all inputs from descending, sensory and spinal circuitry into action potentials to produce movement. In the adult decerebrate cat the ‘excitability’ of spinal motoneurones is enhanced prior to and throughout locomotor tasks via a reconfiguration of their intrinsic electrical properties (Brownstone et al., 1992, Brownstone et al., 1994, Krawitz et al., 2001, Power et al., 2010). For example, during fictive locomotion and scratch motoneurones are characterized by a decreased rheobase current (Krawitz et al. 2001; Power et al. 2010), reduced afterhyperpolarization amplitude (Brownstone et al. 1992; Power et al. 2010), the emergence of intrinsic voltage-dependent depolarizations (Brownstone et al. 1994; Power et al. 2010), and hyperpolarization of the voltage threshold (Vth) for action potential initiation (Krawitz et al. 2001; Power et al. 2010). Importantly, these state-dependent changes in motoneurone excitability are also ‘task-dependent’. Power et al. (2010) examined the excitability of the same motoneurone during two different motor outputs in the adult decerebrate cat - rhythmic scratching and stance, a tonic motor output. They demonstrated that when a motoneurone was engaged in rhythmic scratching the Vth hyperpolarized and the AHP was reduced. When the same motoneurone was active during stance the Vth depolarized and the AHP
amplitude was unchanged. This suggests that there are different neural control mechanisms regulating spinal motoneurone excitability between scratch and stance in the cat.

In humans, it is well-known that central nervous system excitability is modulated during rhythmic and alternating motor outputs. Furthermore, indirect evidence from studies examining corticospinal excitability (Capaday et al., 1999, Pyndt and Nielsen, 2003, Carroll et al., 2006), interlimb coordination (Zehr et al., 2009) and spinal reflexes (Zehr and Stein, 1999) in humans indicate that a spinal CPG likely contributes to the production of rhythmic and alternating motor outputs, such as locomotion and cycling, as it does in quadrupeds. The majority of studies involving assessment of nervous system excitability during locomotor tasks in humans tend to focus on the modulation of supraspinal and/or spinal reflex excitability. For example, in the lower-limb, Capaday et al. (1999) demonstrated that transcranial magnetic stimulation (TMS) induced motor evoked potentials (MEPs) in the soleus were smaller during the stance phase of locomotion when compared to an intensity-matched tonic contraction, whereas the tibialis anterior showed larger MEPs in the same period. They suggested that cortical input was less to the soleus during walking when compared to a tonic contraction, whereas the opposite occurred for the tibialis anterior. In the upper-limb, Carroll and colleagues (2006), showed a decrease in corticospinal excitability (i.e. decreased MEP amplitude) during the flexion phase of rhythmic arm movement and a subthreshold TMS evoked facilitation of spinal reflexes during tonic contraction but not arm cycling (Carroll et. al. 2006). They concluded that a spinal CPG was likely involved in the production of arm cycling. It is presently unclear, however, whether the observed changes in TMS evoked MEPs and/or H-reflex excitability in these studies could be partially explained by changes in the excitability of the final common path, the spinal motoneurone.
Only one study has assessed both supraspinal excitability and spinal motoneurone excitability during a rhythmic and alternating motor output in humans (Sidhu et al., 2012). Since TMS evoked MEPs can be influenced by changes in cortical and spinal motoneurones, Sidhu and colleagues (2012) used TMS and transmastoid electrical stimulation (TMES) to assess supraspinal excitability and spinal excitability, respectively, of the knee extensors during leg cycling. TMES directly activates the corticospinal axons producing cervicomedullary motor evoked potentials (i.e. CMEPs) that are independent of changes in supraspinal excitability (Taylor et al., 2002) and are thought to be an effective way to assess spinal motoneurone excitability (McNeil et al., 2013). They demonstrated that the activation of inhibitory interneurones in the cortex via subthreshold TMS stimulation intensities led to suppression of the EMG during cycling. They also demonstrated that both MEPs and CMEPs were modulated similarly across all phases of cycling, with MEPs mainly driven by changes at the spinal level. Based on these findings they concluded that supraspinal centres were directly involved in the generation of cycling and that spinal factors dominated the phase-dependent modulation of corticospinal excitability. They did not compare CMEPs during cycling to CMEPs during an intensity-matched tonic contraction, however, thus it is unclear whether the observed changes in CMEPs were cycling-dependent.

To date, human studies have not assessed supraspinal excitability and/or spinal motoneurone excitability of an upper-limb prime mover during a rhythmic and alternating, cyclical motor output (i.e. arm cycling). Thus, the primary objective of the present study was to determine whether corticospinal excitability of the biceps brachii was different during arm cycling when compared to an intensity-matched tonic contraction, and if so, to determine whether the observed differences were due to supraspinal and/or spinal mechanisms. We chose
to compare these two motor outputs given that the generation of the basic rhythmic and alternating pattern of muscle activity during arm cycling is generated, in part, by spinal interneuronal networks (Zehr et al., 2004). Tonic activation of a key muscle involved in arm cycling (i.e. biceps brachii) was chosen to represent a similar level of motoneurone pool activation, but with reduced or absent activation of spinal interneuronal groups contributing to the generation of rhythmic activation during arm cycling. A second objective that arose from the results of our primary study was to determine whether biceps brachii motoneurone excitability was enhanced during the extension phase of arm cycling when compared to rest. We hypothesized that (1) supraspinal excitability of the biceps brachii would be lower and spinal motoneurone excitability higher during arm cycling when compared to an intensity-matched tonic contraction (experiment #1) and (2) that motoneurone excitability would be increased during the extension phase of arm cycling when compared to rest (experiment #2). Portions of the results presented herein have been reported in abstract form (Power et al., 2013).
ABBREVIATIONS LIST

½ Max - 50% of maximum CMEP stimulation intensity
µs - microseconds
AHP - after hyperpolarization
CMEP - cervicomedullary motor evoked potential
EMG - electromyography
FCR - flexor carpi radialis
Max - Maximum CMEP stimulation intensity
MEP - motor evoked potential
M_max - maximum amplitude of the compound muscle action potential
ms - millisecond
MSO - maximum stimulator output
M-wave - compound muscle action potential
rms - root mean square
rpm - revolutions per minute
s - second
T - Threshold CMEP stimulation intensity
TMES - transmastoid electrical stimulation
TMS - transcranial magnetic stimulation
Vth - voltage threshold
W - Watts
METHODS

Ethical Approval

Participants were verbally informed of the procedures and gave informed, written consent to participate in the study. The study conformed to the standards set by the latest revision of the Declaration of Helsinki and the experimental procedures were approved by the Research Ethics Board at the University of Ontario Institute of Technology (REB# 12-008) and at Memorial University of Newfoundland (ICEHR#: 20140358-HK). Experiments were in accordance with the Tri-Council guideline in Canada with full disclosure of potential risks to participants.

Participants

Eleven male volunteers (20-23 years of age) with no known neurological deficits participated in experiment #1. Four separate male volunteers participated in experiment #2 (20-22 years of age). Prior to the experiments all participants completed a magnetic stimulation safety checklist designed to screen for potential contraindications with magnetic stimulation procedures (Rossi et al., 2009).

Experimental Set-up

The two motor tasks, cycling and tonic contraction (described below) were performed using an arm cycle ergometer (Monark Rehab Trainer, Model 881E) mounted on a table (see Fig. 1). Participants were instructed to sit upright at a comfortable distance from the ergometer so that when cycling trials began there was no variation in trunk posture (leaning forward or backward) and when the elbow was fully extended at the 3 o’clock position participants were not ‘reaching.’ The participants were not restrained during cycling. The arm cranks were fixed 180 degrees out
of phase and the centre of the arm crank shaft was aligned at approximately shoulder height. Participants lightly gripped the ergometer handles with the forearms pronated and wore a brace on the right wrist for all trials in order to restrict movement of the joint during cycling given the heteronymous reflex connections between wrist flexors and extensors and the biceps brachii (Manning and Bawa, 2011).

Crank positions were made relative to a clock face (12, 3, 6 and 9 o’clock, as viewed from the right) with the “top dead centre” position of the crank arm defined as 12 o’clock and “bottom dead centre” as 6 o’clock. The biceps brachii was the major muscle group of interest and as such, we employed terminology to describe the cycling movement based on elbow joint position. Thus, elbow flexion was defined as movement from the 3 to the 9 o’clock position, while the hand was moving toward the body. Elbow extension was defined as movement from the 9 to the 3 o’clock position, while the hand was moving away from the body. For the cycling trials, participants were instructed to cycle at a constant power output of 25W and a cadence of 60 rpm. Responses were evoked at three equidistant positions in the cycle (12, 3 and 6 o’clock). These positions were chosen to ensure that responses in the biceps brachii were evoked during the period of maximal and minimal EMG activity (6 and 12 o’clock, respectively) and just prior to the maximum EMG burst (3 o’clock). The timing of stimulation was determined by the position of the right arm crank. Responses were triggered automatically as the crank arm passed one of the three predetermined positions (i.e. 12, 3 and 6 o’clock). Each position was tested separately in its own trial and the order was randomized across participants.

Electromyography Recordings
EMG activity of the biceps brachii and triceps brachii were recorded using pairs of surface electrodes (MeditraceTM 130 ECG conductive adhesive electrodes) positioned over the midline of the biceps brachii and the triceps brachii muscles. EMG was recorded from the right arm of each participant using a bipolar configuration (Ag-AgCl, 2-cm inter-electrode distance). A ground electrode was placed on the lateral epicondyle. Thorough skin preparation for recording electrodes included removal of dead epithelial cells with abrasive (sand) paper around the designated area followed by cleansing with an isopropyl alcohol swab. Data was collected on-line at 5 KHz for off-line analysis using the CED 1401 interface and the Signal 4 (Cambridge Electronic Design Ltd., Cambridge, UK) software program. Signals were amplified (CED 1902) and filtered using a 3-pole Butterworth with cutoff frequencies of 10-1000 Hz.

Stimulation Conditions

Motor responses from the biceps brachii were elicited via 1) brachial plexus electrical stimulation at Erb’s point, 2) transcranial magnetic stimulation (TMS) and 3) transmastoid electrical stimulation (TMES). TMS and TMES stimulation intensities were set to match the peak-to-peak amplitudes of the resulting MEPs and CMEPs, respectively, to approximately 5-10% of the maximal M-wave (M_max) recorded during an isometric elbow flexion contraction (see below). All participants had prior experience with TMS, TMES and Erb’s point stimulation procedures (see below).

Prior to setting the stimulation intensities for TMS and TMES, the maximum EMG of the biceps brachii was determined by having participant’s cycle for 1 min at a workload of 75 W and a cadence of 60 rpm. The maximum EMG activity was determined by averaging the RMS amplitudes of the middle six EMG bursts during the 60 second trial. Participants next sat in an
upright position with hips, knees and elbow flexed at 90° and the upper arm supported on a table. The wrist of the right arm was then inserted into a padded strap, attached by a high-tension wire to force transducer. Participants were then required to produce an isometric elbow flexion force equal to 20% of the max cycling rectified EMG determined in the previous step. This was ensured by having the participant’s match their biceps brachii EMG to a horizontal line placed on a computer screen. During this intensity of contraction the stimulation intensities required for TMS and TMES to produce evoked potentials with peak-to-peak amplitudes of approximately 5-10% of $M_{\text{max}}$ was determined. These stimulation intensities were then used for the remainder of the study.

**Brachial Plexus Stimulation**

Resting $M_{\text{max}}$ of the biceps was first determined by eliciting M-waves by electrical stimulation of the brachial plexus at Erb’s point (200µs duration, 50-250mA) with a Digitimer stimulator (DS7AH, Digitimer Ltd, Welwyn Garden City, Hertfordshire, UK). The cathode was placed in the supraclavicular fossa and the anode over the acromion process. The electrical stimulation was gradually increased until the M-wave of the biceps brachii reached a plateau and there was noticeable elbow flexion. At this point the stimulation intensity was increased by 20% to ensure maximal M-waves were elicited throughout the study. MEP and CMEP amplitudes were normalized to $M_{\text{max}}$ to account for changes in peripheral neuromuscular propagation (Taylor, 2006).

**Transcranial Magnetic Stimulation**
The stimulation intensity for TMS was always performed following TMES because it was easier to match MEPs to CMEPs than vice versa. Stimulation was applied over the vertex using a circular coil (13.5 cm outside diameter) attached to a Magstim 200 (Magstim, Dyfed, UK). To locate vertex, the distances from nasion to inion and from tragus to tragus were measured and marks were placed halfway directly on the scalp for both measurements. The intersection of both halfway marks was defined as vertex (Power and Copithorne, 2013). The coil was held parallel to the floor with the direction of the current flow preferentially activating the left motor cortex. The coil was held firmly in place by the investigator and care was taken to ensure the coil position was accurately aligned with the scalp marking during every trial. Stimulation intensity was then increased until the peak-to-peak amplitude of the MEP was matched to that of the CMEP (~5-10% $M_{\text{max}}$). This intensity of magnetic stimulator output (MSO) was then used for the remainder of the experiment.

**Transmastoid Electrical Stimulation**

Stimulation was applied via adhesive Ag-AgCl electrodes fixed to the skin slightly inferior to the mastoid processes and current passed between them (100µs duration, 125-350 mA; model DS7AH, Digitimer Ltd, Welwyn Garden City, UK). Stimulation intensity was increased until the cervicomedullary motor-evoked potential (CMEP) peak-to-peak amplitude reached a value of approximately 5-10% of the peak-to-peak amplitude of the participant’s $M_{\text{max}}$. This intensity of stimulator output was then used for the remainder of the experiment. We were confident that the corticospinal tract and not the ventral roots were stimulated because the latency of the CMEP was typically 4-5ms shorter than the MEP latency and ~4ms longer than the maximal M-wave latency.
Experiment 1: Corticospinal changes during arm cycling and tonic contractions

Once the TMS and TMES intensities were determined, participants were instructed to relax their arms (hands off of the pedals) and their resting MEP and CMEP amplitude values were assessed. The participants were then re-positioned in front of the arm cycle ergometer. While cycling at 25W and maintaining 60 rpm, each participant was tested at each of the three positions using the three different forms of stimulation. A total of 10 MEPs, 10 CMEPs, and 5 M-waves were delivered at each position. To prevent anticipation of the stimulation an additional 10 frames without stimulation were added for the MEP and CMEP trials and an additional 5 frames for M-wave. Participants cycled continuously and received stimulation in 50% of the trials, which were pseudo-randomized and evoked every 5-10s. Because $M_{\text{max}}$ is muscle length dependent (Simonsen and Dyhre-Poulsen, 1999) and may change over the course of an experiment (Crone et al., 1999) all $M_{\text{max}}$ measurements to which MEPs and CMEPs were normalized were made at the same muscle length and at the same time during the experiments as the MEPs and CMEPs.

In order to compare corticospinal excitability between the two different tasks it was vital that the intensity of the contractions be matched as closely as possible given the large influence of contraction intensity on evoked potentials. To accomplish this, the average background rmsEMG of the biceps brachii from 50 to 0ms before stimulation at each of the three positions during cycling was assessed as an indication of contraction intensity. EMG was measured prior to the stimulation to avoid the stimulation artifact. The average pre-stimulus rmsEMG at the selected position was then rectified and displayed on a computer screen via a horizontal line. With the arm crank set at the position corresponding to the cycling trial the participant was then
required to produce a tonic contraction whereby the EMG produced in the biceps brachii was equal to the horizontal line displayed on the screen, as was done in Carroll et al., (2006) and Pyndt and Nielsen, (2003). During the tonic contractions the pedals were locked in place and the participants were instructed to flex at the elbow for the 3 and 6 o’clock positions and to extend at the 12 o’clock position to further simulate the cycling condition. The number of stimulations was the same as that during cycling.

**Experiment 2: Spinal motoneurone excitability of the biceps brachii using different stimulation intensities at rest and during the extension phase of arm cycling**

We next compared the effects of varying stimulation intensities on CMEP amplitudes during rest and arm cycling (n=4). A total of three stimulation intensities were used: 1) CMEP threshold (T), maximum CMEP (Max) and half max CMEP (1/2 Max)). CMEP threshold stimulation (T), was defined as the lowest stimulator output that resulted in a CMEP of 50 microvolts in ≥ 50% of trials. For maximum CMEP (Max), the stimulation intensity was continually increased until the peak-to-peak amplitude plateaued or there was a decrease in the onset latency, indicative of cervical ventral root stimulation rather than the desired corticospinal tract (Taylor and Gandevia 2004). If the later occurred, the stimulation intensity was decreased until the appropriate onset latency was restored. Finally, 50% of maximum CMEP (1/2 Max) was defined as the stimulator intensity required to make CMEP amplitude equal to approximately 50% of maximum CMEP. Once the stimulation intensities were determined, the trials began in which all subsequent measurements were recorded with the arm at the 12 o’clock position: 1) rest, 2) 60 rpm and 3) 90 rpm. The selected cycling velocities were chose because 60 rpm was used in a similar study (Carroll et al., 2006) and 90 rpm has been used in an incremental test to maximum to assess
physiological measures such as peak oxygen consumption (Price et al., 2007). All three stimulation intensities were given during each trial. The trial order and stimulation intensities were randomized.

For the resting condition a total of 10 CMEPs were elicited for each of the T and ½ Max stimulation intensities every 8-10 seconds. For Max stimulations, 5 CMEPs instead of 10 were performed as has been done by others (Taylor et al. 2002) because the stimulation intensities used for Max CMEPs were less variable in amplitude and transiently painful. For the two cycling tasks, CMEPs were elicited automatically when the right hand pedal passed the 12 o’clock position.

**Measurements**

Data were analyzed off-line using Signal 4 software (CED, UK). Measurements of magnetic and electrical responses included the peak-to-peak amplitude and onset latency for MEPs, CMEPs and M-waves. Peak-to-peak amplitudes for all potentials in all subjects elicited in the biceps brachii were assessed from the initial deflection of the voltage trace from baseline to its’ third crossing point on the horizontal axis. Changes in MEP and/or CMEP amplitude or latency could be due to changes at the peripheral level, i.e. muscle. Thus, MEPs and CMEPs were normalized to M_{max}. The latency of evoked response onset was defined as the time between the stimulation artifact and the point at which the voltage trace became tangent to a straight line drawn along the horizontal voltage trace at baseline. Measurements for latency were taken from the averaged un-rectified traces for each type of response. In experiment 1, the rectified, pre-stimulus EMG (50ms) was averaged for each of the trials. All data for cycling and tonic contraction conditions and for each experiment were analyzed in the same manner.
Statistics

Experiment 1: A two-way (task x position) repeated-measures ANOVA was used to determine whether statistically significant differences occurred in MEP, CMEP and M-wave amplitudes and the average of the pre-stimulus EMG between the three cycling and tonic positions. Separate paired t-tests were utilized to determine changes in excitability for each arm position between arm cycling and intensity matched tonic contraction. All tests were performed on grouped data. For all comparisons a significance level of P < 0.05 was used. Group data are reported as means ± SD and shown as ± SE in the figures.

Experiment 2: A two-way (condition x stimulation intensity) repeated-measures ANOVA was used to determine whether statistically significant differences occurred in CMEP amplitudes and the average of the pre-stimulus EMG between the three conditions (rest, 12 o’clock, 60 and 90 rpms). Separate paired t-tests were utilized to determine changes in excitability for each condition. All tests were performed on grouped data. Additional participants were not recruited given the convincing findings obtained (see Results).

All data for both experiments were analyzed using IBM SPSS Statistics Version 19. For all comparisons a significance level of P < 0.05 was used. Group data are reported as means ± SD and shown as means ± SE in the figures.

RESULTS

Experiment 1: Corticospinal excitability changes during arm cycling and tonic contractions

EMG patterns of the biceps brachii during arm cycling

Average values of cycling EMG from all subjects during arm cycling are plotted in Fig. 1B. Averages were calculated from files that did not involve stimulation. This was done for all
positions which were made relative to a clock face. The values for each individual were expressed as a percentage of their maximum biceps brachii EMG recorded during the maximum cycling trial.

**Corticospinal excitability**

**MEPs:** The TMS stimulation intensities used to evoke MEPs equal to approximately 5-10% of \( M_{\text{max}} \) are influenced by a range of both excitatory and inhibitory circuits at the cortical and spinal levels and provide information regarding excitability of the corticospinal pathway. Fig. 2 (left column) shows an example of the differences in MEP amplitude between cycling and tonic tasks at each of the three positions assessed. The left column shows MEPs. In this example, MEPs expressed as a percentage of \( M_{\text{max}} \) were, 1.8, 7.0 and 44.2 % during cycling and 0.6, 1.0 and 22.7% during tonic contraction. As a group, MEP amplitudes were 7.2 and 8.8% \( M_{\text{max}} \) larger during arm cycling when compared to a tonic contraction at the 3 (cycle, 9.8 ± 1.6% \( M_{\text{max}} \); tonic, 2.6 ± 0.7% \( M_{\text{max}} \); \( P < 0.001 \)) and 6 o’clock (cycle, 29.7 ± 4.0% \( M_{\text{max}} \); tonic, 20.9 ± 2.6% \( M_{\text{max}} \); \( P < 0.001 \)) positions (Fig. 3a). These positions correspond to the end of extension/initiation of flexion (3 o’clock) and mid-flexion (6 o’clock) phases of arm cycling. There was no difference in MEP amplitude between cycling and tonic conditions when the arm was extending at the 12 o’clock position (cycle, 0.75 ± 0.22% \( M_{\text{max}} \); tonic, 0.41 ± 0.15% \( M_{\text{max}} \); \( P = 0.25 \)). Transcranial magnetic stimulation intensity was the same for arm cycling and tonic conditions for each subject. MEPs were normalized to M-wave for statistical comparisons.

**Background EMG:** There was no difference in the background EMG of the biceps brachii between the tasks at any of the three positions assessed (\( P = 0.21 \)). In contrast, the background
EMG of the triceps brachii was significantly larger during cycling at the 12 o’clock position ($P < 0.001$) and during the tonic contraction at the 6 o’clock position ($P < 0.001$). There was a trend towards significantly higher triceps brachii EMG during the tonic contraction at the 3 o’clock position ($P = 0.059$). See Figs. 3B and 3C for pre-stimulus group data.

**Spinal motoneurone excitability**

**CMEPs**: Because changes in MEP amplitude could represent changes at the supraspinal and/or spinal level, we examined spinal changes in excitability using the TMES technique (see Methods). Fig. 2 (right column) shows an example of the differences in CMEP amplitude between cycling and tonic tasks at each of the three positions assessed. In this example, CMEPs expressed as a percentage of $M_{\text{max}}$, were 2.4, 13.2 and 50.4 % during cycling and 5.5, 1.7 and 38.1% during tonic contraction. As a group, CMEP amplitudes were 5.2% $M_{\text{max}}$ larger during arm cycling when compared to a tonic contraction at the 3 o’clock position (cycle, 9.3 ± 1.9% $M_{\text{max}}$; tonic, 4.1 ± 0.9% $M_{\text{max}}$; $P < 0.001$) (Fig. 4a). This position corresponds to the end of extension/initiation of the flexion phase of arm cycling. There was no difference in CMEP amplitude between cycling and tonic conditions when the arm was in mid-flexion at the 6 o’clock position (cycle, 19.4 ± 4.5% $M_{\text{max}}$; tonic, 18.4 ± 3.8% $M_{\text{max}}$; $P = 0.73$) or during the extension phase at the 12 o’clock position (cycle, 1.3 ± 0.4% $M_{\text{max}}$; tonic, 2.2 ± 0.6% $M_{\text{max}}$; $P = 0.10$). Transmastoid electrical stimulation intensity was the same for arm cycling and tonic conditions for each subject. Data for each condition was an average of 10 frames. As was done with MEPs (see above), CMEPs were normalized to M wave for statistical comparisons.
Background EMG: There was no difference in the background EMG of the biceps brachii between the tasks at any of the three positions assessed ($P = 0.35$). In contrast, the background EMG of the triceps brachii was significantly larger during the tonic contractions at the 3 ($P = 0.004$) and 6 o’clock ($P < 0.001$) positions. See Figs. 4B and 4C for pre-stimulus group data.

**Background EMG of biceps brachii and triceps brachii between stimulation paradigms**

Cycling: There was no difference in the background EMG for either biceps brachii or triceps brachii when comparing the background EMG within the cycling task between stimulation paradigms (i.e. TMS and TMES) (biceps brachii: $P = 0.41$; triceps brachii: $P = 0.17$).

Tonic contraction: There was no difference in the background EMG for either biceps brachii or triceps brachii when comparing the background EMG within the cycling task between stimulation paradigms (i.e. TMS and TMES) (biceps brachii: $P = 0.43$; triceps brachii: $P = 0.32$).

**Experiment 2: Spinal motoneurone excitability of the biceps brachii during the extension phase of arm cycling**

CMEPs: The amplitudes of the CMEPs were assessed using three different stimulation intensities (T, ½ Max and Max) with the arm at the 12 o’clock position at rest and while cycling at both 60 and 90 rpms. Data for each condition was an average of 10 frames. As was done with MEPs (see above), CMEPs were normalized to M-wave for statistical comparisons. Fig. 5 shows an example of the differences in CMEP amplitude between the resting and two cycling tasks at each of the three stimulation intensities. In this example, CMEPs expressed as a percentage of $M_{\text{max}}$, were 3.5, 2.3% and 0.7% at T stimulation, 37.5, 9.1 and 4% at ½ Max stimulation, and 60.4, 29.3, and 21.1% at Max CMEP stimulation. In Fig. 6, CMEP amplitudes increased as the
stimulation intensity increased for each of the three conditions, as expected. CMEP amplitudes were smaller for all three stimulating intensities during both cycling conditions when compared to rest (P = 0.001). T CMEPs taken at rest were 37 and 135% larger than T CMEPs taken during cycling at 60 (P = 0.04) and 90 (P = 0.03) rpm, respectively. ½ Max CMEPs during rest were 319 and 420% larger than ½ Max CMEPs measured while cycling at 60 (P = 0.01) and 90 (P = 0.02) rpm, respectively. The Max CMEP stimulation resulted in resting amplitudes that were 117 and 135% greater than the amplitudes seen during cycling at 60 (P = 0.02) and 90 (P = 0.03) rpm, respectively. There were no differences in CMEP amplitudes between cycling at 60 and 90 rpms for any of the three stimulation intensities utilized (T, P = 0.1; ½ Max, P = 0.25; and Max, P = 0.38).

We initially compared CMEPs with each of the three stimulation intensities during two resting conditions: 1) rest with the hand pronated and resting on the participant’s leg and 2) rest at the 12 o’clock position as described above. CMEP amplitudes nor the pre-stimulation EMG (as described in experiment one) of the biceps brachii were different between the ‘resting’ conditions (P = 0.88 and P = 0.37, respectively; data not shown). Due to the similarities of background EMG and CMEP amplitudes between the resting conditions we are reasonably confident that at the 12 o’clock position the arm was ‘at rest.’ For clarity of results we only present the rest at 12 o’clock data so as to only compare CMEPs at the same joint position between conditions (rest and cycling at 60 and/or 90 rpms).
DISCUSSION

This report is the first to show that corticospinal excitability of an upper-limb prime mover, the biceps brachii, is higher during arm cycling than an intensity-matched tonic contraction and that enhanced spinal motoneurone excitability can partially account for this finding. The results from experiment #2 indicate that spinal motoneurone excitability of the biceps brachii is not increased during the extension phase of arm cycling when compared to rest at the same position.

Increased supraspinal excitability during arm cycling

In the present study, corticospinal excitability of the biceps brachii was significantly greater at the initiation (i.e. 3 o’clock) and middle (i.e. 6 o’clock) of elbow flexion during arm cycling when compared to tonic contraction, as demonstrated via larger MEP amplitudes (Figs. 2 and 3). There was no difference in spinal excitability at the 6 o’clock position (Fig. 4A) between tasks, indicating that the increased MEP amplitude was mediated via changes at the supraspinal level. Corticospinal excitability of the arms during arm cycling has been previously investigated, however. Zehr and colleagues (2006) demonstrated a decrease in corticospinal excitability during the flexion phase of arm cycling in the flexor carpi radialis (FCR) accompanied by a decrease in the H-reflex amplitude. This led to the conclusion that during arm cycling there is a suppression of excitatory drive from descending and peripheral pathways to the wrist flexors. Differences between our data and those of Carroll et al. (2006) may be related to muscle function during arm cycling. The main role of the FCR muscle during arm cycling is to stabilize the wrist to allow for a steady grip of the crank handles which means that the FCR is continuously active during cycling with relatively minor phase-dependent activation changes and no strong
propulsive phase (see Figs. 3C and 4C in Carroll et al. 2006). In contrast, the biceps brachii demonstrates strong phase-dependence and propulsive activation during arm cycling (Fig. 1B).

Though different from the results of Carroll et al. (2006), our results are in-line with those of Pyndt and Nielsen (2003). Pyndt and Nielsen (2003) demonstrated an increase in MEP amplitude of the soleus during the propulsion phase of leg cycling. As is the case for the biceps brachii, the soleus is considered a main power producer during cycling (Hug and Dorel, 2009).

We thus suggest, as did Pyndt and Nielsen (2003), that the increased MEP observed in the present study was due to enhanced excitability of the cortical neurones projecting to the spinal cord, thus making them more susceptible to activation via TMS. This increased descending drive to the spinal motor pools would perhaps be necessary to increase the recruitment and firing rate of spinal motoneurones to ensure adequate muscle activation and power production is obtained during the propulsive phase. Intermuscle differences in corticospinal excitability as measured via TMS-induced MEPs have been previously reported in the ankle muscles during locomotion (Capaday et al., 1999, Sidhu et al., 2012). Capaday and colleagues (1999) showed that soleus MEPs were smaller during stance compared to an intensity-matched tonic contraction whereas tibialis anterior MEPs were larger.

**Increased spinal excitability during arm cycling**

Changes in MEP amplitude can result from changes in neuronal excitability at supraspinal and/or spinal locations. Thus, an alternative explanation for the increase in supraspinal excitability at 3 and 6 o’clock during cycling relative to the tonic contraction is an increase in spinal excitability. We thus employed TMES (see methods) to assess spinal excitability. Collision experiments have shown that TMES directly activates the corticospinal
axons (Gandevia et al., 1999) producing CMEPs that are independent of changes in supraspinal excitability (Taylor et al., 2002), have a large monosynaptic component in biceps brachii motoneurone pool (Petersen et al., 2002) and are free from classical afferent-mediated presynaptic inhibition (Nielsen and Petersen, 1994). This technique was particularly important to the current study because one of our main objectives was to determine whether spinal motoneurone excitability was different between arm cycling and tonic contraction. This objective was based on previous work by Power et al. (2010) who showed that the excitability of the same spinal motoneurone was higher during rhythmic scratch as compared to stance in the adult decerebrate cat.

In the present study we show that spinal motoneurone excitability was higher during arm cycling than an intensity-matched tonic contraction, at the initiation of flexion (3 o’clock; Fig. 4A). Task-dependent changes in human motoneurone excitability have been reported using motor unit recordings during either isometric or isotonic contractions. Recruitment thresholds and firing patterns may vary depending on the direction (Thomas et al., 1987), velocity (Desmedt and Godaux, 1977) and type (Tax et al., 1990) of contraction. Studies examining human spinal motoneurone excitability during rhythmic and alternating motor outputs thought to be partially generated by spinal CPGs are limited, mainly due to technical limitations. Sidhu and colleagues (2012) examined supraspinal and spinal excitability and concluded that changes in corticospinal excitability were driven mainly by changes at the spinal level. The authors did not discuss the potential ‘spinal’ mechanisms responsible and were focussed on determining the supraspinal and spinal contributions to leg cycling. As such, they did not compare spinal excitability between cycling and a tonic contraction. In the present study it is possible that motoneurone excitability was higher during cycling even though the background EMG was similar. Background EMG is a
crude measure of motoneurone excitability and does not take into account factors such as the
activation of voltage-dependent excitation which may be active in one task and not the other.
This could lead to a non-linear increase in the amplitude of evoked responses even with similar
background EMG. However, it is important to recognize that although CMEP amplitudes do
represent the excitability of the spinal motoneurone pool, they do not necessarily indicate
changes in intrinsic electrical properties such as those observed in cat motoneurones during
scratch (see Introduction). The motoneurone pool may have been relatively more excitable
during arm cycling due to differences in the balance of excitatory and inhibitory synaptic inputs.

It would have been preferable to match the EMG activity in multiple motor pools
between tasks to eliminate possible heteronymous influences. Triceps brachii activity, however,
was higher during the tonic contraction at 3 and 6 o’clock during both MEP and CMEP
recordings (Figs. 3C and 4C). Thus, reciprocal inhibition may have reduced the amplitude of the
evoked potentials during tonic contraction even though biceps brachii EMG was matched across
tasks and positions. Because reciprocal inhibition affects motoneurone responsiveness, however,
it would be expected that both MEPs and CMEPs would demonstrate a similar pattern of
between-task modulation. However, MEPs were larger at the 3 and 6 o’clock positions during
arm cycling whereas CMEPs were larger only at the 3 o’clock position. If reciprocal inhibition
were reducing motoneurone excitability during the tonic contraction it would perhaps be
expected that CMEPs would be smaller during tonic contraction at the 6 o’clock position, as was
the case with MEPs. This did not occur (Figs. 3 and 4). Differences between the pattern of MEP
and CMEP modulation in the biceps brachii are not likely explained by differences in the degree
of triceps brachii activity when comparing MEPs or CMEPs at 3 or 6 o’clock during tonic
contraction given that the background EMG was similar. Regardless, we cannot conclude with
certainty whether reciprocal inhibition contributed to the observed differences in the present study as it was not directly assessed.

Methodological considerations

There are a number of other factors to consider in the interpretation of the present results. A major difference between the motor outputs examined is that cycling involves limb movement while tonic contraction does not. This leads to task-dependent changes in afferent feedback which could affect both MEPs and CMEPs given that sensory feedback contributes significantly to muscle activity during movement (Nielsen, 2004). For example, the muscle is rapidly elongated at the 3 o’clock position during cycling (Fig. 1B) likely activating Ia afferents which could lead to increased excitatory input to the spinal motoneurones and thus increased MEP and CMEP amplitudes compared to the tonic contraction. Furthermore, as with previous studies comparing neural responses between cycling and to that of a tonic contraction (Carroll et al. 2006, Pyndt and Nielsen, 2003) the influence of a rapidly changing EMG pattern during cycling (Fig. 1B) as compared to a stable EMG during tonic contraction may have differentially modulated the sensitivity of spinal motoneurones to synaptic input. It is also possible that recurrent inhibition from other upper limb muscles such as the brachioradialis and/or extensor carpi radialis (Katz et al., 1993) influenced CMEP amplitudes. These muscles were not assessed in the present study and we are therefore unable to comment on their potential role in the observed differences. Finally, it is possible that the observed findings are not cycling-dependent, and thus not due to potential involvement of a spinal CPG, but rather movement related. Tax and colleagues (1989) reported a lower recruitment threshold for biceps brachii motor units during
slow isotonic versus isometric contractions. They subsequently determined that differences in recruitment, however, were due to central and not peripheral factors (Tax et al., 1990).

**Spinal motoneurone excitability of the biceps brachii is greater at rest than during the extension phase of arm cycling**

An additional question we posed and assessed via our second experiment (see Methods), was “Is biceps brachii motoneurone excitability higher during the extension phase of arm cycling when compared to rest?” This question was based on previous findings in the cat (Power et al., 2010). During the approach (flexion) phase of fictive scratch in the adult decerebrate cat, extensor motoneurones are tonically hyperpolarized (Power et al., 2010) while both flexor and extensor motoneurones receive reciprocal inhibitory input during the hyperpolarized phase of rhythmic scratch (i.e. when the antagonist is active) (Geertsen et al., 2011). Interestingly, the state-dependent enhancement of spinal motoneurone excitability (i.e. hyperpolarized Vth and AHP suppression) occurred during the inhibitory phase despite these two inhibitory factors (Power et al., 2010). We thus expected, based on the results from the cat, that spinal motoneurone excitability of the biceps brachii would be increased during the extension phase of arm cycling (12 o’clock) relative to rest in experiment #1. This didn’t appear to be the case so we furthered this question in experiment #2 (see Methods) to determine if motoneurone excitability of the biceps brachii was higher during the extension phase of arm cycling when compared to rest, as it was in the cat. As noted in the cat study, even though motoneurone excitability was increased, an increase in intracellular current injection was required to make the cell fire, likely to overcome this background inhibition. In addition, Pyndt and Nielsen (2003) showed that reciprocal inhibition was reduced during lower limb cycling as the pedaling frequency increased.
We thus reasoned that if we increased the TMES intensity and/or increased the pedalling frequency of arm cycling we would see a large increase in CMEP amplitude during the extension phase of arm cycling (12 o’clock) when compared to rest if similar changes in motoneurone excitability occurred as those that occurred in the cat. Our results indicate that even with a maximal TMES intensity and a 90 rpm pedaling velocity, spinal motoneurones were less responsive than at rest in the 12 o’clock position. It is concluded that spinal motoneurone excitability of the biceps brachii is not increased during the extension phase of arm cycling with an increase in pedalling frequency and is in fact reduced when compared to rest.

CONCLUSION

Increased supraspinal excitability of the biceps brachii occurred during the flexion phase of arm cycling while spinal motoneurone excitability was increased at the initiation of flexion, when compared to an intensity-matched tonic contraction. Perhaps spinal motoneurone excitability is enhanced prior to the propulsive phase (6 o’clock) of the biceps brachii in an attempt to reduce the supraspinal input required to reach firing threshold. It is currently unclear whether the larger CMEP amplitude at the 3 o’clock position during cycling was due to intrinsic changes in spinal motoneurone excitability and/or differences in synaptic input. Our results from experiment #2 indicate that unlike the cat, spinal motoneurone excitability of the biceps brachii is not increased during the extension phase of a rhythmic and alternating motor output when compared to rest.
References


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**Competing Interests**

The authors have no competing interests to declare.

**Author Contributions**

Experiment #1 formed part of the Master’s thesis for A.R and a research practicum for D.F., both of whom were responsible for data collection and analysis for experiment #1. D.F. collected and analyzed data for experiment #2. The studies were conceptually designed by K.E.P. All authors contributed equally to the final versions of the paper.

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Figure Legends

Figure 1. Experimental set-up and averaged EMG in the biceps brachii during arm cycling. A: Participants were seated in a comfortable chair. Positions of the right arm were made relative to a clock face. In this example, the participant is grasping the handle of a cycle ergometer at the 6 o’clock position using their right hand. Transcranial magnetic stimulation (TMS) was applied over vertex to preferentially active the left motor cortex. Transmastoid electrical stimulation (TMES) was applied between the mastoid processes and nerve stimulation at Erb’s point. Evoked potentials were recorded from the right biceps brachii using surface EMG. B: Averaged EMG values throughout arm cycling. EMG amplitudes were normalized to the maximum EMG recorded during the maximum cycling trial (mean ± SE, n =11).

Figure 2. Average MEP and CMEP traces after 10 stimuli during arm cycling (solid black lines) and tonic contraction (dashed grey lines) at each of the three arm positions (12, 3 and 6 o’clock) from one participant.

Figure 3. Group data (mean ± SE, n =11) for MEP amplitude (A) and pre-stimulus EMG of the biceps brachii (B) and triceps brachii (C) during arm cycling (black bars) and tonic contraction (white bars) at each of the three arm crank positions (12, 3 and 6 o’clock). MEP amplitudes are expressed relative to Mmax amplitudes at the same arm crank position. Pre-stimulus EMG is expressed relative to the maximum EMG obtained during the max cycle test. Asterisks indicate a significant difference (P < 0.001) between the arm cycling and tonic contraction conditions.

Figure 4. Group data (mean ± SE, n =11) for CMEP amplitude (A) and pre-stimulus EMG (B) during arm cycling (black bars) and tonic contraction (white bars) at each of the three arm crank positions (12, 3 and 6 o’clock). CMEP amplitudes are expressed relative to Mmax amplitudes at the same arm crank position. Pre-stimulus EMG is expressed relative to the maximum EMG obtained during the max cycle test. Asterisks indicate a significant difference (P < 0.001) between the arm cycling and tonic contraction conditions.

Figure 5. Average CMEP traces following 10 stimuli for (A) CMEP threshold stimulation and (B) 50% of max CMEP stimulation and 5 stimuli for (C) max CMEP stimulation of a single participant. Measurements for the three tasks (Rest; solid black lines, 60 rpm; solid grey lines, 90 rpm; dashed black lines) were taken at the 12 o’clock position.

Figure 6. Group data (mean ± SE, n =4) for CMEP amplitudes during each of the three conditions [rest (black bars), 60 rpm (grey bars) and 90 rpm (white bars)] and three different stimulation intensities, Threshold, Threshold + 50%, and Max. CMEPs were evoked at the 12 o’clock position for each of the three conditions. CMEP amplitudes are expressed relative to Mmax amplitudes at the same arm crank position. Asterisks indicate a significant difference between rest and both 60 and 90 rpm. There were no differences between 60 and 90 rpms at either of the three stimulation intensities.
Figure 1

A

Transcranial
Transmastoid
Erb’s Point
Biceps Brachii EMG
Triceps Brachii EMG

B

Cycling EMG Amplitude (% of Max Cycle)

Crank Position
Figure 2

MEPs

CMEPs

20 ms

10% Mmax

12 o’clock

10% Mmax

25 ms

10%

Mmax

3 o’clock

6 o’clock

Cycling

Tonic

Mathematical notation and unit symbols are used in the text, such as 10% Mmax and 25 ms.
Figure 3

A. MEPs

Crank Position

B. Biceps Pre-stimulus EMG

C. Triceps Pre-stimulus EMG
Figure 4

A. CMEPs

B. Biceps Pre-stimulus EMG

C. Triceps Pre-stimulus EMG
Figure 5

A

1% Mmax

B

10% Mmax

C

20% Mmax

Rest  60 rpm  90 rpm
Figure 6

CMEPs

Amplitude (% M-max)

Threshold
Threshold + 50%
Max

Rest
60 rpm
90 rpm

* indicates significant difference from baseline (Rest)