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Acute and Chronic Changes in Neural Excitability During Physical Activity in Non-Pathological States

Edited by
Kevin Power

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Special Issue Editor

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Contents

About the Special Issue Editor

Kevin Power (PhD in Neurophysiology) is a Full Professor in the School of Human Kinetics and Recreation at Memorial University of Newfoundland. He received his bachelor's (2001) and master's degrees (2003) from the School of Human Kinetics and Recreation at Memorial University and his PhD in neuroscience from the University of Manitoba (2009).

Dr. Power is the Co-Director of the Human Neurophysiology Lab at Memorial University with his colleague, Dr. Duane Button. Together with his team of students, Dr. Power studies the neural control of arm cycling, a rhythmic motor output that has similar neural control strategies to locomotion. Using this model, Dr. Power examines how the motor cortex and spinal motoneurones produce movement and adapt to exercise interventions, the findings of which have implications for guiding neurorehabilitation programs for persons following stroke or traumatic brain injury. Dr. Power has served as an Associate Editor for Applied Physiology Nutrition and Metabolism since 2017 and is the Vice-Chair of Research with the Canadian Society for Exercise Physiology (CSEP) and is himself a Certified Exercise Physiologist® and course examiner for CSEP.

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Article

Neuromuscular Mechanisms Underlying Changes in Force Production during an Attentional Focus Task

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Abstract: We examined the effects of attentional focus cues on maximal voluntary force output of the elbow flexors and the underlying physiological mechanisms. Eleven males participated in two randomized experimental sessions. In each session, four randomized blocks of three maximal voluntary contractions (MVC) were performed. The blocks consisted of two externally and two internally attentional focus cued blocks. In one of the sessions, corticospinal excitability (CSE) was measured. During the stimulation session transcranial magnetic, transmastoid and Erb's point stimulations were used to induce motor evoked potentials (MEPs), cervicomedullary MEP (CMEPs) and maximal muscle action potential (Mmax), respectively in the biceps brachii. Across both sessions forces were lower ($p = 0.024$) under the internal (282.4 ± 60.3 N) compared to the external condition (310.7 ± 11.3 N). Muscle co-activation was greater ($p = 0.016$) under the internal (26.3 ± 11.5%) compared with the external condition (21.5 ± 9.4%). There was no change in CSE. Across both sessions, force measurements were lower ($p = 0.033$) during the stimulation (279.0 ± 47.1 N) compared with the no-stimulation session (314.1 ± 57.5 N). In conclusion, external focus increased force, likely due to reduced co-activation. Stimulating the corticospinal pathway may confound attentional focus. The stimulations may distract participants from the cues and/or disrupt areas of the cortex responsible for attention and focus.

Keywords: corticospinal excitability; attentional focus; co-contraction

1. Introduction

The effects of attentional focus instructions on motor learning and performance have been extensively studied in the past 20 years [1,2]. Specifically, two types of instructions have been compared: those that elicit an internal focus (IF) and external focus (EF) of attention [1,2]. EF leads one to focus on the intended effects of movements on the environment. For example, focusing on the bull's eye during a dart throwing task. Conversely, IF leads one to focus on a body part or muscle group. For example, focusing on wrist movement during a dart throwing task [3]. Many studies report that EF enhances motor learning and physical performance compared to IF [1,4–7]. This includes tasks that require accuracy, balance, strength and speed. The effects are consistent across children, adults, older adults, and those suffering from mental disease [8–10]. These effects are arguably some of the most established ones identified in human movement science.

Despite the impressive number of studies comparing attentional focus strategies across tasks and populations, little is known about the underpinning neuromuscular mechanisms that can explain the observed effects. A handful of studies examined if attentional focus strategies lead to different brain activation patterns using electroencephalography (EEG) and functional magnetic resonance imaging (fMRI) [11–14]. EEG alpha power is generally lower during EF and associated with more ideal alpha frequencies [15]. An fMRI study observed higher activation in the motor cortex during EF compared to IF. Thus, some evidence shows that various parts of the brain are activated differently between EF and IF which may account for enhanced neuromuscular performance during EF. The most commonly used tool to shed light on the mechanistic pathway explaining the superiority of EF is surface electromyography (EMG), which is a general measure of central nervous system excitation. A repeated—although not consistent—pattern is that EF leads to decreased muscle EMG activity from both the agonist and antagonist muscle groups involved in the task execution compared to IF [16]. The reduction in EMG activity during EF may promote effective and efficient movement patterns [17]. However, EEG, fMRI and EMG alone cannot pinpoint the motor pathways leading to the enhanced movement patterns associated with EF. Indeed, EF can promote superior motor performance by eliciting greater nervous system excitation, less inhibition, or a combination thereof from the brain to the spinal cord.

Nervous system excitation and inhibition can be examined through measuring corticospinal excitability via transcranial magnetic stimulation (TMS), and transmastoid electrical stimulation (TMES) [18,19]. TMS elicits a motor evoked potential (MEP) in a muscle of interest, while TMES elicits a cervicomedullary MEP. TMS-evoked MEP amplitudes are used to quantify corticospinal excitability (CSE) [20]. Alterations in CSE could occur anywhere along the corticospinal pathway (i.e., from cortex to motoneuron). The combined use of the aforementioned techniques is used to determine whether the modulation of CSE is predominantly supraspinal or spinal [18]. The corticospinal tract is examined due to its importance in the organization of single and multi-jointed movements. The corticospinal fibers control the spinal motoneurons that innervate the muscles of the trunk and limbs [21]. Many modulators have been shown to influence CSE from caffeine indigestion [22] to arousal imagery [23]. It is possible that EF may increase corticospinal excitability, decrease corticospinal inhibition, or a combination of both which would account, in part, for the increase in motor performance seen. This would further our understanding of the pathways and underlying mechanisms to address the changes in motor performance and learning seen with attentional focus feedback.

In view of the insight that can be gained using corticospinal excitability measurement techniques, and the limited knowledge accumulated to date on the pathways accounting for attentional focus instructions effects, combining the two in a single experiment is a worthwhile endeavor. Therefore, the aim of this study was to (1) compare CSE to the biceps brachii between EF and IF cued maximal voluntary contractions (MVC) of the elbow flexors and (2) compare co-activation patterns of the biceps brachii and triceps brachii between EF and IF cued MVC of the elbow flexors. We hypothesized that (1) CSE would be modulated differently between EF and IF cued condition and (2) co-activation would be greater with an IF cued condition.

2. Materials and Methods

Eleven resistance-trained males (1.77 ± 0.02 m, 84.32 ± 3.22 kg, 23.8 ± 2.36 years) participated in the experimental study. Resistance-trained status was determined as meeting the Canadian Society of Exercise Physiology guidelines of two hours a week of resistance training for at least a year. We chose to recruit only resistance-trained participants because corticospinal excitability is training dependent [24–27]. Participants completed a magnetic stimulation safety checklist prior to participation in order to screen for potential contraindications with magnetic stimulation procedures [28]. Verbal description about the procedures was given to the participants and if accepted they gave their informed written consent. The study was approved by The Memorial University of Newfoundland

Interdisciplinary Committee on Ethics in Human Research and was in accordance with the Tri-Council guidelines in Canada with full disclosure of potential risks to participants (HK-20190008).

2.1. Elbow Flexor Force

Participants were seated in a custom-built chair (Technical Services, Memorial University of Newfoundland, St. John's, NL, Canada) in an upright position, with chest and head strapped in place to minimize movement, with hips and knees flexed at 90°. The forearm was held horizontal, positioned supine with the shoulders resting against the back of the chair, and placed in a custom-made orthosis that was connected to a load cell (S-beam hanging load cells, model number LC101-500, Omegadyne, Inc., Sunbury, OH, USA) which was calibrated prior to the measurement. The load cell detected force output, which was amplified (×1000) (CED 1902, Cambridge Electronic Design Ltd., Cambridge, UK) and displayed on a computer screen. Data was sampled at 5000 Hz. Participants were instructed to maintain an upright position with their head in a neutral position during contractions. Visual feedback was given to all participants during all contractions prior to and during the conditions as a line on a computer screen in front of them showing when to begin and end the contraction. Participants were only able to view the amount of their force production and biceps brachii EMG activity.

2.2. Electromyography

Electromyography (EMG) activity was recorded from the dominant arm's biceps brachii and triceps brachii using surface EMG (10 mm MediTrace Ag-AgCl, Graphic Controls Ltd., Buffalo, NY, USA). Electrodes were placed 2 cm apart (center to center) over the midpoint of the muscle belly of the participant's biceps brachii and triceps brachii lateral head. A ground electrode was placed over the lateral epicondyle of the dominant knee. Skin preparation for all recording electrodes included shaving to remove excess hair and cleaning with an isopropyl alcohol swab to remove dry epithelial cells. An inter-electrode impedance of <5 kΩ was obtained prior to recording to ensure an adequate signal-to-noise ratio. EMG signals were amplified (×1000) (CED 1902) and filtered using a 3-pole Butterworth filter with cut-off frequencies of 10–1000 Hz. All signals were analog-digitally converted at a sampling rate of 5 kHz using a CED 1401 (Cambridge Electronic Design Ltd., Cambridge, UK) interface.

2.3. Stimulation Conditions

Motor responses from the bicep brachii were elicited via (1) transcranial magnetic stimulation (TMS), (2) transmastoid electrical stimulation (TMES) and (3) brachial plexus electrical stimulation at Erb's point. Stimulation intensities used for TMS and TMES were adjusted similar to that of Pearcey et al. (2014) so that the evoked potentials produced by each, evoked motor evoked potentials (MEPs), and CMEPs, respectively, were of similar amplitude and normalized to a maximal M-wave (Mmax) [26]. Stimulation intensities were then set during an isometric elbow flexion contraction equal to 5% of MVC.

2.3.1. Transcranial Magnetic Stimulation (TMS)

TMS- (MEPs) were used to measure corticospinal excitability. A TMS (Magstim 200, maximal output 2.0 Tesla) circular coil (13 cm outside diameter) was placed directly over the vertex of the head to induce MEPs in the active (5% MVC) biceps brachii muscle. The vertex was located by marking the measured halfway points between the nasion to inion and tragus to tragus. The coil was flipped to ensure the induced current flow was anterior to posterior in the target motor cortex (A side up for right side, B side up for left) to activate the dominant biceps brachii [29–31]. The optimum stimulation site was determined by initially placing the coil over the vertex and slightly moving the coil towards the non-dominant hemisphere in three directions, i.e., anteriorly, posteriorly, and laterally. Once the highest MEP response was elicited, that position was determined as the optimum stimulation site. Stimulation intensity was set to elicit a MEP 10%–20% of Mmax taken as an average of eight trials in the biceps brachii during a 5% MVC.

2.3.2. Transmastoid Electrical Stimulation (TMES)

Stimulation (Model DS7AH, Digitimer Ltd., Welwyn Garden City, Hertfordshire, UK) was applied via surface electrodes placed over the mastoid processes and current was passed between them (200 μs duration, 80–200 mA). Stimulation intensity was adjusted to prevent ventral root activation by closely monitoring CMEP responses for any decrease in onset latency (~2 ms), which shows cervical ventral root activation [32]. Stimulation intensity was adjusted to elicit a response that matched the size of MEP amplitude, taken as an average of eight trials, in the biceps brachii during a 5% MVC.

2.3.3. Brachial Plexus Stimulation

Stimulation (Model DS7AH, Digitimer Ltd., Welwyn Garden City, Hertfordshire, UK) of the brachial plexus was used to measure maximal compound muscle action potential (Mmax). Erb's point was electrically stimulated via a cathode and anode placed on the skin over the supraclavicular fossa and the acromion process, respectively. Current pulses were delivered as a singlet (200 μs duration, 90–185 mA, 400 V). The electrical current was gradually increased until Mmax of the biceps brachii at a 5% MVC was observed.

2.4. Experimental Protocol

Participants completed a familiarization session and two experimental sessions that were randomized. Each session took place on separate days with 48 h between each session.

2.4.1. Familiarization Session

Participants performed two 5 s MVCs of the dominant elbow flexors, with 2 min of rest between contractions. If the force from the two MVCs differed by greater than 5%, a third MVC was performed. Following completion of the MVCs, participants practiced holding the 5% MVC contraction for 10 s at each position. Participants then received the three different types of stimulations at various intensities to ensure that they were comfortable to endure the stimulation paradigm involved in each experimental session.

2.4.2. Stimulation Session

Upon arrival, the participants were prepared for EMG and asked to perform two elbow flexor MVCs. A 10 min rest period was then issued to ensure no effect of the MVC on the CSE measurements [33]. Following the rest period, the experimental procedures began and the stimulation intensities for the Mmax, MEP, and CMEP of the biceps brachii during 5% MVC were determined. Participants then moved on to perform a semi-randomized protocol where they completed four blocks of 3 MVCs of the elbow flexors with 3 min of rest between MVCs. Five minutes of rest was given between each block of conditions. A total of 12 MVCs were performed. Participants were verbally encouraged with the same attentional focus cue provided immediately before each contraction in each block of conditions. Participants were either asked to "focus on pulling up on the handle as hard and as quickly as you possibly can" (external cue) or to "focus on contracting your biceps as hard and as quickly as you possibly can" (internal cue). In total participants were EF cued six times or IF cued six times. These cues were countered balanced between sets. During each condition participants received counter-balanced TMSs and TMESs at 1.5 and 3 s and an M-wave was given at the 4.5 s mark. See Figures 1 and 2 for the experimental set-up.

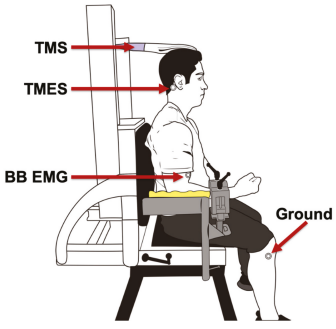

Figure 1. Participant's experimental set-up. Participants were positioned up right in an elevated chair with shoulders at 0 degrees and elbows at 90 degrees. TMS: transcranial magnetic stimulation, TMES: transmastoid electrical stimulation, BB EMG: biceps brachii electromyography. Ground: ground electrode.

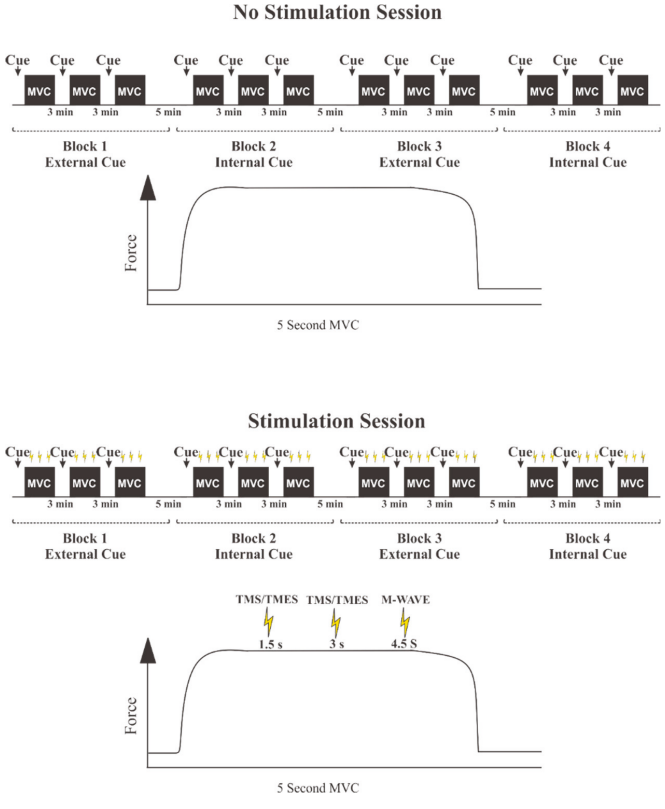

Figure 2. Experimental protocol. Each participant completed two experimental sessions (no stimulation session above, stimulation session below) which were randomized. Within each session, participants completed two blocks of three externally cued condition and two blocks of internally cued condition which were also randomized. Maximal voluntary contractions (MVC) were held for 5 s, beginning and ending at 2 and 7 s respectively, and during the stimulation session a transcranial magnetic stimulation (TMS) and transmastoid electrical stimulation (TMES) pulse was randomly delivered at 1.5 and 3.0 s marks with an M-Wave delivered each time at the 4.5 s mark. The traces underneath the sequence blocks represents the timing of the no-stimulation/stimulation during a 5 s MVC contraction.

2.4.3. Non-Stimulation Session

If the stimulation session was completed first, then the non-stimulation session was completed 48 h after the first session. This session was identical to the stimulation session except no stimulations were used. This session was included in the study to examine if stimulations impact a participant's ability to perform an MVC and their ability to focus on the attentional focus cues.

2.5. Data Analysis

Force, EMG, and CSE data were measured offline using Signal 4.0 software (Cambridge Electronic Design Ltd., Cambridge, Hertfordshire, UK). All offline computations were conducted using Microsoft Office Excel 2016 (Microsoft Corporation, Redmond, WA, USA).

Maximum voluntary isometric force. Peak elbow flexor's force was obtained from all six MVCs under each condition (external cued condition and internal cued condition) during both no-stimulation and stimulation sessions. MVC force output was measured as the peak amplitude from no force to maximum force.

Electromyography (EMG). Root mean square EMG (rmsEMG) from the biceps brachii and triceps brachii muscles was calculated from $t = 1$ s to $t = 2$ s interval of each MVC once the force reached its peak output under each condition (external cued condition and internal cued condition) and during each session (no-stimulation and stimulation). Additionally, muscle co-activation was quantified by computing the percentage of triceps brachii rmsEMG/biceps brachii rmsEMG [34]. To illustrate the amount of co-activation per unit of force production and illustrate the relationship between force and coactivation, the percentage ratio of muscle co-activation per Newton of force was calculated for MVCs from both the external and internal cued conditions during both no-stimulation and stimulation sessions.

Corticospinal excitability (CSE). During the stimulation session only, biceps brachii MEP, CMEP, and Mmax peak-to-peak amplitudes (mV) were extracted during all six MVCs under each condition (external cued condition and internal cued condition). See Figure 3 for raw data for MEP, CMEP and Mmax responses during a MVC. Since amplitudes and areas give similar results, we used MEP, CMEP, and Mmax amplitudes for comparisons [35]. MEP and CMEP peak-to-peak amplitudes were normalized to Mmax amplitudes (%Mmax), given Mmax is a stable measure of muscle activity during maximal muscle fibre recruitment [36]. Simply put, the MEP and CMEP values were divided by the M-wave produced from the same trial. As well, ratios of normalized MEP/CMEP amplitude were calculated [37]. The MEP/CMEP would indicate the level of supraspinal or spinal excitability. Levels above 1 are indicative of higher supraspinal excitability compared to spinal excitability [30].

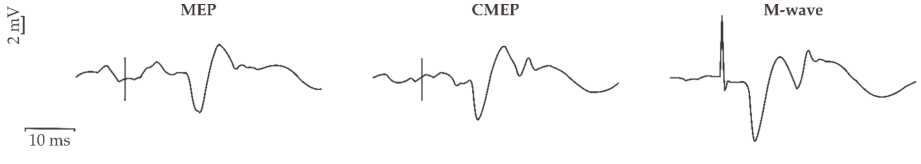

Figure 3. Raw data sample for MEP, CMEP and Mmax responses during an MVC from a single participant.

2.6. Statistical Analysis

Prior to statistical analyses all data underwent quality control checks in Microsoft Office Excel 2016 (Microsoft Corporation, Redmond, WA, USA) for missing data points and outliers. In terms of missing data, only one participant was unable to complete the stimulation session (P09). This participant was not included in CSE analyses; however, their MVC peak force and rmsEMG data (trial 1 to trial 6) for both the external condition and internal conditions (12 trials) were subsequently imputed for the stimulation session to enable groupwise comparisons across sessions. Additionally,

two participants (P10, P11) were missing force data for one MVC trial each (trial 4), under both the internal and external conditions, for the stimulation session alone (four trials). The missing datapoints were in a time series where data for both sessions (i.e., stimulation and non-stimulation sessions) were not available. In total, 16 datapoints were missing for MVC peak force (6.1%) and 12 datapoints each were missing for rmsEMG of both biceps brachii (4.5%) and triceps brachii (4.5%). Missing data were imputed by determining the series average for the entire sample, including both conditions (external condition, internal condition), at their respective timepoints and sessions using the Missing Values Analysis and Transform functions in SPSS (V26.0, IBM Corporation, Armonk, NY, USA). Outliers were considered datapoints that exceeded the sample mean by ± three standard deviations (SD). No outliers were identified.

Statistical analyses were completed using SPSS (V26.0, IBM Corporation, Armonk, NY, USA). Assumptions of normality (Shapiro–Wilk test), sphericity (Mauchly's test), and homogeneity of variances (Levene's test) were tested for all outcome measures where appropriate. For the Shapiro–Wilk test, statistical significance was set at $p < 0.001$ [38]. In the event of a violation of the assumption of sphericity, p-values were adjusted using the Greenhouse–Geisser correction. If the assumption of homogeneity of variances was violated, p-values were adjusted (equal variances not assumed).

To rule out whether measures of MVC peak force, rmsEMG, or CSE changed over subsequent trials (trial 1 to trial 6), separate one-way repeated-measures analyses of variance (ANOVAs) with the factor TRIAL (6 levels) were conducted on all data independently for internal and external conditions, as well as stimulation and no-stimulation sessions. This test was used to guide subsequent analyses in terms of whether trials were pooled or tested separately. For MVC peak force, the main effect of TRIAL was statistically significant in all cases ($F_{(5, 50)} \geq 3.982$, $p \leq 0.022$). Similarly, with reference to rmsEMG data, the main effect of TRIAL was statistically significant in most cases ($F_{(5, 50)} \leq 6.690$, $p \geq 0001$). However, regarding CSE, the main effect of TRIAL was not statistically significant in any case ($F_{(5, 45)} \leq 2.137$, $p \geq 0.150$). Consequently, in main statistical tests, TRIAL was considered a separate factor for MVC peak force and rmsEMG data, whereas all levels of the factor TRIAL were pooled for CSE.

For main statistical tests, repeated-measures ANOVAs and paired-samples t-tests were used, with designs depending on the result of the above one-way repeated-measures ANOVAs. Peak force measurements from MVCs were compared across trials (trial 1 to trial 6), conditions (external cued condition and internal cued condition), and sessions (no-stimulation and stimulation) using a $6 \times 2 \times 2$ three-way repeated-measures ANOVA with the factors TRIAL, CONDITION, and SESSION, respectively. Due to variability in EMG recordings, raw rmsEMG values for biceps brachii and triceps brachii were examined separately for each session (no-stimulation and stimulation) across trials (trial 1 to trial 6) and conditions (external cued condition and internal cued condition) using 2×2 two-way repeated measures ANOVAs with the factors TRIAL and CONDITION, respectively, given they were not normalized [33]. Because triceps brachii/biceps brachii co-activation values were normalized, they were compared as square root transformed values across trials (trial 1 to trial 6), conditions (external cued condition and internal cued condition), and sessions (no-stimulation and stimulation) using separate $6 \times 2 \times 2$ three-way repeated measures ANOVAs with the factors TRIAL, CONDITION, and SESSION, respectively [33]. For CSE, average values across all trials (trial 1 to trial 6) for Mmax amplitude (mV), as well as MEP/Mmax, CMEP/Mmax, and square root transformed CMEP/MEP ratios, were compared across conditions (external cued condition and internal cued condition) using separate paired-samples t-tests. Finally, to investigate the relationship between changes in peak force and co-activation across stimulation conditions, two analyses were performed. First, a 2×2 two-way repeated-measures ANOVA with the factors CONDITION and SESSION was conducted on the percentage ratios of muscle co-activation per Newton of force calculated from MVCs from both the external and internal conditions during both no-stimulation and stimulation sessions. Last, simple bivariate correlations (Pearson's r) were calculated between changes in MVC peak force and triceps brachii/biceps brachii co-activation from external to internal cued condition in the no-stimulation and

stimulation sessions separately. Strength of the correlation coefficients (r) was interpreted as <0.3 (negligible), 0.3–0.5 (weak), 0.5–0.7 (moderate), 0.7–0.9 (strong), and > 0.9 (very strong) [39].

Statistical significance for main tests was set at $p \leq 0.05$. In the event of a statistically significant ANOVA outcome, pairwise comparisons were completed post hoc using the Bonferroni correction. In the text, tables' and figures' data are reported as mean ± SD.

3. Results

3.1. Data Distribution

All data were normally distributed (MVC: $W_{(11)}$ = 0.821–0.966, p = 0.018–0.848; rmsEMG: $W_{(11)}$ = 0.699–0.982, p = 0.001–0.976; CSE: $W_{(10)}$ = 0.684–0.934, p = 0.001–0.490), with the exception of MEP/CMEP ratio values under the internal condition of the stimulation session ($W_{(10)}$ = 0.628, p = 0.0001) and muscle co-activation (% triceps/biceps brachii rmsEMG) under the internal condition of the stimulation session ($W_{(11)}$ = 0.639–0.858, p = 0.0001–0.054). Thus, all MEP/CMEP ratio and muscle co-activation values were square root transformed using the Transform function in SPSS, resulting in normal distributions (MEP/CMEP: $W_{(10)}$ = 0.740–0.879, p = 0.003–0.126; co-activation: $W_{(11)}$ = 0.745–0.956, p = 0.002–0.715).

3.2. Peak Force

MVC peak force are shown in Figure 4, Tables 1 and 2. The three-way repeated-measures ANOVA on peak force measurements from elbow flexor MVCs revealed three statistically significant main effects. First, a statistically significant main effect of CONDITION ($F_{(1, 10)}$ = 7.033, p = 0.024) showed that force was significantly less under the internal conditions (282.4 ± 60.3 N) versus the external condition (310.7 ± 11.3 N) (Figure 4A). Next, a statistically significant main effect of SESSION ($F_{(1, 10)}$ = 6.076, p = 0.033) demonstrated that force measurements were significantly smaller during the stimulation session (279.0 ± 47.1 N) than the no-stimulation session (314.1 ± 57.5 N) (Figure 4B). Finally, there was a statistically significant main effect of TRIAL ($F_{(5, 50)}$ = 14.262, p = 0.00001) (see Table 2 for multiple comparisons). Neither the TRIAL × CONDITION ($F_{(5, 50)}$ = 1.701, p = 0.152), TRIAL × SESSION ($F_{(5, 50)}$ = 0.211, p = 0.891), CONDITION × SESSION ($F_{(5, 50)}$ = 1.365, p = 0.270), nor TRIAL × CONDITION × SESSION interactions ($F_{(5, 50)}$ = 1.344, p = 0.281) were statistically significant.

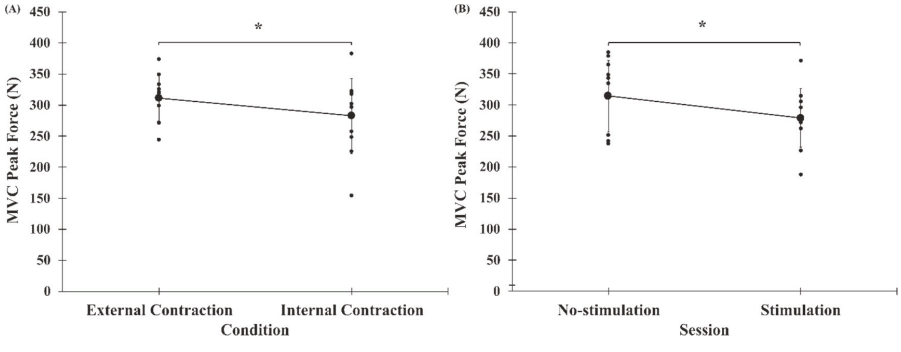

Figure 4. Peak force values for MVCs, measured in Newtons (N). Smaller points represent individual participant data, larger points represent mean, and error bars represent one standard deviation. (**A**) Peak force values for external versus internal conditions, collapsed across all trials (trial 1 to trial 6) and sessions (no-stimulation, stimulation), demonstrating the significant main effect of CONDITION. (**B**) Peak force values for no-stimulation versus stimulation session, collapsed across all trials (trial 1 to trial 6) and conditions (external condition, internal condition), signifying the significant main effect of SESSION. *, statistically significant at $p < 0.05$.

Table 1. Mean ± SD of MVC force and electromyographic (EMG) data, collapsed across trials (trial 1 to trial 6), presented for conditions (external cue, internal cue) during both no-stimulation and stimulation sessions.

	No-Stimulation		Stimulation	
	External Cue (Range)	**Internal Cue (Range)**	**External Cue (Range)**	**Internal Cue (Range)**
MVC Peak Force (N)	333.5 ± 43.7, (242.6–375.5)	294.8 ± 76.7, (184.2–398.6)	287.9 ± 38.7, (245.3–376.0)	270.1 ± 62.4, (124.3–367.1)
Biceps Brachii rmsEMG	0.73 ± 0.51, (0.27–1.88)	0.60 ± 0.38, (0.16–1.47)	0.59 ± 0.33, (0.26–1.41)	0.53 ± 0.25, (0.21–1.04)
Triceps Brachii rmsEMG	0.12 ± 0.03, (0.07–0.18)	0.15 ± 0.09, (0.05–0.34)	0.11 ± 0.04, (0.05–0.17)	0.12 ± 0.04, (0.05–0.17)
Co-activation (% Triceps/Biceps rmsEMG)	22.2 ± 13.8, (8.6–49.6)	31.5 ± 19.5, (12.1–77.2)	24.3 ± 15.7, (11.3–63.0)	26.8 ± 17.5, (12.7–74.4)
% Co-activation per Newton Force	0.06 ± 0.04, (0.03–0.14)	0.12 ± 0.08, (0.03–0.24)	0.09 ± 0.06, (0.03–0.23)	0.11 ± 0.08, (0.03–0.29)

N: Newton, rmsEMG: Root mean square of EMG signal.

Table 2. Mean ± SD of MVC force and electromyographic data, collapsed across sessions (no-stimulation, stimulation) and conditions (external condition, internal condition), for MVC trial 1 to trial 6.

		1 (Range)	2 (Range)	3 (Range)	4 (Range)	5 (Range)	6 (Range)
MVC Peak Force (N)		313.8 ± 45.8, (229.9–373.8) ^{d,e}	304.7 ± 42.1, (224.4–372.2) ^{d,e}	308.1 ± 36.2, (258.9–377.7) ^{d,e}	276.0 ± 34.1, (227.5–336.1) ^{a,b,c}	284.0 ± 30.4, (23.4–320.9) ^{a,b,c}	288.6 ± 34.8, (219.4–335.6)
Biceps Brachii rmsEMG	**S**	0.72 ± 0.46, (0.25–1.83)	0.70 ± 0.46, (0.22–1.76) ^{e}	0.69 ± 0.45, (0.21–1.71) ^{d}	0.64 ± 0.43, (0.21–1.59) ^{c}	0.64 ± 0.45, (0.23–1.67) ^{b}	0.61 ± 0.40, (0.17–1.50)
	NS	0.63 ± 0.28, (0.31–1.10)	0.57 ± 0.31, (0.18–1.27)	0.58 ± 0.33, (0.20–1.38)	0.53 ± 0.33, (0.18–1.35)	0.52 ± 0.26, (0.21–1.14)	0.53 ± 0.27, (0.20–1.15)
Triceps Brachii rmsEMG (mV·s)	**S**	0.14 ± 0.05, (0.07–0.23)	0.15 ± 0.06, (0.07–0.26)	0.14 ± 0.06, (0.06–0.25)	0.14 ± 0.08, (0.06–0.35)	0.13 ± 0.06 (0.06–0.25)	0.12 ± 0.05, (0.06–0.20)
	NS	0.12 ± 0.04, (0.05–0.16)	0.11 ± 0.04, (0.05–0.17)	0.12 ± 0.04, (0.06–0.17)	0.11 ± 0.04, (0.05–0.18)	0.12 ± 0.05, (0.05–0.19)	0.11 ± 0.04, (0.05–0.16)
Co-activation (%Triceps/Biceps rmsEMG) (mV·s)		23.9 ± 9.8, (12.8–40.5)	27.0 ± 13.4, (13.1–53.9)	25.5 ± 12.1, (12.3–48.7)	27.6 ± 12.6, (12.1–55.8)	26.8 ± 12.9, (12.5–54.5)	26.3 ± 14.2, (11.8–55.9)
%Co-activation per Newton Force		0.08 ± 0.03, (0.03–0.13)	0.09 ± 0.04, (0.04–0.16)	0.08 ± 0.04, (0.03–0.15)	0.10 ± 0.04, (0.04–0.18)	0.09 ± 0.04, (0.04–0.17)	0.09 ± 0.05, (0.04–0.18)

N: Newton, rmsEMG: Root mean square of EMG signal, S: Stimulation, NS: No stimulation. ^{a}, statistically significant difference versus trial 1, $p < 0.05$. ^{b}, statistically significant difference versus trial 2, $p < 0.05$. ^{c}, statistically significant difference versus trial 3, $p < 0.05$. ^{d}, statistically significant difference versus trial 4, $p < 0.05$. ^{e}, statistically significant difference versus trial 5, $p < 0.05$.

3.3. Electromyography (EMG)

Biceps brachii and triceps brachii rmsEMG data are displayed in Tables 1 and 2.

3.3.1. Biceps Brachii

No-stimulation session. For biceps brachii rmsEMG during the no-stimulation session there was a statistically significant main effect of TRIAL ($F_{(5, 50)} = 7.341$, $p = 0.001$) (see Table 2 for multiple comparisons). The main effect of CONDITION trended towards significance ($F_{(1, 10)} = 3.958$, $p = 0.075$) and indicated that biceps brachii rmsEMG tended to be greater under the external condition (0.73 ± 0.51% MVC) compared to internal condition (0.60 ± 0.38% MVC). The TRIAL × CONDITION interaction effect was not statistically significant ($F_{(5, 50)} = 1.83$, $p = 0.133$) (Table 2).

Stimulation session. During the stimulation session, the main effect of TRIAL trended towards significance ($F_{(5, 50)} = 3.317$, $p = 0.068$) and suggested that rmsEMG tended to be greater under trial 3 (0.58 ± 0.33% MVC) versus trial 4 (0.53 ± 0.33% MVC) (see Table 2 for multiple comparisons). Otherwise, there was neither a statistically significant main effect of CONDITION ($F_{(1, 10)} = 2.407$, $p = 0.152$) nor TRIAL × CONDITION interaction effect ($F_{(5, 50)} = 0.506$, $p = 0.565$) (Table 2).

3.3.2. Triceps Brachii

No-stimulation session. With reference to triceps brachii rmsEMG throughout the no-stimulation session, there were no statistically significant main effects of TRIAL ($F_{(5, 50)}$ = 1.722, p = 0.210) or CONDITION ($F_{(1, 10)}$ = 2.178, p = 0.171), nor a two-way TRIAL × CONDITION interaction effect ($F_{(5, 50)}$ = 0.510, p = 0.528).

Stimulation session. In the stimulation session, there were no statistically significant effects of TRIAL ($F_{(5, 50)}$ = 1.443, p = 0.226), CONDITION ($F_{(1, 10)}$ = 0.141, p = 0.716), or TRIAL × CONDITION ($F_{(5, 50)}$ = 0.642, p = 0.583), for triceps brachii rmsEMG.

3.3.3. Co-Activation

Muscle co-activation data (expressed as % triceps/biceps rmsEMG) are shown in Figure 5A and Tables 1 and 2. The three-way repeated-measures ANOVA on percentage values of co-activation demonstrated a statistically significant main effect of CONDITION ($F_{(1, 10)}$ = 8.438, p = 0.016), whereby muscle co-activation was significantly greater under the internal condition (26.3 ± 11.5%) versus external contraction condition (21.5 ± 9.4%) (Figure 5A).

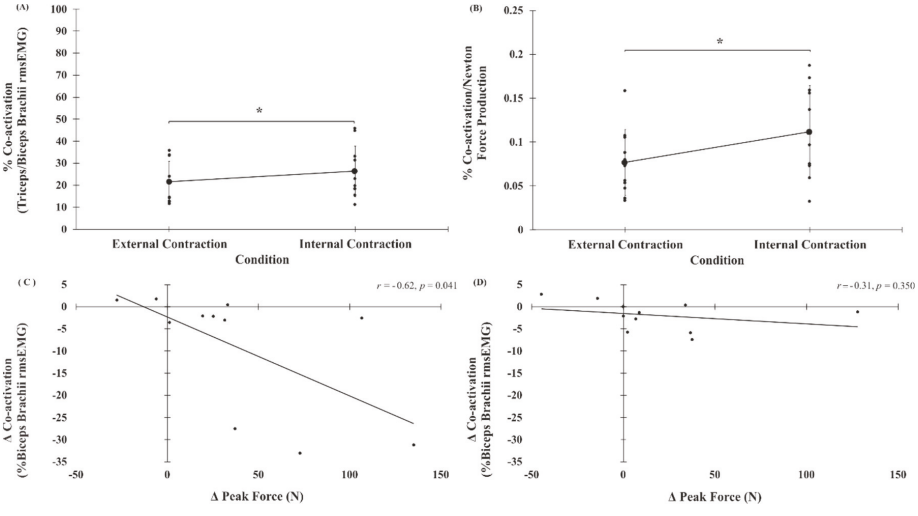

Figure 5. Data expressing the relationship between muscle co-activation and MVC peak force. In panels A–B, smaller points represent individual participant data, larger points represent mean, and error bars represent one standard deviation. In panels D–E, points represent individual data. (**A**) Muscle co-activation values for external and internal conditions, collapsed across trials (trial 1 to trial 6) and sessions (no-stimulation, stimulation), demonstrating the significant main effect of CONDITION. (**B**) Percentage of muscle co-activation/MVC peak force (co-activation per Newton force production) for external versus internal conditions, collapsed across session (no-stimulation, stimulation), illustrating the significant main effect of CONDITION. (C-D) Scatterplots demonstrating relationship between changes in MVC peak force and triceps brachii/biceps brachii co-activation across external and internal conditions during the (**C**) no-stimulation and (**D**) stimulation sessions. *, statistically significant at $p < 0.05$.

The main effects of TRIAL ($F_{(5, 50)}$ = 2.123, p = 0.136) and SESSION ($F_{(1, 10)}$ = 0.029, p = 0.869) were not statistically significant. Likewise, neither the TRIAL × CONDITION ($F_{(5, 50)}$ = 0.175, p = 0.971), TRIAL × SESSION ($F_{(5, 50)}$ = 0.419, p = 0.833), CONDITION × SESSION ($F_{(5, 50)}$ = 1.969, p = 0.191), nor TRIAL × CONDITION × SESSION ($F_{(5, 50)}$ = 2.072, p = 0.144) interaction effects were statistically significant.

3.4. Corticospinal Excitability (CSE)

CSE data are presented for each condition (external cue, internal cue), collapsed across trials (trial 1 to trial 6) in Table 3.

Table 3. Mean ± SD of the corticospinal excitability (CSE), collapsed across trials (trial 1 to trial 6), for external and internal contraction conditions. Data presented as mean (M), standard deviation (SD), and range.

	External Cue (Range)	Internal Cue (Range)
Mmax Amplitude (mV)	8.62 ± 4.97, (3.0–20.1)	8.48 ± 5.0, (1.63–17.47)
MEP Amplitude (Ratio of Mmax)	0.83 ± 0.36, (0.48–1.65)	1.01 ± 0.78, (0.48–2.95)
CMEP Amplitude (Ratio of Mmax)	0.64 ± 0.38, (0.30–1.76)	0.79 ± 0.71, (0.23–2.20)
MEP/CMEP Ratio	1.64 ± 0.99, (0.92–3.64)	1.97 ± 1.77, (0.82–6.44)

Mmax: Maximal compound motor unit action potential, MEP: motor evoked potential, CMEP: Cervicomedullary MEP.

There was no statistically significant difference ($t_{(9)} = -0.508$, $p = 0.624$; $t_{(9)} = 0.598$, $p = 0.565$; $t_{(9)} = 0.340$, $p = 0.742$; and $t_{(9)} = -1.215$, $p = 0.255$) in Mmax, MEP, or CMEP amplitudes or MEP/CMEP ratios, respectively across external cued condition and internal cued conditions. A sample of MEP, CMEP, and M-wave responses during stimulation is presented Figure 2.

3.5. Co-Activation/MVC Peak Force

There was a statistically significant main effect of CONDITION for ratios of co-activation/Newton force produced in MVCs ($F_{(1, 10)} = 11.307$, $p = 0.007$), which indicated that under the external condition (0.08 ± 0.04%) less muscle co-activation occurred per Newton of force production compared to the internal condition (0.11 ± 0.05%; $p = 0.007$) (Figure 5B). Neither the main effect of SESSION ($F_{(1, 10)} = 0.131$, $p = 0.725$) nor the CONDITION × SESSION two-way interaction effect ($F_{(5, 50)} = 1.333$, $p = 0.275$) reached statistical significance.

3.6. Relationship between Change in Peak Force and Co-Activation

Values of percent muscle co-activation per Newton of force production in MVCs, and correlations between changes in MVC peak force and triceps brachii/biceps brachii co-activation, are shown in Figure 5B–D and Tables 1 and 2.

Correlations

No-stimulation session. During the no-stimulation session there was a statistically significant negative correlation between changes in MVC peak force (38.8 ± 48.6 N) and triceps brachii/biceps brachii co-activation (−9.2 ± 13.9%) across external and internal conditions ($r_{(9)} = -0.623$, $p = 0.041$, moderate correlation), suggesting increased co-activation was related to reduced MVC force production in the internal condition (Figure 5C).

Stimulation session. In the stimulation session the relationship between changes across external and internal conditions in MVC peak force and triceps brachii/biceps brachii co-activation was not present ($r_{(9)} = -0.312$, $p = 0.350$, weak correlation) (Figure 5D).

4. Discussion

The purpose of this study was to examine if consistent superior motor performance observed with an external, compared with an internal focus instruction, is mediated by different corticospinal excitability processes. We observed three key findings. First, consistent with the literature, force production was greater with external focus instructions. Second, the greater force outputs were accompanied with lower co-contraction ratios between the biceps and triceps brachii (measured as

rmsEMG Triceps Brachii/rmsEMG Biceps Brachii) under the external focus condition, leading to a more effective contractions strategy. Third, the neuromuscular strategy identified with the EMG patterns did not coincide with a change in corticospinal excitability. This finding likely stems from an interaction between the stimulation techniques for measuring CSE and attentional focus. We speculate that the stimulation negated the effect of an external focused cue. This assumption is supported by the higher forces produced under the external focus condition in the non-stimulation session.

Maximal elbow flexor force is affected by the type of attentional focus cue. Participants were able to produce more force when provided an external focus cue (310.7 ± 11.3 N) compared to internal cue (282.4 ± 60.3 N) condition during the non-stimulation session. This is consistent with previous research which showed enhanced force production when given an external cue over no cue and internal focus cues. For example, Marchant et al. (2009) found that during concentric elbow flexion completed at a set speed, an external cue led to a higher peak net torque (102.10 ± 2.42% MVC) than the internal condition (95.33 ± 2.08% MVC) [6]. Halperin et al. (2016) reiterated these results showing that when given an external focus cue during an isometric mid-thigh pull, trained athletes applied 9% more force compared to those that received an internal cue, and 5% more force than control [40]. This supports that external focus cues enhance force output compared to internal ones.

While there was an observed difference in force production between conditions in the non-stimulation session, there were no significant changes in force production between conditions during the stimulation session. This finding is not consistent with previous research as it is well documented that attentional focus alters force production [1,5–7,40], which can be accounted for by a number of possible reasons. First, the stimulation may have distracted the participants from focusing on the provided cue. For example, the stimulation may have caused discomfort, led to fear, or attracted interest, all of which could distract participants from the provided instruction. Additionally, the notion that the stimulations are provided at random intervals may channeled their attention to anticipation, amplifying the distraction from the cues. Second, the use of the stimulation techniques disrupted areas of the cortex responsible for attention. It is known that transcranial magnetic stimulation (TMS) can disrupt cortical function. For example, Ashbridge et al. (1997) suggested that TMS disrupts an area in the front parietal lobe responsible for the focal attention necessary for feature binding in a conjunction search task [41]. Another study showed that repetitive TMS of the intraparietal sulcus and the frontal eye fields during an auditory spatial attention task impaired visually cued auditory attention [42]. With each stimulation pulse, it is possible that more than just the cortical area of interest was being stimulated [43], and therefore it is possible that cortical areas involved in attention were unintentionally disrupted. It has been studied that attentional focus potentiate short-term plasticity in the motor cortex through the premotor-to-motor connection, which is why TMS stimulation of the motor cortex disrupts attentional focus [44]. Either of the mentioned reasons, or a combination thereof would confound the effects of attentional focus instructions on force production and explain the differences between sessions.

Moreover, force produced in the stimulation session (279.0 ± 47.1 N) was lower compared to the non-stimulation session (314.1 ± 57.5 N), collapsed across the two instruction conditions. This finding is aligned with Button and Behm [45], who showed that the expectation of an interpolated twitch stimulation reduced voluntary force production by 9.5%. However, to date there appears to be a lack of research showing how stimulation of the nervous system using TMS and TMES influences force production. This result should be replicated and expanded upon in future studies as it implies that the use of stimulations could confound a study involving force production

Mechanisms underlying changes in elbow flexor maximal force with attentional focus cues.

Electromyography. Our results showed greater co-activation with an internal compared to an external cue. This is consistent with a previous study by Lohse et al. (2011), who reported greater co-contraction between the lateral aspect of the soleus and the tibialis anterior with an internal focus cue during a submaximal plantar flexion task [46]. Greater co-activation of the agonist and antagonist musculature is a possible mechanism underpinning why maximal force production is

lower during internal focused cues. Based on the current EMG findings, and aligned with Wulf's [1] "Constrained action hypothesis", it appears that force production is impaired with an internal cue due to disruption of natural automatized movement as supported by increases in co-activation by increasing the antagonist and decreasing the agonist muscle activity, whereas the greater EMG pattern of the agonists observed during the external cue could be a result of greater motor unit recruitment and/or rate coding. In contrast to some studies, we did not find a significant difference in EMG activity between focus cues. However, a large, albeit not statistically significant, effect was observed in which greater neuromuscular activity of the biceps brachii was associated with an external focus cue, suggesting that external cue leads to greater motor unit recruitment and/or rate coding.

Corticospinal Excitability. Corticospinal tract output can be altered by multiple variables, such as exercise, injury, disuse and disease and potentially attentional cues. As force output is increased, both supraspinal and spinal excitability also increased illustrating that changes in the excitability of cortical neurons and/or spinal motoneurons are occurring [26,27]. Measures of corticospinal excitability, specifically supraspinal and spinal excitability were used during one of the two sessions (stimulation session). This allowed us to determine whether or not the increase in maximal elbow flexor force with an external focus cue was due, in part, to enhanced corticospinal excitability at the supraspinal or spinal level(s) or combination thereof, of the biceps brachii. We expected to see an increase or change in corticospinal excitability at the supraspinal or spinal level(s) of the biceps brachii with an external focus cue as increased central drive is a well-known mechanism underlying increases in force production [26,27,34]. However, we were unable to support this possibility with the current study due to a lack of differences in corticospinal excitability responses of the biceps brachii during elbow flexor MVCs when receiving external versus internal focus cues. There are a couple technical considerations that must be noted in relation to the measurement of corticospinal excitability. First, because MEP [26,47] and CMEP [26] amplitudes are dependent on background EMG during isometric contractions and EMG was different between conditions (i.e., attentional cues), it is possible that the MEPs and CMEPs between conditions may not be comparable. Second, MEP and CMEP amplitudes were matched during a 5% MVC to equal 10%–20% of Mmax. The motor output and activation of cortical neurons and spinal motoneurons required to produce a 5% MVC is different than 100% MVC. Because the same stimulation intensities were used during a 5% and a 100% MVC, we cannot rule out that the corticospinal tract (TMS and TMES) during 100% MVC was not optimally stimulated. Thus, the MEP and CMEP responses may have been suboptimal. In fact, during 100% MVC the MEP and CMEP amplitudes were much higher than 10%–20% of Mmax amplitude. Nonetheless, force and EMG and MEP, CMEP and Mmax amplitudes of the biceps brachii were not significantly different between attentional focus cues. To our knowledge, this appears to be the only study examining corticospinal excitability and attentional focus feedback. In view of the possible reasons raised earlier that can account for the lack of effects under the stimulation condition, it seems like a worthwhile attempt to conceptually replicate the current study with participants who are more experienced with isometric contractions and corticospinal excitability techniques, which may reduce its distracting effects, and thus possibly lead to different results.

5. Conclusions

In conclusion, force production during a MVC of the elbow flexors followed the known pattern in which external cue leads to superior performance compared to internal cue. This finding was accompanied by greater co-activation of the triceps brachii and biceps brachii which appear to be an underlying mechanism for this impairment. Interestingly, and in contrast to the non-stimulation sessions, the use of stimulation techniques impaired attention by way of distraction or impairment of certain areas in the brain, which nullified the established effects of attentional focus instruction on maximal force production.

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N.J.S.; Writing—Original Draft Preparation, S.W., S.A., I.H., B.L., N.J.S., K.E.P. and D.C.B.; Writing—Review & Editing, S.W., S.A., I.H. and D.C.B.; Visualization, S.W., S.A., I.H., N.J.S., K.E.P. and D.C.B.; Supervision, D.C.B.; Project Administration, D.C.B.; Funding Acquisition, D.C.B. All authors have read and agreed to the published version of the manuscript.

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The Inhibitory Tendon-Evoked Reflex Is Increased in the Torque-Enhanced State Following Active Lengthening Compared to a Purely Isometric Contraction

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Abstract: Residual torque enhancement (rTE) is a history-dependent property of muscle, which results in an increase in steady-state isometric torque production following an active lengthening contraction as compared to a purely isometric (ISO) contraction at the same muscle length and level of activation. Once thought to be only an intrinsic property of muscle, recent evidence during voluntary contractions indicates a neuromechanical coupling between motor neuron excitability and the contractile state of the muscle. However, the mechanism by which this occurs has yet to be elucidated. The purpose of this study was to investigate inhibition arising from tendon-mediated feedback (e.g., Golgi tendon organ; GTO) through tendon electrical stimulation (TStim) in the ISO and rTE states during activation-matching and torque-matching tasks. Fourteen male participants (22 ± 2 years) performed 10 activation-matching contractions at 40% of their maximum tibialis anterior electromyography amplitude (5 ISO/5 rTE) and 10 torque-matching contractions at 40% of their maximum dorsiflexion torque (5 ISO/5 rTE). During both tasks, 10 TStim were delivered during the isometric steady state of all contractions, and the resulting tendon-evoked inhibitory reflexes were averaged and analyzed. Reflex amplitude increased by ~23% in the rTE state compared to the ISO state for the activation-matching task, and no differences were detected for the torque-matching task. The current data indicate an important relationship between afferent feedback in the torque-enhanced state and voluntary control of submaximal contractions. The history-dependent properties of muscle is likely to alter motor neuron excitability through modifications in tension- or torque-mediated afferent feedback arising from the tendon.

Keywords: electromyography; history dependence of force; residual force enhancement; eccentric; golgi tendon organ; afferent

1. Introduction

Residual torque enhancement (rTE) is a history-dependent muscle property that results in an increase in steady-state isometric (ISO) torque production following an active lengthening contraction as compared to a purely isometric contraction at the same muscle length and level of activation [1–3]. The presence of rTE has been demonstrated in vitro, from the level of the sarcomere [4] to whole muscle preparations [5], and at the single muscle fiber level [6] to the whole muscle level, via electrical stimulation [7,8] and submaximal and maximal voluntary isometric contractions (MVCs) in humans [2,9,10]. Although numerous reports have focused on the basic mechanical mechanisms of rTE [5,11–14], the neural consequences of rTE have been largely underappreciated. Recent studies

have reported that, during voluntary contractions, rTE may be linked to excitability modifications within the corticospinal pathway [9,15]. For example, rTE led to an increase in corticospinal excitability during plantar flexion MVCs [15] and a decrease in spinal excitability during submaximal steady-state dorsiflexion contractions [15]. However, it is not clear what factors may be modulating these changes in excitability.

Sypkes et al. [15] proposed that reduced spinal excitability during a condition of enhanced muscle force production capacity may be corresponding to greater inhibition of the agonist motor neuron pool arising from tendon-mediated feedback or, more specifically, the Golgi tendon organ (GTO) [15]. The GTOs are located in series with extrafusal muscle fibers at the interface of the musculotendinous junction (MTJ) and act to sense tension produced by its corresponding activated muscle [16–18]. Rising muscle tension increases the firing of the Ib afferent neurons that project from the GTO to a variety of targets within the central nervous system [17,19], including inhibitory interneurons synapsing onto the agonist motor neuron pool [20,21]. An effective mode to assess the efficacy of inhibitory pathways onto the motor neuron pool is using tendon electrical stimulation (TStim) to elicit a short latency reflex [22,23]. Applying TStim during times of increased muscle tension (i.e., rTE state) may elicit increased Ib inhibitory reflex parameters [22,24,25]. Recently, using this technique, we have shown that the tendon-evoked inhibitory reflex is reduced in the shortening-induced residual torque depressed state [23]. The opposite may be true for a condition of enhanced torque production (e.g., rTE). For a review on residual torque depression, please see Chen et al. [26].

Residual torque enhancement can be measured as the increase in torque following active lengthening in the isometric steady-state phase compared to purely ISO contractions during activation-matched tasks (i.e., electromyography), and it has been observed during both submaximal and maximal voluntary activation [27–29]. It is proposed that rTE occurs due to a stiffening and shortening of titin's free spring length in the presence of calcium and cross-bridge cycling [27], effectively increasing the contribution of passive force to total force following active lengthening. Therefore, while matching torque, the increase in passive tension associated with rTE [5,30] requires less muscle activation as compared with a purely ISO contraction [28,29]. This activation reduction is regularly measured through a decrease in electromyography (EMG) and has been observed in both submaximally and maximally activated contractions [3,7,28,31]. These two methods of evaluating rTE—activation matching and torque matching—provide a unique approach to investigate the role of tension-mediated afferents on previously observed reductions in spinal excitability.

The purpose of this study was to determine how the tendon-evoked inhibitory reflex is modified between the rTE and ISO states and to discuss its implications in the reduction of motor neuron pool excitability in the rTE state. During activation-matched ISO and rTE contractions, muscle tension in the isometric steady state is expected to be greater, whereas muscle tension is expected to be equivalent during torque-matched ISO and rTE contractions. If previously observed reductions in agonist motor neuron pool excitability during rTE contractions are in fact modulated from tendon-mediated feedback, we hypothesize that increases in TStim-elucidated reflex parameters (such as amplitude) should be apparent in rTE contractions during isometric steady state compared to purely ISO contractions for the activation-matching trials but not the torque-matching ones.

2. Materials and Methods

2.1. Participants

Fourteen healthy male participants with a mean age of 22 ± 2 years, height of 180 ± 6 cm, and mass of 81.8 ± 13.2 kg with no prior history of neuromuscular disease or ankle joint injuries were recruited from the university population. Participants gave written informed consent prior to testing. All procedures were approved by the Human Research Ethics Board of the University of Guelph (REB: 15NV008).

2.2. Experimental Set Up

A HUMAC NORM dynamometer (CSMi Medical Solutions, Stoughton, MA, USA) was used to record torque, angular velocity, and position. Each participant sat with their right hip and knee angles set at 110° and 140° (180° = full extension), respectively. Joint angles were measured using a goniometer. The right knee was immobilized just proximal to the patella with the dynamometer's leg restraint preventing hip flexion and a cushion positioned beneath the distal hamstrings preventing hip extension, while movement at the torso was restricted with a four-point seatbelt harness. The right foot was fixed to the dorsi/plantar flexor adaptor with one inelastic strap secured over the ankle and another across the mid-distal portion of the metatarsals. The dynamometer's maximum ankle dorsi- and plantar flexion angles were set to 0° and 40° plantar flexion (PF; 0° = neutral), respectively, allowing for 40° of ankle excursion.

Locations for the surface EMG electrodes (Ag/AgCl, 1.5 × 1 cm: Kendall, Mansfield, MA, USA) were prepared by shaving and cleaning the skin with alcohol swabs. The active electrode was placed over the tibialis anterior (TA) approximately 7 cm inferior and 2 cm lateral to the tibial tuberosity, and a reference electrode was placed inferiorly, adjacent to the active electrode in line with the muscle fibers. To record antagonist activity, the active electrode was placed on the soleus, along the midline of the leg approximately 2 cm inferior to the border of the heads of the gastrocnemii, and a reference electrode was placed inferiorly, bordering the active electrode. A single ground electrode was positioned over the patella.

Surface EMG, torque, angular velocity, dynamometer position, and stimulus trigger data were digitized using a 12-bit analog-to-digital converter (PowerLab System 16/35, ADInstruments, Bella Vista, Australia) and analyzed with Labchart software (Labchart, Pro Modules 2014, version 8). Torque angular velocity and position as well as EMG data were recorded at a sampling rate of 1000 and 2000 Hz, respectively. The EMG data were bandpass filtered using a digital filter (10–1000 Hz).

2.3. Peripheral Nerve Stimulation

To test the voluntary activation of the dorsiflexors (see next section) and obtain compound muscle action potentials (M-waves) from the TA and soleus, peripheral nerve stimulation was delivered transcutaneously with a standard clinical bar electrode (Empi, St. Paul, MN, USA) coated in conductive gel. The deep fibular nerve, innervating the dorsiflexor muscles, was located by palpating the head of the fibula and moving posteroinferiorly until the nerve was identified. Stimulation distal to the bifurcation of the common fibular nerve was ensured in order to limit activation of the peroneal muscles. The tibial nerve was stimulated via a bar electrode positioned within the popliteal fossa to maximize the M-wave. All stimulations were delivered as a single square-wave pulse from a constant-current, high-voltage stimulator (model DS7AH, Digitimer, Welwyn Garden City, Hertfordshire, UK). Voltage and pulse width were set to a maximum of 400 V and 200 μs, respectively. The current was increased incrementally until a plateau was reached for the peak-to-peak amplitude of the resting M-wave (Mmax). To ensure consistent activation of all motor neurons throughout the experiment, the current was then increased to a supramaximal level, equivalent to 110% of that required to generate Mmax (range: 20–200 mA and 25–250 mA for deep fibular and tibial nerves, respectively). An overview of all experimental procedures is provided in Figure 1A.

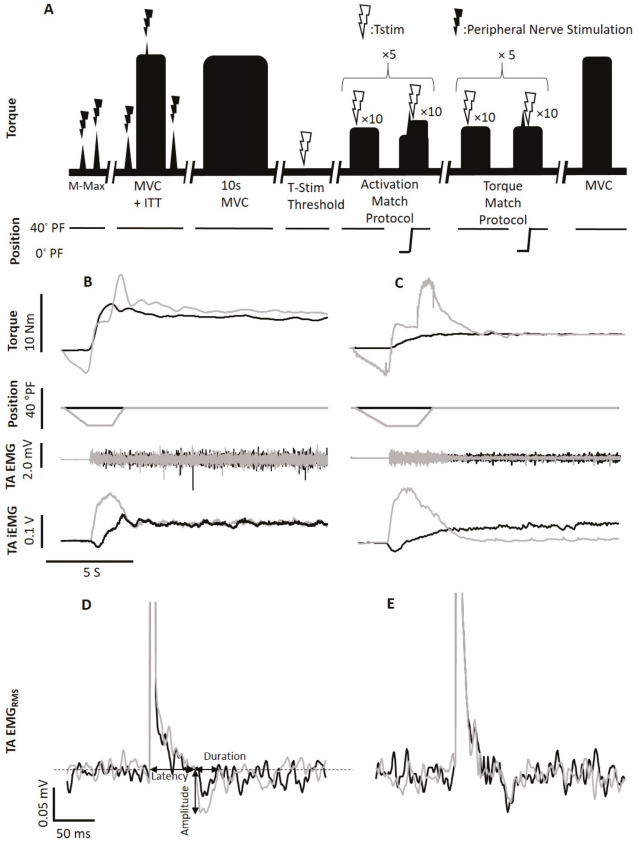

Figure 1. Timeline and raw data traces. Schematic timeline of experimental procedures (**A**). To establish maximum compound muscle action potentials (Mmax) for the tibialis anterior (TA) and soleus, peripheral nerve stimulation was performed on the deep branch of the common fibular and tibial nerves, respectively. A 10 s maximum voluntary contraction (MVC) was followed by an initial MVC performed with the interpolated twitch technique (ITT) to assess maximum torque and voluntary activation. Tendon electrical stimulation (TStim) was applied at increasing currents until perceptual threshold (PT) was found, which was defined as the minimum current intensity that induced a tingling or tapping sensation that was detectable to the participant. Stimulation intensity was then increased to 6 × PT, a current at which participants reported a muscular sensation, including a tugging or pulling at the muscular insertion, a deep tingling near the cathode, or a muscle twitch. If a visible muscle twitch was induced, stimulation intensity was reduced to the maximum current that failed to produce a visible muscle twitch. This stimulation intensity was used for all subsequent trials. Participants then performed 10 pairs of activation-matched dorsiflexion trials (**B**) consisting of an isometric (ISO) trial (black trace) followed by a residual torque-enhanced (rTE) trial (gray trace). These contractions were performed at 40 ± 5% of the participant's maximum TA activation, and 10 TStims were applied during the isometric steady state of each contraction. Participants then performed 10 pairs of torque-matched dorsiflexion trials (**C**) consisting of an ISO trial (black trace) followed by an rTE trial (gray trace). These contractions were performed at 40% of the participants maximum dorsiflexion torque, and 10 TStims were applied during the isometric steady state of each contraction to evoke an inhibitory reflex (activation-matched trial (**D**), torque-matched trial (**E**)). Lastly, participants were instructed to perform a final MVC to assess for fatigue. A resting period of 5 min was given to participants after all contractions, and each contraction was performed with visual feedback as well as verbal encouragement.

2.4. Maximum Voluntary Contraction and Voluntary Activation

Voluntary activation of the dorsiflexors was assessed during brief MVCs (~5 s) performed twice, separated by 4 min of rest both prior to and following the experimental trials. The interpolated twitch technique was used to evaluate voluntary activation [32]. The torque resulting from peripheral nerve stimulation delivered during the plateau phase of the MVC was compared to a resting twitch evoked 1–2 s after relaxation. The level of voluntary activation was calculated as follows: voluntary activation (%) = [1 −(interpolated twitch torque/resting twitch torque)] × 100%. Participants were encouraged verbally and provided visual feedback of torque output during all MVC attempts [33]. All participants were required to reach a minimum of 95% voluntary activation in order to be included in the study and were given five minutes of rest following the qualifying MVCs before continuing with the experiment.

2.5. Determining Submaximal Muscle Activation

To determine the submaximal integrated EMG (iEMG) and torque targets, participants were instructed to perform a 10 s dorsiflexion MVC at an ankle angle of 40° PF. The average iEMG and torque collected between 6 and 8 s was then used to determine the 40% submaximal iEMG and torque targets [23] (Figure 1B). For activation-matched contractions, a ± 5% window was calculated about the 40% iEMG target, and participants were instructed to maintain their iEMG amplitude within set guidelines marking this target window [23].

2.6. Tendon Electrical Stimulation

Percutaneous TStim was used to induce tendon-evoked inhibitory agonist reflexes. This technique involved percutaneous square-wave electrical stimulation of the tendon near the MTJ and evoked a short-latency (<50 ms) reflexive inhibition in the agonist muscle. This reflex has been demonstrated for several upper and lower limb muscles [22–25] and is thought to be mediated via Ib spinal pathways owing to the short latency and the polarity of the response, which is consistent with Ib autogenic inhibition [22,23]. However, contributions from other sources, such as muscle or tendon type III afferents, cannot be ruled out completely [25]. Still, non-GTO origins of TStim, specifically cutaneous receptors overlying the tendon or muscle stimulation via current spread, were excluded in prior studies [24]. Previous reports [22,34] have demonstrated that indwelling electrical stimulation of the tendon evokes an inhibitory response with similar characteristics as percutaneous stimulation, indicating that the percutaneous tendon-evoked reflex technique used here is most likely tension-mediated via GTO afferents. Ag/AgCl electrodes (1.5 × 1 cm: Kendall, Mansfield, MA, USA) were used for TStim in order to generate tendon-evoked inhibitory reflexes. The cathode was placed near the MTJ of the TA, and the anode was placed over the distal tendon at the level of the malleoli [23]. Single stimuli were presented with a constant-current, high-voltage stimulator (DS7AH). Voltage was set to a maximum of 400 V and pulse width to 200 μs. The stimulation protocol was initiated with the detection of perceptual threshold (PT), which was defined as the minimum current intensity that induced a tingling or tapping sensation that was detectable to the participant (6.63 ± 2.13 mA) [23]. Stimulation intensity was then increased to 6 × PT, a current at which participants have reported a muscular sensation, including a tugging or pulling at the muscular insertion, a deep tingling near the cathode, or a muscle twitch [9,23]. If a visible muscle twitch was induced, stimulation intensity was reduced to the maximum current that failed to produce a visible muscle twitch [23]. This stimulation intensity was used for all subsequent trials (5.2 ± 0.90 × PT).

2.7. Experimental Procedures

Each rTE trial was preceded by an ISO trial, and protocol A was followed by protocol B. Five rTE trials and five ISO trials were performed for each of the two protocols for a total of 20 contractions. Participants were provided visual feedback of the iEMG and torque amplitudes on a computer monitor

and were verbally encouraged to match the target as closely as possible during all submaximal contractions. Four minutes of rest separated all submaximal contractions.

2.8. Protocol "A": Activation-Matching Condition

For each rTE trial, the protocol consisted of a 40% iEMG contraction involving a 2 s isometric phase at an ankle angle of 90°, a 1 s isokinetic lengthening phase (angular velocity: 40°/s) and ~20 s isometric phase at 40° PF (Figure 1B). A series of 10 TStim pulses were delivered at random 1–4 s intervals [9,23] during the isometric phase at 40° PF. During the ISO trials, an isometric dorsiflexion contraction corresponding to 40% iEMG was performed for ~23 s at an ankle angle of 40° PF, with a similar pattern of stimuli delivered as described for the rTE trials.

2.9. Protocol "B": Torque-Matching Condition

For each rTE and ISO trial, participants were instructed to maintain 40% MVC torque. The movement and stimulation protocols were identical to those of protocol A (Figure 1C).

2.10. Data Analysis and Statistics

Mean torque from each protocol contraction was calculated from 500 ms prior to the first stimulation to the end of each contraction [23]. Root mean squared EMG (EMG_{RMS}) amplitude was calculated in a 500 ms window that occurred between 6 and 8 s after contraction initiation following the achievement of an isometric steady state. It was ensured that the window selected was matched between corresponding rTE and ISO trials [23]. The EMG_{RMS} of the resting Mmax recorded at the TA and soleus was used to normalize the voluntary TA and soleus EMG, respectively. A paired *t*-test was performed to compare the torque and EMG data between rTE and ISO trials to validate the presence of rTE and activation reduction.

To obtain the reflex parameters, a stimulus-triggered average of the raw TA EMG_{RMS} was generated using Labchart software (Labchart, Pro Modules 2014, version 8). For each protocol, separate rTE and ISO averages were constructed from all stimuli delivered in each condition; therefore, each average was composed of 50 stimuli delivered over 5 contractions (Figure 1D,E). A paired *t*-test was performed to compare reflex characteristics between the rTE and ISO states, including latency, duration, amplitude, and change in average EMG_{RMS} from baseline, in order to characterize changes in the tendon-evoked inhibitory reflex in the rTE and AR state [23]. Baseline was measured as the average EMG_{RMS} in a 300–500 ms window occurring before TStim, and the onset of the stimulation artifact was defined as 0 s. Latency was measured as the time from the initiation of the stimulus artifact to the sharp decrease in baseline EMG_{RMS} occurring at the start of the reflex. Duration was measured as the time occurring from the initial decrease in EMG_{RMS} from baseline at the start of the reflex to when baseline EMG_{RMS} was once again reached. Amplitude was measured as the magnitude of EMG_{RMS} from baseline to the lowest trough in the reflex [23].

Change in average EMG_{RMS} from baseline was measured as the difference between baseline EMG_{RMS} and the average EMG_{RMS} from the duration of the reflex. Paired *t*-tests were also used to detect any differences in the torque produced during MVCs performed before and after the experiment in order to assess any effects of fatigue during the experimental protocol. Descriptive data found in text are reported as means ± standard deviation, while data presented in figures are reported as means ± standard error of the mean. Significance was determined based on $\alpha < 0.05$.

3. Results

3.1. Maximum Voluntary Contraction and Voluntary Activation

The mean pretrial MVC torque was 27.6 ± 6.4 Nm, and all participants were capable of achieving near-maximal values for voluntary activation (98.9 ± 1.5%). Following the 20 submaximal contractions, MVC torque was not different from the pretrial values (27.1 ± 6.6 Nm).

3.2. Dorsiflexion Torque and Muscle Activity

3.2.1. Activation Matching

Normalized EMG_{RMS} of both TA ($p = 0.8$) and soleus ($p = 0.6$) were not different between rTE and ISO contractions (Figure 2B,C). Following active lengthening, steady-state isometric torque was 15.0 ± 9.7% ($p < 0.0001$) greater than that produced during the purely isometric contractions at the corresponding muscle length and level of activation (Figure 2A). Participants successfully maintained the EMG target level such that iEMG of the TA did not differ in the rTE and ISO contractions ($p = 0.1$; Figure 1B). This indicates indirectly that motor neuron output was similar in both the rTE and ISO states. For the tendon-evoked inhibitory reflex, reflex latency was not significantly different between the rTE and ISO states, with the onset of inhibition occurring at 47.8 ± 7.8 ms following TStim in rTE trials and 48.6 ± 4.8 ms following TStim in ISO trials ($p = 0.6$; Figure 2E). Further, reflex duration ($p = 0.5$; Figure 2F) and reduction in average reflex EMG_{RMS} from baseline were not different when rTE and ISO contractions were compared ($p = 0.2$). However, inhibitory reflex amplitude differed by 22.6 ± 41.8% ($p < 0.05$; Figure 2D) in the rTE state compared to the ISO state.

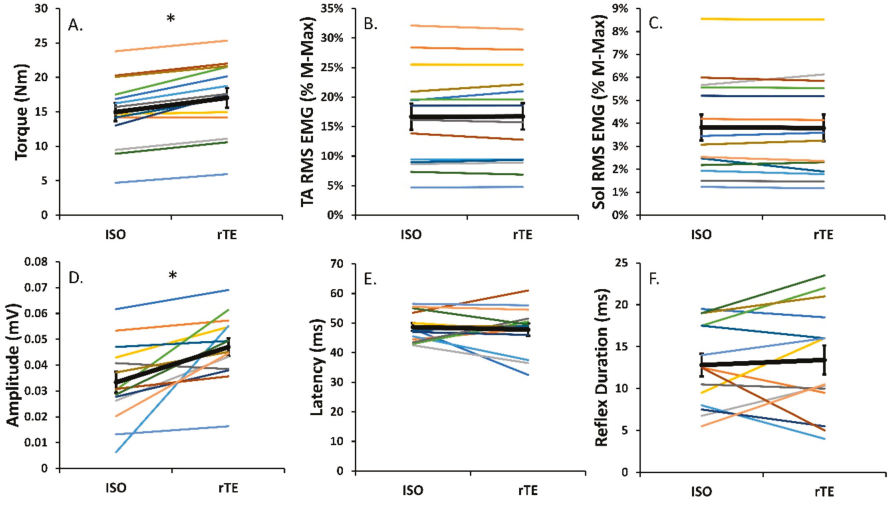

Figure 2. Activation-matching trial. Mean values for each participant across measures (colored lines) and the group mean (black line; error bars indicate standard error of the mean) in the rTE and ISO states. For the activation-matching trial, in the rTE state as compared to the ISO state, there was a 15.0% increase in torque (**A**) and a 22.6% increase in reflex amplitude (**D**) (* $p < 0.05$). There was no significant difference in EMG_{RMS} collected from the tibialis anterior (**B**) or soleus (**C**) reflex duration (**F**) or reflex latency (**E**) between the two states.

3.2.2. Torque Matching

Steady-state isometric torque was not different following active lengthening when the rTE and ISO states were compared ($p = 0.2$ Figure 3A). In the rTE state, however, there was a significant 14.4 ± 13.3% decrease in normalized EMG_{RMS} ($p < 0.01$; Figure 3B) and a 26.9 ± 23.7% decrease in iEMG ($p < 0.01$) for the TA compared to ISO contractions, with no change in antagonist coactivation (Figure 3C). For the tendon-evoked inhibitory reflex, reflex latency was not significantly different between the rTE and ISO states, with the onset of inhibition occurring at 48.0 ± 6.8 ms following TStim in rTE trials and 50.6 ± 5.6 ms following TStim in ISO trials ($p = 0.1$; Figure 3E). Further, reflex duration ($p = 0.2$; Figure 3F), reflex amplitude ($p = 0.2$; Figure 3D), and reduction in average reflex EMG_{RMS} from baseline ($p = 0.2$) were not different when rTE and ISO contractions were compared.

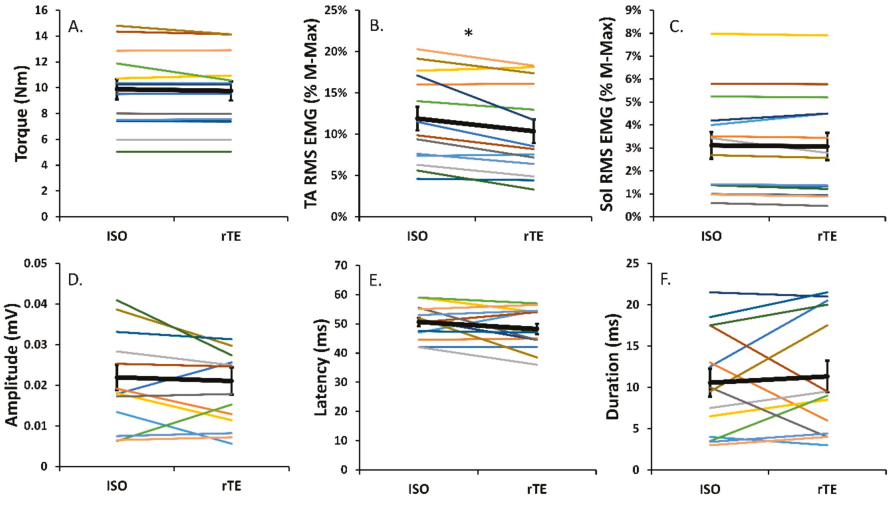

Figure 3. Torque-matching trial. Mean values for each participant across measures (colored lines) and the group mean (black line; error bars indicate standard error of the mean) in rTE and ISO states. For the torque-matching trial, in the rTE state as compared to the ISO state, as expected, there was a 14.4% decrease in TA activation (**B**) (*$p < 0.05$). There were no differences in torque (**A**), antagonist coactivation (**C**), or any reflex parameter (**D**–**F**) between the two states.

4. Discussion

The purpose of this study was to determine how the tendon-mediated inhibitory reflex is modified between the rTE and ISO states through TStim of the tibialis anterior during submaximal activation-matched and torque-matched dorsiflexions. The activation-matched task successfully elicited an ~15% increase in torque in the rTE state compared to ISO, while the torque-matching task resulted in an ~14% decrease in EMG. Our hypothesis of an increased tendon-mediated inhibitory reflex in the rTE state was supported by a 23% increase in inhibitory reflex magnitude in the rTE state compared to the ISO state during the activation-matching task but not the torque-matching task. Therefore, these results support an underlying tension-mediated factor as a plausible explanation for the previously reported decrease in agonist motor neuron pool excitability in the rTE state [15].

In the force-enhanced isomeric steady state, there is greater relative contribution of passive force to total force production, possibly owing to stiffening of the giant molecular spring titin [27]. This increase in passive tension can manifest in rTE contractions in two ways when compared to purely ISO contractions. The first is an activation reduction in torque-matching tasks in which no change in muscle tension occurs [3,29]. The second is an increase in torque, and consequently muscle tension, during activation matching [29,35]. Critical to the present study was the use of both paradigms: activation matching and torque matching. Previous reports of decreases in agonist motor neuron pool [15] and increases in corticospinal [9] excitability have been observed for submaximal and maximal rTE contractions compared to purely ISO contractions. Given the aforementioned investigations [9,15] had similar motor neuron outputs for both ISO and rTE contractions, the alteration in spinal excitability was most likely owing to peripheral sensory inputs. One source of peripheral input upon the motor neuron pool are muscle spindles. While activating their respective Ia afferents via Achilles tendon vibration, no modulatory effect on rTE was observed [35]. Therefore, a tension-mediated factor was speculated as the most likely factor driving the previously reported results [15]. Golgi tendon organs increase Ib afferent firing during periods of increased muscle tension [16] and excite inhibitory interneurons within the spinal cord [17,19]. Excitation of these inhibitory interneurons results in decreased neural output of the agonist motor neuron pool, which can be observed within the surface

EMG signal [24]. Through single pulse electrical stimulation of the TA tendon, an inhibitory reflex can be observed in the EMG_{RMS} ~50 ms following stimulation [22,24,25]. Following active lengthening, during an activation-matching task, rTE torque increased by ~15% compared to ISO, which resulted in an ~23% increase in tendon-evoked inhibitory reflex magnitude. The torque-matching task served as a control, i.e., with no changes in torque or muscle tension between rTE and ISO states, no changes in tendon-evoked reflexes should occur. This is particularly important because it ensures any changes observed in the activation-matching trials are indeed due to GTO-mediated reflexes. While there was an ~14% reduction in TA activation (normalized EMG_{RMS}), indicating the presence of rTE, negligible differences in muscle tension were detected, and as expected, there was no significant differences in the tendon-evoked inhibitory reflex across the rTE and ISO tasks. The lack of changes in reflex characteristics during the torque-matching task indicates our findings were due to a tension-mediated factor. Therefore, the tension-dependent GTO and the Ib afferent is most likely a key contributor in modulating agonist motor neuron excitability during voluntary control of submaximal contractions in the rTE state.

The results of our investigation are similar to previous reports on rTE and activation reduction. While we found ~15% increase in torque across all 40% activation-matched rTE trials in comparison to ISO, Pinniger and Cresswell [36] found a 12% increase in torque at 25% of maximally activated TA dorsiflexion. The 40% MVC torque-matching task induced an ~14% reduction in activation of the tibialis anterior EMG_{RMS}. Other studies reported a similar activation reduction ranging from 5% to 20% [28,29,31]. Additionally, the aim of this study was to elucidate a measurable change in tendon-evoked reflex parameters between ISO and rTE states, where we hypothesized an increase in inhibitory reflex parameters in the rTE state compared to the ISO during the activation-matching protocol but not the torque-matching one. In support of this hypothesis, we found an ~23% increase in inhibitory reflex magnitude in the rTE state compared to the ISO state during the activation-matching task but not the torque-matching one. Under similar premises—decreased torque production in the torque-depressed state eliciting a decrease in tendon-evoked reflex parameters—Sypkes et al. [23] found a 16% reduction in reflex magnitude. The symmetry in the findings of Sypkes et al. [23] and the present study demonstrate a clear relationship between these history-dependent properties of force and the GTO mechanoreceptor. The resulting neural reflex pathway consisting of Ib afferent neurons and spinal inhibitory interneurons provide a plausible explanation to decreased motor neuron pool excitability reported previously [15].

A limitation to the present study was the lack of randomization for the order of protocols A and B. This had the potential to change perceptual threshold and reduce stimulus efficacy throughout the testing session. However, when protocol A ISO was compared to protocol B ISO, there were no differences in reflex parameters (Reflex Latency, Reflex Duration, Reflex Magnitude; Change in TA EMG_{RMS} from Baseline). Thus, the lack of randomization does not appear to influence the results.

5. Conclusions

Residual torque enhancement, a history-dependent property of muscle, was present during submaximal activation- and torque-matching tasks. In the isometric steady state following an active lengthening contraction, there was an increase in tendon-evoked inhibitory reflex magnitude compared to purely isometric contractions for the activation-matching task but not the torque-matching task. This observation likely characterizes a tension-dependent increased contribution of tendon-mediated inhibitory feedback on the agonist motor neuron pool and as such may explain—at least partially—the documented decrease in agonist motor neuron excitability during an rTE state [15]. This study provides novel insight into the peripheral contributions of the history dependence of force and bridges the gap between the central nervous system and a property that was once thought to be purely intrinsic to muscle.

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supervision, B.H.D. and G.A.P.; writing—original draft, V.S.C., B.H.D., and G.A.P.; writing—review & editing, V.S.C., B.H.D., and G.A.P. All authors have read and agreed to the published version of the manuscript.

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The Task at Hand: Fatigue-Associated Changes in Cortical Excitability During Writing

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Abstract: Measures of corticospinal excitability (CSE) made via transcranial magnetic stimulation (TMS) depend on the task performed during stimulation. Our purpose was to determine whether fatigue-induced changes in CSE made during a conventional laboratory task (isometric finger abduction) reflect the changes measured during a natural motor task (writing). We assessed single-and paired-pulse motor evoked potentials (MEPs) recorded from the first dorsal interosseous (FDI) of 19 participants before and after a fatigue protocol (submaximal isometric contractions) on two randomized days. The fatigue protocol was identical on the two days, but the tasks used to assess CSE before and after fatigue differed. Specifically, MEPs were evoked during a writing task on one day and during isometric finger abduction to a low-level target that matched muscle activation during writing on the other day. There was greater variability in MEP amplitude ($F(1,18) = 13.55$, $p < 0.01$) during writing compared to abduction. When participants were divided into groups according to writing style (printers, $n = 8$; cursive writers, $n = 8$), a task x fatigue x style interaction was revealed for intracortical facilitation ($F(1,14) = 9.90$, $p < 0.01$), which increased by 28% after fatigue in printers but did not change in cursive writers nor during the abduction task. This study is the first to assess CSE during hand-writing. Our finding that fatigue-induced changes in intracortical facilitation depend on the motor task used during TMS, highlights the need to consider the task-dependent nature of CSE when applying results to movement outside of the laboratory.

Keywords: corticospinal excitability; task-dependent; transcranial magnetic stimulation; fatigue; writing

1. Introduction

Single-pulse transcranial magnetic stimulation (TMS) elicits motor evoked potentials (MEPs) that are recorded from the muscle of interest using surface electromyography (EMG). MEPs are used as a measure of corticospinal excitability (CSE) that reflect the excitability of the pathway from the site of stimulation to the site of recording, such that both cortical and spinal mechanisms contribute to changes in the MEP evoked using single-pulse TMS. Intracortical mechanisms that may contribute to CSE are assessed using conditioned MEPs elicited via paired-pulse TMS. For example, paired-pulses with a brief interpulse interval (1–5 msec) provide a measure of short interval intracortical inhibition (SICI), whereas longer interpulse intervals (10–15 msec) provide a measure of intracortical facilitation (ICF) [1]. One challenge associated with using single- and paired-pulse TMS to assess CSE is the variability in MEP amplitude, both within and between participants. To minimize variability, most TMS protocols are conducted with the muscle at rest or during submaximal isometric muscle contractions to standardize levels of muscle activation and control for joint position, muscle length, movement, and other factors. Although these laboratory tasks are intended to minimize variability, they do not reflect activities of daily living to which the results may be extrapolated. This is problematic because

CSE depends on the net excitability of the targeted brain region and net excitatory and inhibitory input to spinal motor neurons at the time of stimulation, all of which vary with different motor tasks and states [2–4]. Thus, the conclusions drawn from TMS studies depend on the motor task employed during stimulation and therefore may not translate to movement outside of laboratory settings.

Muscles of the hand (e.g., first dorsal interosseous (FDI) and adductor pollicis (AP)) are frequently used in studies of CSE because of the ease with which MEPs are elicited in distal muscles of the upper extremity. In such studies, MEPs are typically evoked with the hand at rest or during submaximal isometric contractions. For example, FDI MEPs are often assessed with the hand pronated with the wrist and hand secured to allow movement only at the metacarpal phalangeal joint of the index finger. Outside the lab, however, we use intrinsic muscles of the hands in a variety of positions, including power and precision grips. Measures of CSE differ between these grips. For example, FDI MEP amplitude is greater during conventional abduction tasks compared to power, pincer, or grasping tasks [5–7], whereas APB MEPs are greater during pincer tasks compared to during power tasks or at rest [8]. More direct measures made in the monkey reveal that corticospinal neurons are more active during a precision grip compared to a power grip, despite increased EMG activity in the latter [9]. This suggests that CSE is not simply related to the force of contraction, but also to the specific task. Pearce and Kidgell (2010) demonstrate that CSE is modulated when the precision required for a given task is increased [10] Furthermore, more complex tasks will require the involvement of proximal muscles for stability during finger movements. Flament and colleagues [11] compared index finger abduction to simple manual tasks including precision grip, power grip, grasping of a petri dish, and rotation of a bottle cap. Compared to isolated finger abduction, which was restricted to FDI use, all other tasks required activation of at least one additional muscle, which was speculated to contribute to the increased CSE found during the complex tasks [11].

Conventional laboratory tasks, such as matching force output to a static target displayed on a computer monitor, require very different cognitive demands compared to more natural motor tasks such as hand writing, use of a keyboard, or object manipulation. Nonetheless, there is a tendency to attribute the TMS results solely to the changes within or downstream to the primary motor cortex, and to overlook the influence of upstream cognitive processes transmitted to the primary motor cortex [12]. For example, internally guided movements, such as writing or drawing, have been shown to generate greater activation in the pre-supplementary motor area and dorsal premotor cortex as compared to externally guided movements, such as tracing [13]. Using the hand to convey language introduces additional cognitive influences on the motor system. In fact, mere observation of letters and words can alter CSE measured in the FDI when at rest [14,15]. Interestingly, this effect is specific to handwritten text, including handwritten "non-words", and is not observed with typed text [14,15], indicating that the recognition of hand-writing represents a unique cognitive demand that is conveyed to the motor system, likely via the mirror neuron system [14]. Accordingly, it is unlikely that estimates of CSE obtained during simple, isometric laboratory tasks would translate directly to CSE during more complex tasks outside the lab. In this study, we compared a hand-writing task, which represents an internally generated and complex task that is familiar and relevant outside the laboratory, to a conventional externally guided, isometric force-matching task often used in TMS research.

We wanted to determine whether changes in CSE in response to a well-studied intervention (neuromuscular fatigue) would depend on the motor task that is used to produce background muscle activity during the delivery of TMS. TMS has been used for many years to understand the role of the central nervous system in neuromuscular fatigue (for review, see [16,17]). Such studies have revealed that CSE is briefly facilitated and then undergoes a more prolonged period of depression following fatigue when MEPs are evoked in resting muscles [18–21]. Post-exercise depression is also observed after a fatigue protocol when MEPs are evoked at rest just prior to a voluntary contraction [22]. On the other hand, MEPs recover much more quickly when assessed during a strong muscle contraction after fatigue [23,24], suggesting that reductions in CSE after fatigue are overcome with sufficient voluntary drive. Given that measures of CSE depend on limb position, muscle activation level, and other aspects

of the motor task, such as different cognitive demands, we speculate that fatigue-induced changes in CSE made during a conventional laboratory task may not represent fatigue-induced changes in CSE made during the more relevant and complex task of writing. Therefore, the purpose of this study is to elicit neuromuscular fatigue and then compare fatigue-associated changes in CSE during writing to the same measures made during a conventional isometric finger abduction task. We hypothesized that CSE would be greater during the writing task compared to the simple isometric abduction task. Furthermore, we hypothesized that the effect of fatigue on CSE would depend on the motor task employed during stimulation, even when the fatigue task itself was the same.

2. Materials and Methods

2.1. Participants

Twenty participants with a mean age of 22.6 ± 1.1 were recruited from the Wilfrid Laurier University student population. This sample size was based on a power calculation made using data from an earlier study of intracortical mechanisms of fatigue from our lab [22]. All participants were right-handed as determined by the Annett Handedness Questionnaire [25]. One participant was excluded from analysis due to an inability to complete the full testing protocol, leaving 19 participants (six male). Exclusion criteria included any neurological conditions, and orthopaedic conditions or pain of the hand, wrist, or arm. The study was approved by the Wilfrid Laurier University Research Ethics Board (REB #5381), and all participants provided written informed consent prior to participating.

2.2. Experimental Design

In this repeated-measures study design, each participant attended two testing sessions occurring no less than 48 h apart and was tested at the same time on both days to decrease between session variability [26]. On one day, participants completed a writing task and on the other day, participants completed an abduction task. The order of the two days was randomized and counterbalanced between participants. On each day participants began with a short familiarization to each task. Following familiarization, participants completed 90 pre-fatigue trials of the selected task (writing or abduction), a fatigue protocol of intermittent isometric abduction contractions, and finally 90 post-fatigue trials of the same task (writing or abduction) (Figure 1).

On each testing day participants completed a familiarization protocol including the writing task, the abduction task, and maximal voluntary contractions (MVCs). The writing task included 20 trials where the participant repetitively wrote the word “name” on a iPad tablet with a Adonit Pro3 precision disc stylus pen. The word “name” was specifically selected for the writing task because it is familiar, it is a short enough word to be written within the 5-s recording frame, and because the letters n, a, m, and e do not contain ascenders or descenders that would require greater finger movement. The tablet screen presented a blank 7 × 2 cm rectangle on a series of Powerpoint slides that were refreshed every 5 s. The rectangle served as the boundaries within which the participant was asked to write the word. These writing boundaries and the refreshing of the writing “page” on the tablet before each trial, allowed the participants to maintain a constant hand position and to minimize wrist deviations between trials. The participants were allowed to place the tablet in any position and orientation on the table in front of them. This position was marked and maintained between trials, blocks, and testing days. Participants were instructed to maintain the same self-selected grip of the pen (e.g., dynamic tripod, lateral tripod, quadrupod, etc.) for the duration of the protocol. In the abduction task, TMS was applied during isometric abduction of the right index finger against a force transducer for 3 s with a 2-s rest. The target level of contraction during the abduction task was set to the average RMS amplitude of FDI EMG activity over the 20 familiarization writing trials so that the contraction intensity was matched to activity required during writing for each participant (Figure 2). This level of activity was marked with horizontal cursors (±2.5%) to allow the participant to match it using a real-time smoothed and rectified EMG biofeedback channel. The location of the force transducer was adjusted

to be in line with the self-selected graphic tablet location, such that shoulder and elbow angle were maintained between the writing and abduction tasks (Figure 3). The hand was in a pronated position for abduction with the third finger secured by a strap and wooden dowels placed on either side of the wrist. Participants then performed three isometric MVCs from which the highest was taken as maximal finger abduction force.

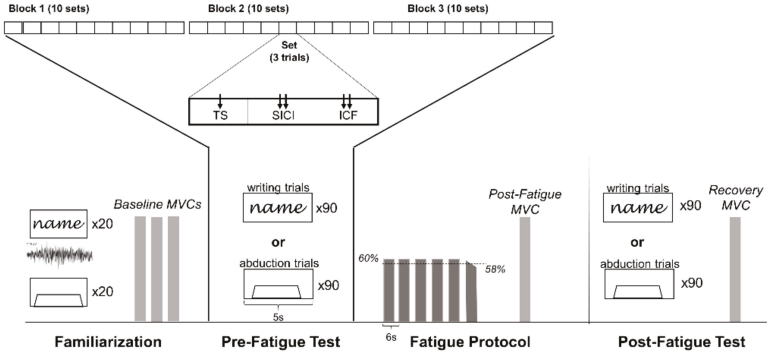

Figure 1. Each day began with a familiarization period that included 20 repetitions of each task to record muscle activation during writing, familiarizing participants with the force-tracing task, and an assessment of baseline maximal voluntary contraction (MVC). Participants then completed the Pre-Fatigue Test, which included 90 trials (abduction or writing task), followed by a Fatigue Protocol that was comprised of submaximal (60% MVC target) abduction contractions until failure (force fell below 58% MVC force for >3 s during attempts to hold the 60% target), followed by a post-fatigue MVC. The protocol ended with the Post-Fatigue Test that was identical to the Pre-Fatigue Test followed by a recovery MVC. The 90 pre-fatigue and post-fatigue trials were separated into 3 blocks of 10 sets each. Each set contained 3 randomized trials: (1) test MEP (TS), (2) short-interval intracortical inhibition (SICI) paired-pulse, and (3) intracortical facilitation (ICF) paired-pulse stimulation, resulting in pseudo-randomization of TS, SICI, and ICF across each block.

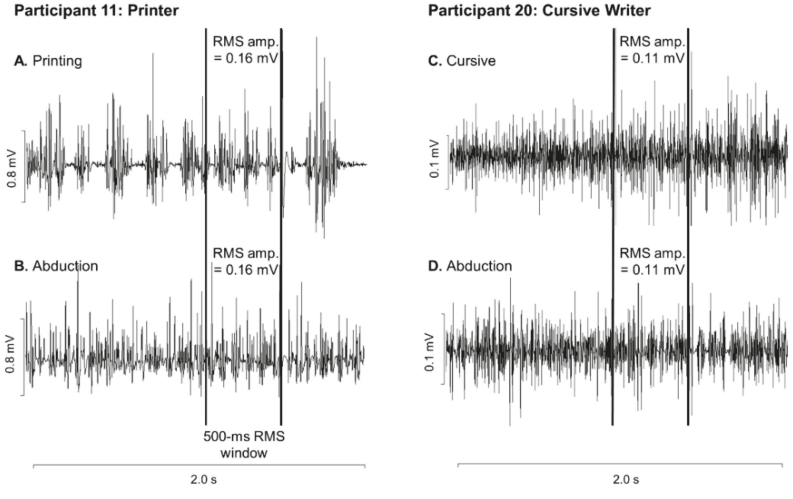

Figure 2. First dorsal interosseous (FDI) electromyographic activity in two representative participants: one who printed (**A**,**B**) and one who wrote in cursive (**C**,**D**). FDI EMG activity is shown during writing (**A**,**C**) and abduction (**B**,**D**). The vertical lines denote the 500-msec window prior to TMS stimulation from which the root-mean-square activity was calculated.

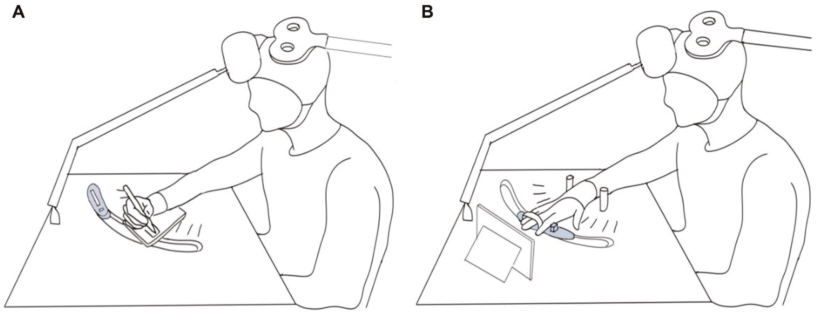

Figure 3. Experimental set-up of participant positioning during the writing (**A**) and abduction (**B**) tasks. The table design allowed the position of the force transducer to match the self-selected writing position of the graphic tablet.

Following familiarization, the participant was set up for TMS. The pre-fatigue and post-fatigue tests included a total of 90 trials, which consisted of 30 pulses each of TMS stimulation type, including single-pulse test MEP (TS), and paired-pulse stimuli to assess short-interval intracortical inhibition (SICI), and intracortical facilitation (ICF). The 90 trials were performed in 3 blocks, with 10 sets per block and 3 trials per set to pseudo-randomize the TMS stimulation such that each set would include a single-pulse TS, SICI, and ICF evoked in random order (Figure 1). Each trial was 5 seconds in duration. On the writing day, the timing of the screen refresh was set to ensure that the stimulus was delivered as the participant wrote the letter "a" or "m". Timing between pulses (5 s) was never changed. On the abduction day, the TMS stimulation occurred 2 seconds into the 5-second trial to ensure the participant was in the plateau portion of the submaximal, isometric, finger abduction contraction.

The fatigue protocol consisted of repeated 4-second isometric finger abduction contractions at 60% of the participant's MVC, with two seconds between each contraction. Task failure was defined as the point at which force fell below 58% MVC for more than three seconds despite encouragement to meet and hold the 60% target. Immediately following task failure, participants completed a post-fatigue MVC before beginning the post-fatigue test (Figure 1). It is important to note that on both days, regardless of the condition (abduction or writing), participants performed the same isometric finger abduction fatigue protocol. In this way, we examined the task-dependent nature of TMS measures of CSE rather than the task-dependent nature of neuromuscular fatigue.

2.3. Experimental Set-Up and Recordings

2.3.1. Electromyography and Force Recordings

The skin over the right FDI muscle was cleaned with isopropyl alcohol and two Ag/AgCl EMG electrodes were affixed in a bipolar configuration (0.5 cm recording surface, 1 cm interelectrode distance) over the muscle belly. A ground electrode was placed on the dorsal aspect of the right hand after being shaven and cleaned with alcohol. To allow the hand to rest comfortably on the graphic tablet, a glove covering the hand and the fourth and fifth digits was worn by participants. The skin over the extensor carpi radialis (ECR) and the flexor carpi radialis (FCR) was prepared for EMG by shaving, abrading, and cleansing with alcohol. Parallel bar surface EMG electrodes (10 × 1 mm Ag contacts, 1 cm interelectrode distance, DE-2.1 DELSYS Inc., Natick, MA, USA) were affixed over the muscle belly of the ECR and FCR, and a ground electrode was placed on the elbow. EMG and force signals were digitized at 2000 Hz using the Micro 1401-3 data acquisition unit and Signal 6.0 waveform acquisition software (Cambridge Electronics Design, Cambridge, UK). The FDI EMG signal was pre-amplified 300× and band-pass filtered from 15 to 450 Hz (Motion Lab Systems, Inc. Los Angeles, CA, USA). The

ECR and FCR EMG data was amplified 1000× and band-pass filtered from 20 Hz to 450 Hz (Bagnoli-16, DELSYS Inc., Natick, MA, USA).

2.3.2. Transcranial Magnetic Stimulation

Participants were seated at a table with an adjustable headrest situated in front of the forehead to allow the body and head to be supported in a comfortable writing position (Figure 3). A figure-eight magnetic stimulating coil (D70 coil, Magstim Company Ltd., Whitland, UK) was positioned over the primary motor cortex with the handle positioned posteriorly 45° to the midsagittal line and the induced current in a posterior to anterior direction and held in place using a lighting support arm and clamp (Manfrotto Supports, Cassola, Italy) with additional support and position maintenance by the investigator. The TMS coil was moved in small increments in order to determine the optimal site for generating a motor evoked potential (MEP) in the FDI via suprathreshold stimulations from the BiStim2 system (Magstim Company Ltd., Whitland, UK). Once located, this spot was marked on the cap worn by participants. Stimulator output was then adjusted to find the active motor threshold (AMT), determined as the minimum intensity that elicited a 200 μV MEP in 5 out of 10 of trials while the participant maintained a target matched to the average EMG over 20 writing trials recorded during the familiarization period at the beginning of each experimental day. Pulses were delivered at intervals of at least 5 s during threshold hunting. Paired-pulse TMS was used to assess SICI and ICF with a conditioning stimulus of 80% AMT preceding the test MEP set at 120% AMT. To elicit SICI, the conditioning stimulus preceded the test MEP by 3 ms To elicit ICF, the conditioning stimulus preceded the test MEP by 12 ms [27].

2.3.3. Motor Evoked Potentials (MEPs)

Motor evoked potential (MEP) peak-to-peak amplitudes were measured offline using Signal 6.0 (Cambridge Electronics Design, Cambridge, UK). Trials were excluded if (a) the participant responded incorrectly (e.g., no contraction), (b) TMS stimulation occurred between EMG bursts during writing, and (c) no MEP was elicited (MEP < 200 μV) [28]. Exclusion criteria a, b, and c were based on trial by trial inspection of the waveform data. SICI and ICF MEPs were normalized to the corresponding test MEP within the same set (each series of three randomized trials that include TS, SICI, and ICF). If a test MEP was excluded, MEPs were normalized to the test MEP in the next closest set such that the conditioned MEP was always normalized to an unconditioned MEP that was no further than three trials away. Test MEP peak-to-peak amplitudes are reported in millivolts (mV). The coefficient of variation of the test MEP amplitude was calculated for each participant to determine the consistency of the measure. Conditioned MEP peak-to-peak amplitudes (SICI and ICF) were normalized to the test MEP amplitude and are therefore reported as ratio values where <1 would indicate inhibition and >1 would indicate facilitation. Sample data from one participant are shown in Figure 4.

2.3.4. Cortical Silent Period

The duration of the test MEP CSP was measured offline using a custom script in MATLAB (MathWorks, Inc., Natick, MA, USA). CSP duration was calculated from the point of the test pulse stimulus delivery to the point at which EMG activity returned to prestimulus activity (i.e., average RMS amplitude calculated over a 300-ms period prior to stimulation). CSP was calculated as the average for the 30 test pulse trials only (CSP following paired-pulses were not included).

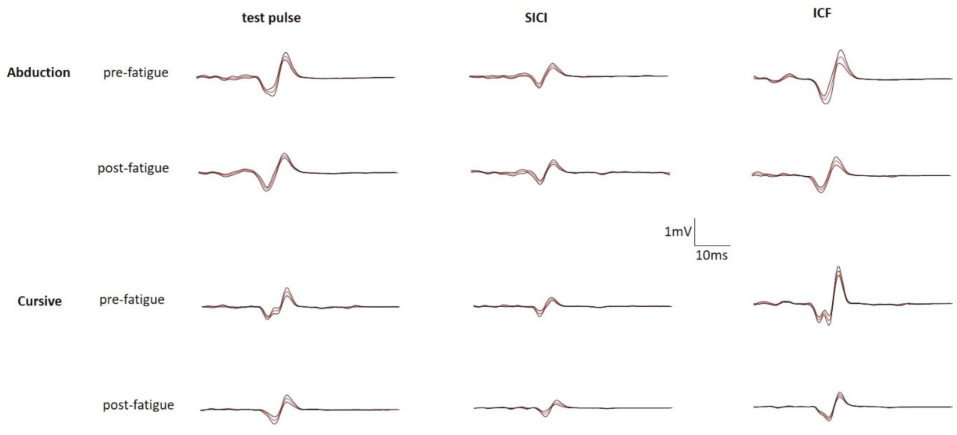

Figure 4. Block averages (red line) and error (black lines) of evoked potentials for one participant. Each block included 10 single test pulses, 10 SICI paired-pulses, and 10 ICF paired-pulses. This participant completed the writing task in cursive. Pre-fatigue averages are shown above the post-fatigue averages.

2.4. Statistical Analysis

Analysis was performed using Statistica 13.2 (TIBCO Software Inc., Palo Alto, CA, USA). For TMS pulses, trial outliers were removed if they fell >2 standard deviations from the average within each individual participant. Two participants were excluded from the SICI analysis due to a large number of outliers occurring within a single block of trials. One participant was removed from the CSP analysis due to an inability return to the target level of EMG activity following stimulation. Assumptions for the analysis of variance (ANOVA) were tested for each variable using the Shapiro–Wilk test for normality. Test MEP amplitudes were not normally distributed, thus a 1/sqrt transformation was applied for statistical analysis. Preliminary analysis was performed to ensure that there was no effect of block (i.e., the 3 blocks of 30 trials pre- and post-fatigue). No block main effect was present for any variable, therefore all pre-fatigue trials and all post-fatigue trials were averaged for each participant.

To examine potential differences between testing days a dependent samples *t*-test was conducted on baseline measures (see Table 1). To determine the effect of task on dependent measures, a one-way repeated measures ANOVA was performed comparing pre-fatigue values between writing and the abduction task. To determine the effect of task on the dependent measures during fatigue, a 2 (task: abduction versus writing) × 2 (fatigue: pre versus post) repeated measures ANOVA was performed for each dependent variable. The number of participants included in each analysis is noted in Tables 2 and 3.

Table 1. Baseline measures made during the familiarization period on the abduction and writing days. These measures included maximal index finger abduction force and first dorsal interosseous (FDI) activity during MVCs, the level of FDI activity during 20 writing trials, the number of fatiguing sets completed, and active motor threshold (AMT) for TMS (Mean ± SD).

	Abduction Day	**Writing Day**
Maximal Force (*N*)	19.49 ± 5.80	24.05 ± 11.38
Maximal FDI RMS amplitude (mV)	0.91 ± 0.38	0.94 ± 0.40
Writing EMG (% max)	18.7 ± 6.88	20.5 ± 9.62
AMT (%MSO)	39.1 ± 8.20	41.0 ± 7.30
# of Fatiguing Trials	43.50 ± 26.34	48.83 ± 45.34

FDI: first dorsal interosseous; MVC: maximal voluntary contraction; AMT: active motor threshold; RMS: root mean squared; EMG: electromyography; MSO: maximum stimulator output.

Table 2. Motor evoked potentials recorded from the FDI muscle and results of the repeated measures ANOVAs comparing Task (abduction versus writing) and Task × Fatigue conducted with all 19 participants.

	Abduction Task			Writing Task			Effects	
Measure	**Pre-Fatigue**	**Post-Fatigue**	**% Change Effect Size**	**Pre-Ftg**	**Post-Ftg**	**% Change Effect Size**	***F* Value [§] (Task)**	***F* Value (Task × Fatigue)**
Test MEP (*n* = 19)	1.64 ± 1.11	1.54 ± 1.13	−5.9% $d = 0.09$	1.85 ± 1.53	1.64 ± 1.65	−11.2% $d = 0.13$	F(1,18) = 0.60, $p = 0.45$, $\eta_p^2 = 0.03$	F(1,18) = 2.22, $p = 0.15$, $\eta_p^2 = 0.11$
SICI (*n* = 17)	0.95 ± 0.22	0.94 ± 0.30	−0.7% $d = 0.03$	0.82 ± 0.22	0.86 ± 0.21	4.1% $d = 0.16$	F(1,16) = 4.40, $p = 0.052$, $\eta_p^2 = 0.22$	F(1,16) = 0.36, $p = 0.56$, $\eta_p^2 = 0.02$
ICF (*n* = 19)	1.25 ± 0.33	1.19 ± 0.30	−4.2% $d = 0.16$	1.13 ± 0.28	1.26 ± 0.33	11% $d = 0.41$	F(1,18) = 1.69, $p = 0.21$, $\eta_p^2 = 0.09$	F(1,18) = 3.60, $p = 0.07$, $\eta_p^2 = 0.17$

[§] Calculated on the pre-fatigued values only. % Change is calculated as ((post-pre)/pre) × 100 from the unrounded data. Cohen's *d* is calculated for the pre- to post-fatigue means. Abbreviations: SICI: short-interval intracortical inhibition; ICF: intracortical facilitation.

Table 3. Results of the ANOVA comparing Task × Style (printing versus cursive) and Task × Fatigue × Style conducted with the subset of participants (*n* = 16) that could be classified as printers (bold font) or cursive writers.

		Abduction Task			Writing Task			Effects	
Measure	**Method (*n*)**	**Pre-Fatigue**	**Post-Fatigue**	**% Change Effect Size**	**Pre-Fatigue**	**Post-Fatigue**	**% Change Effect Size**	***F* Value [§] (Task × Style)**	***F* Value (Task × Fatigue × Style)**
Test MEP	**Printers (8)**	**1.49 ± 1.16**	**1.54 ± 0.82**	**3.0% $d = 0.05$**	**1.96 ± 1.54**	**1.77 ± 1.50**	−10.0% $d = 0.13$	F(1,14) = 2.95, $p = 0.11$, $\eta_p^2 = 0.17$	F(1,14) = 0.77, $p = 0.40$, $\eta_p^2 = 0.05$
	Cursive writers (8)	1.93 ± 1.16	1.90 ± 1.43	−1.4% $d = 0.02$	2.09 ± 1.78	1.86 ± 2.08	−10.9% $d = 0.12$		
SICI	**Printers (7)**	**1.03 ± 0.28**	**1.05 ± 0.25**	**1.5% $d = 0.06$**	**0.71 ± 0.13**	**0.86 ± 0.16**	20.4% $d = 1.02$	$F_{(1,13)} = 8.00$, $p = 0.01$, $\eta_p^2 = 0.38$	F(1,13) = 0.56, $p = 0.47$, $\eta_p^2 = 0.04$
	Cursive writers (8)	0.87 ± 0.17	0.81 ± 0.33	−6.6% $d = 0.22$	0.86 ± 0.26	0.82 ± 0.26	−4.1% $d = 0.13$		
ICF	**Printers (8)**	**1.37 ± 0.35**	**1.21 ± 0.41**	**−12.0% $d = 0.43$**	**1.09 ± 0.29**	**1.40 ± 0.36**	28.1% $d = 0.94$	F(1,14) = 2.71, $p = 0.12$, $\eta_p^2 = 0.16$	F(1,14) = 9.90, $p = 0.007$, $\eta_p^2 = 0.41$
	Cursive Writers (8)	1.15 ± 0.33	1.14 ± 0.21	−0.4% $d = 0.02$	1.19 ± 0.33	1.12 ± 0.31	−5.6% $d = 0.21$		

[§] Calculated on the pre-fatigued values only. % Change is the (post − pre)/pre fatigue values. Cohen's *d* is calculated for the pre- to post-fatigue means. Abbreviations: SICI: short-interval intracortical inhibition; ICF: intracortical facilitation.

As the study progressed, we found that of the 19 participants, 8 wrote in cursive, 8 printed, and 3 used a combination of cursive and printing. Accordingly, we conducted an additional post hoc analysis of writing style. This separate-groups post hoc analysis included writing style (cursive vs. printing) as a categorical predictor to determine the baseline effect (task × writing style) and the effect of task and style on fatigue (task × fatigue × writing style) in a posteriori analysis. The three individuals who used a combination of printing and cursive writing strategies to complete the task were excluded from this analysis. Because this analysis of writing style was not planned a priori, it is important to note that it is underpowered.

Tukey's HSD testing was used for post hoc analysis where applicable. Partial eta-squared was calculated in Statistica for the ANOVA models. Cohen's d was calculated for the pre-fatigue to post-fatigue values where applicable [29].

3. Results

3.1. Baseline Measures

The baseline measures recorded during familiarization (force and FDI RMS amplitude during MVCs, FDI activity of writing, and AMT) were not different between the abduction and writing days. Additionally, the number of fatiguing trials performed was not different between the two days. The amount of fatigue elicited was not significantly different (Task × Style: $F(1,13) = 0.88$, $p = 0.37$) between printers on the writing day (−26.0% MVC force), cursive writers on the writing day (−18.6%), printers on the abduction day (−27.5%), and cursive writers on the abduction day (−22.6%).

3.2. Effect of Task

Motor Evoked Potentials

In the pre-fatigue trials, there was no effect of task (writing versus abduction) on the amplitude of the test MEP ($F(1,18) = 0.60$, $p = 0.45$, $\eta_p^2 = 0.03$, Figure 5A), ICF ($F(1,18) = 1.69$, $p = 0.21$, $\eta_p^2 = 0.09$, Figure 5C), or CSP ($F(1,17) = 1.14$, $p = 0.30$, $\eta_p^2 = 0.06$). However, the coefficient of variation of test MEP amplitude was 23% greater in the writing task compared to the abduction task ($F(1,18) = 13.55$, $p < 0.01$, $\eta_p^2 = 0.43$). The coefficient of variation of the test MEP was higher in both the printing (48.4 ± 7.3, $n = 8$) and cursive writing (42.4 ± 5.5, $n = 8$) styles, as compared to abduction in printers (38.3 ± 7.9, $n = 8$) and abduction in cursive writers (35.1 ± 8.2, $n = 8$). Although there appeared to be a trend toward a greater level of inhibition (SICI) during writing compared to abduction ($F(1,16) = 4.40$, $p = 0.052$, $\eta_p^2 = 0.22$, Table 2, Figure 5B), this analysis included only 17 participants and was not adequately powered. When writing style (printing vs. cursive writing) were factored in (task × style: $F(1,13) = 8.00$, $p = 0.01$, $\eta_p^2 = 0.38$, Table 3), post hoc analysis revealed a significant difference ($p < 0.01$) in SICI between printing (0.71 ± 0.13, $n = 7$) and abduction (1.03 ± 0.28, $n = 7$, Figure 6B) in printers, but no difference between cursive writing (0.86 ± 0.26, $n = 8$) and abduction (0.87 ± 0.17, $n = 8$) in cursive writers.

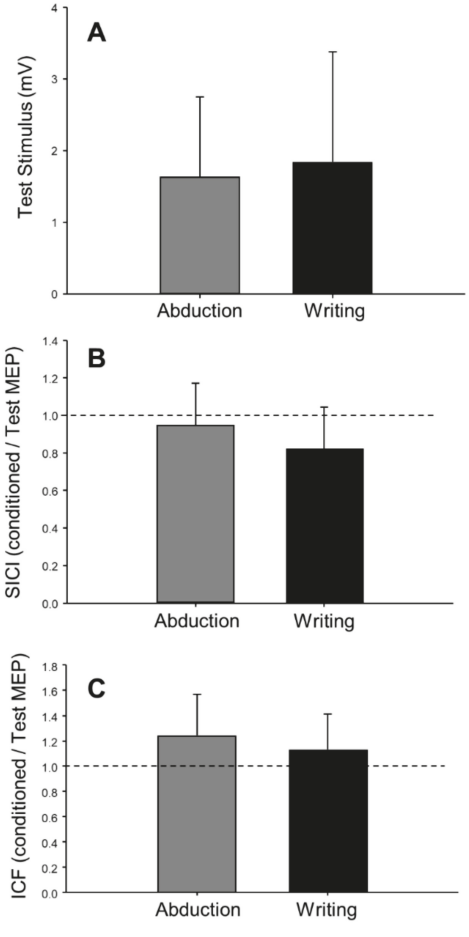

Figure 5. Pre-fatigue levels of test MEP (n = 19), intracortical facilitation (n = 19), and intracortical inhibition (n = 17) during finger abduction and writing (n = 19). There were no differences between test stimulus (**A**), short-interval intracortical inhibition (SICI; **B**), and intracortical facilitation (ICF; **C**) between the two tasks. In panels B and C, dashed lines represent no effect of the conditioning stimulus. Values below the line represent inhibition (conditioned MEP < test MEP), and values above the line represent facilitation (conditioned MEP > test MEP). Error bars represent standard deviation.

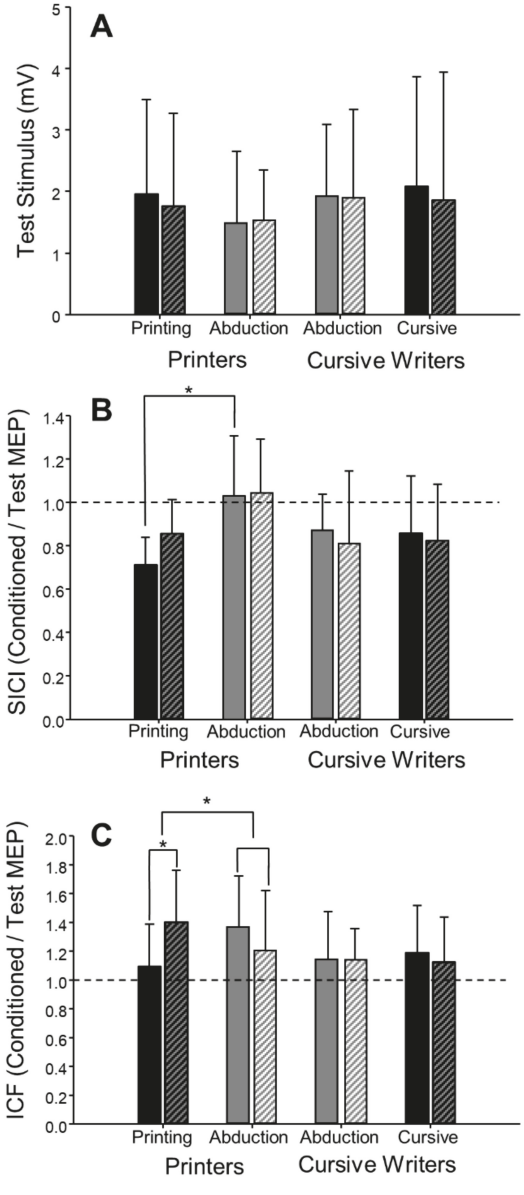

Figure 6. Motor evoked potentials during finger abduction and writing in printers (n = 8) and cursive writers (n = 8). There were no differences in single-pulse (test stimulus) MEPs between writers and printers or between tasks (test stimulus; **A**). Short-interval intracortical inhibition (SICI; **B**) was greater during printing than during abduction pre-fatigue (*, $p < 0.01$). In printers, there was a fatigue-associated increase in intracortical facilitation (ICF; **C**) when assessed during printing, but not during abduction (*, $p < 0.01$). Solid bars represent pre-fatigue values, and hatched bars represent post-fatigue values. In panels B and C, dashed lines represent no effect of the conditioning stimulus. Values below the line represent inhibition (conditioned MEP < test MEP), and values above the line represent facilitation (conditioned MEP > test MEP). Error bars represent standard deviation.

3.3. Muscle Activity

Task × fatigue repeated-measures ANOVAs were conducted for FDI, FCR, and ECR surface EMG measures of muscle activation. Level of FDI muscle activation was successfully maintained between the two tasks ($F(1,18) = 0.60$, $p = 0.45$, $\eta_p^2 = 0.03$). This was expected because average writing RMS amplitude was used as the muscle activation target during the abduction task. The abduction task elicited greater levels of FCR activity ($F(1,18) = 7.24$, $p = 0.02$, $\eta_p^2 = 0.29$) and lower levels of ECR activity ($F(1,18) = 7.73$, $p = 0.01$, $\eta_p^2 = 0.30$) compared to the writing task. Analysis of writing styles (task × style) revealed a significant interaction for both FCR activity ($F(1,14) = 4.70$, $p = 0.048$, $\eta_p^2 = 0.25$; Figure 7A) and ECR activity ($F(1,14) = 5.25$, $p = 0.04$, $\eta_p^2 = 0.27$; Figure 7B). Post hoc analysis identified that cursive writing required significantly less FCR activity (12.58 ± 7.42 μV) than abduction (29.79 ± 17.57 μV) in the cursive writing group. There was no significant difference between FCR activity during printing (18.36 ± 9.10 μV) or abduction (20.92 ± 13.07 μV) in the printing group. In the extensor muscle, post hoc analysis identified that printing (154.22 ± 71.53 μV) required significantly more ECR activity than abduction (70.36 ± 47.40 μV) in the printers. There was no significant difference between ECR activity during cursive writing (99.07 ± 27.63 μV) or abduction (92.84 ± 39.92 μV) in the cursive group.

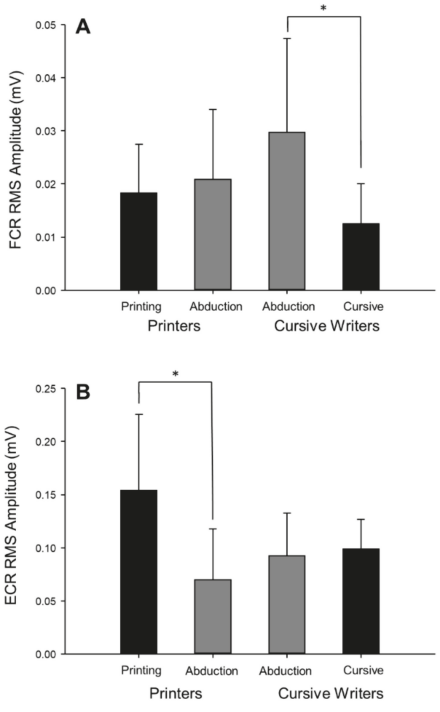

Figure 7. Pre-fatigue levels of wrist flexor (**A**) and wrist extensor (**B**) activity during the abduction and writing tasks in printers and cursive writers. Wrist flexor muscle activity was higher in the writing task than in the abduction task in cursive writers, whereas wrist extensor muscle activity was higher in the writing task than in the abduction task in the printers (*, $p < 0.05$). This analysis included participants who completed the writing task using a pure printing ($n = 8$) or cursive ($n = 8$) strategy. Error bars represent standard deviation.

3.4. Effect of Task on Fatigue

3.4.1. Motor Evoked Potentials

The task × fatigue repeated-measures ANOVA was not significant for any measure of CSE (see Table 2 for test MEP, SICI, and ICF results), including test MEP coefficient of variation ($F(1,18) = 3.59$, $p = 0.07$, $\eta_p^2 = 0.17$) and CSP ($F(1,17) = 0.29$, $p = 0.60$, $\eta_p^2 = 0.02$). However, when writing style was taken into consideration, a 3-way (task × fatigue × style) repeated-measures ANOVA revealed a significant 3-way interaction for ICF ($F(1,14) = 9.90$, $p < 0.01$, $\eta_p^2 = 0.41$) (Table 3). Post hoc analysis revealed a significant increase in ICF from pre-fatigue to post-fatigue (28.1%, d = 0.94, $p = 0.04$) in the printing group. Planned comparisons were conducted using percent change.

Scores (pre- to post-fatigue) to determine the task × style effect on fatigue. The increase in facilitation in printing (28.1%) was significantly different than the decrease in facilitation of printers performing the abduction task (−12.0%, $p < 0.01$, Figure 6C). There were no significant effects of writing style on the test MEP or SICI (see Table 3), test MEP coefficient of variation ($F(1,14) = 0.02$, $p = 0.88$, $\eta_p^2 = 0.002$), or CSP ($F(1,13) = 0.30$, $p = 0.60$, $\eta_p^2 = 0.02$).

3.4.2. Muscle Activity

The task × fatigue repeated measures ANOVA was not significant for FDI activity ($F(1,18) = 1.31$, $p = 0.27$, $\eta_p^2 = 0.07$), FCR activity ($F(1,18) = 1.69$, $p = 0.21$, $\eta_p^2 = 0.09$), or ECR activity ($F(1,18) = 0.86$, $p = 0.37$, $\eta_p^2 = 0.05$). Similarly, an analysis of writing style (task × fatigue × style) did not yield any significant interactions for FDI activity ($F(1,14) = 0.57$, $p = 0.46$, $\eta_p^2 = 0.04$), FCR activity ($F(1,14) = 0.57$, $p = 0.47$, $\eta_p^2 = 0.04$), or ECR activity ($F(1,14) = 2.82$, $p = 0.12$, $\eta_p^2 = 0.17$).

4. Discussion

Our study demonstrates that fatigue-associated changes in CSE depend on the motor task completed during single- and paired-pulse TMS stimulation. Not only did we find differences in fatigue-associated changes in cortical excitability assessed during hand-writing compared to isometric finger abduction, but we also found differences between participants who completed the writing task by printing compared to those who wrote in cursive. Specifically, we found that the same fatigue protocol elicited an increase in ICF during printing, but not during cursive writing. It is important to note that the fatigue protocol itself was the same on each day, and only the motor task during pre-fatigue and post-fatigue assessments of CSE differed. Furthermore, because the level of FDI muscle activation was held constant during the two tasks, the differences in CSE between writing and abduction days were not simply due to differences in voluntary drive to that muscle.

The present study examined CSE during voluntary movement. Because the level of muscle activation during voluntary movement affects levels of CSE [30–32], it was critical in the present study to match FDI EMG between tasks. We accomplished this by measuring FDI muscle activation during the writing task in a familiarization period each day, and then using biofeedback to set a target on the abduction day that matched muscle activation during writing for each participant. This approach to matching muscle activation between the two tasks (writing and abduction) was equally effective in printers and cursive writers. Therefore, differences in intracortical measures of excitability pre- and post-fatigue cannot be attributed to differing FDI EMG activity between the tasks.

During baseline (pre-fatigue) testing, the variability of single-pulse MEP amplitudes was greater in the writing task than the abduction task. This was expected because the abduction task is a single-joint, externally cued task frequently used in TMS studies for the purpose of reducing variability. The abduction task was maintained at a stable force level using visual feedback. To make the writing task more functionally-relevant, we did not control the style, or speed of each participant's writing. Therefore, it is not surprising that the writing task resulted in greater variability than the abduction task, both within and between subjects. Similar results have been found comparing test–retest reliability of

MEP amplitudes between static and dynamic tasks in the lower limb [33], where MEPs evoked during a static task (i.e., plateau at a target force level) were less variable than those evoked during a dynamic task (i.e., continuously increasing force level). The style of writing did not impact the MEP amplitude variability, as we found higher coefficient of variation values for both printing and cursive writing compared to abduction. A future challenge in TMS research will be to utilize laboratory tasks that balance variability with the relevance of the task to motor activities outside of the lab.

We hypothesized that CSE would be greater during writing compared to abduction due to the complexity of the writing task. However, single-pulse MEP amplitude was not different between tasks. Reports of differences in CSE between precision tasks and conventional abduction tasks are inconsistent. Original research in monkeys suggested that corticospinal neurons were more active during a precision task compared to a power task [9], a finding supported by several studies that found greater MEP amplitudes during complex, precision tasks compared to simple, power tasks [4,11,34]. Alternatively, larger MEP amplitudes have been reported during conventional abduction tasks compared to power, pincer, or grasping tasks [5–7]. The discrepancy between our findings and previous research may be that other studies have used precision tasks that were visually guided and externally controlled, whereas our writing task is a dynamic, internally generated task that is retrieved and implemented from memory [35,36]. Furthermore, writing involves higher levels of activation in the dorsal premotor cortex in comparison to simple finger contraction tasks, as well as unique activation in multiple brain regions (e.g., premotor cortex and anterior putamen) not activated during tapping or "zigzagging" finger actions [37]. This association of writing with higher cognitive demands would suggest that writing is a more complex task in comparison to simple finger contractions. On the other hand, because writing is learned and practiced from a young age into adulthood, it is also associated with a degree of automaticity [38]. Accordingly, our finding that writing was not associated with increased CSE compared to abduction, may have been due to either the increased complexity or the automaticity of writing compared to unpracticed, less natural, and externally guided precision-grip tasks used in previous studies.

Despite the fact that baseline (pre-fatigue) CSE did not differ between tasks, levels of intracortical inhibition (SICI) assessed using paired-pulse TMS trended ($p = 0.052$) toward greater inhibition during writing compared to abduction. When participants were subdivided into those who printed and those who wrote in cursive (excluding those who used a combination of printing and cursive within a single word), printers had the greatest intracortical inhibition during the writing task. We hypothesize that this may be due to the increased control required during the more intermittent task of writing distinct letters during printing, compared to the continuous nature of cursive writing (Figure 2). The differences in intracortical inhibition observed between writing styles may also be explained by the activity of the proximal forearm muscles. While FDI EMG activity between abduction and writing did not differ, extensor (ECR) activity was greater and flexor (FCR) activity was lower during the writing task compared to abduction. It has been suggested that activity and position of proximal muscles can have an effect on the corticospinal pathway leading to the distal muscle of interest [39–41]. This relationship has been shown between the FDI and proximal arm muscles (including the ECR, FCR, and deltoid muscles), where proximal muscle activity resulted in facilitation of distal muscle MEPs [39–41]. Similarly, forearm position (pronation vs. semi-supinated) is also known to alter CSE [42]. In our study, however, the forearm was semi-supinated for both printing and cursive writing, and therefore forearm position does not explain the differences in intracortical excitability that we observed when we compared the two different writing styles. The role that proximal muscle activation may play in the CSE of distal muscles highlights the importance of choosing laboratory tasks that more closely resemble natural movements outside of the laboratory to which we aim to extrapolate our results.

TMS is used to assess changes in corticospinal and intracortical excitability in response to many different interventions and perturbations (e.g., fatigue, strength training, skill training, disease, injury, aging, and pharmacological agents). We sought to determine whether changes in CSE following an intervention depend on the motor task used to elicit muscle activity during the delivery of TMS. To this

end, we employed neuromuscular fatigue as an acute intervention, and measured changes in CSE during two different motor tasks before and after two identical fatigue protocols. It is well established that neuromuscular fatigue is associated with changes in CSE, specifically a reduction in unconditioned MEP (test MEP) amplitudes in the target muscle following neuromuscular fatigue [43,44]. This depression has been attributed to central mechanisms of fatigue [44,45]. Paired-pulse TMS techniques have been used to identify intracortical mechanisms of fatigue [22,46,47]. Following fatiguing contractions, SICI decreased [18,46,48]; however, the association between fatigue and ICF is not as clear. For example, ICF measured in the biceps brachii decreases during a sustained fatiguing contraction [48,49], whereas ICF measured in the FDI is elevated at the point of task failure when assessed at rest [22,46]. In other studies, ICF does not change with fatigue [47,49]. It has been suggested that decreased intracortical inhibition [18] and increased facilitation following fatigue [22] may serve as a compensatory mechanism to optimize motor output as fatigue develops.

In our study, there was no task-dependent effect of fatigue on any of the corticospinal and intracortical excitability measures between writing and abduction. However, most of our participants used one of two distinct writing styles: printing or cursive. Therefore, we completed additional a posteriori analyses to determine whether there was an effect of writing styles on fatigue-induced changes in CSE, and found that printers had a significant increase in ICF following fatigue. This is consistent with previous reports of increased ICF following fatigue that employ more conventional laboratory tasks [46,50]. However, this only occurred during printing, and was not found during cursive writing or the conventional finger abduction task. Previous research has suggested that increased ICF post-fatigue may be a mechanism to compensate for peripheral contractile failure or reduced upstream drive to the primary motor cortex [22]. However, this would not explain the increased ICF seen in printing but not in cursive writing or abduction. Possibly the differences in wrist stabilization during printing and cursive, reflected by differences in wrist extensor and wrist flexor muscle activity during the two tasks, had a greater impact on ICF than on SICI. Furthermore, it is possible that printing employs cortical circuits that are affected by isometric finger abduction fatigue task differently than the cortical circuits employed in cursive writing. Although there is very little research regarding the neural control of printing versus cursive writing, one clinical study reports impairment in cursive writing, but not printing, drawing, or the ability to draw continuous loops, following ischemic damage to the parietal lobe [51]. Another clinical case found that the ability to write in cursive was lost with the development of a large cranial tumor impinging on the left frontal lobe, while the ability to print remained intact. In this case, the patient regained the ability to write in cursive following tumor resection [52]. Case studies like these support the idea that printing and cursive writing have different cortical representations, and may therefore be associated with different inputs to the primary motor cortex. This novel finding certainly warrants further exploration, as printing and cursive writing appear to be two different tasks that further demonstrate the task-dependent nature of fatigue-associated changes in CSE.

We started this repeated-measures study with 20 participants based on a power analysis conducted using data from a previous study from our lab that also investigated intracortical mechanisms of fatigue [22]. Of the 20 participants we recruited, 1 participant was unable to complete the study, and 2 participants were excluded from the SICI analysis. Accordingly, this analysis is not robustly powered, and these data should be interpreted cautiously. The analysis of hand writing style was not planned in advance. From the start, we told participants to write as they preferred given that our aim was to assess corticospinal excitability during a natural motor task. As the study progressed, we found that many university students were unable to write in cursive, and we conducted the post hoc analysis of writing style. It should be noted that this a posteriori analysis of writing style (cursive vs. printing) is underpowered. Furthermore, in the present study, we assessed only three intracortical mechanisms (SICI, ICF, and cSP). Each of these measures has been associated with different types of neurotransmission (SICI with GABA-A receptors, ICF with glutamatergic transmission, and the cSP with GABA-B receptors) as reviewed by Reis [1]. It is possible that other intracortical (e.g., long interval

intracortical inhibition) and interhemispheric pathways (e.g., interhemispheric inhibition) may reveal fatigue-associated changes that are dependent on the motor task that is conducted during stimulation. This is an area that requires further investigation

5. Conclusions

This study is the first to assess CSE and the effect of fatigue during a writing task. Although fatigue-associated changes in CSE have been well studied over the last three decades, this study highlights the importance of considering the task used during TMS measures of corticospinal and intracortical excitability. Although controlled laboratory tasks are required to reduce variability of motor evoked potentials to allow for reproducible results, it is important to note that CSE is task-dependent. Because of this, measures of CSE made during a laboratory task may not translate to motor tasks outside of the lab. Despite the fact that the fatigue task and the level of FDI muscle activation during TMS was the same in our study, hand-writing revealed fatigue-associated changes in CSE that were not evident during abduction. Therefore, the task-dependent nature of CSE emphasizes the need for experimental paradigms that better reflect relevant motor tasks or at least acknowledge the differences between the task employed in the laboratory and the movements outside of the lab to which results may be extrapolated. With this in mind, it is essential that studies of CSE consider the "task at hand".

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The Impact of Glucose on Corticospinal and Intracortical Excitability

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Abstract: Neurotransmission is highly dependent on the availability of glucose-derived energy, although it is unclear how glucose availability modulates corticospinal and intracortical excitability as assessed via transcranial magnetic stimulation (TMS). In this double-blinded placebo-controlled study, we tested the effect of acute glucose intake on motor-evoked potential (MEP) recruitment curves, short-interval intracortical inhibition (SICI), short-latency afferent inhibition (SAI) and long-latency afferent inhibition (LAI). Eighteen healthy males participated in four sessions. Session 1 involved acquisition of an individualized blood glucose response curve. This allowed measurements to be time-locked to an individualized glucose peak after consuming one of three drinks during the subsequent three sessions. Participants were administered a 300 mL concealed solution containing 75 g of glucose, sucralose, or water in separate sessions. Dependent measures were assessed at baseline and twice after drinking the solution. Secondary measures included blood glucose and mean arterial pressure. Corticospinal excitability and blood pressure increased following the drink across all treatments. No changes were observed in SICI, SAI or LAI. There was no rise in corticospinal excitability that was specific to the glucose drink, suggesting that acute changes in glucose levels do not necessarily alter TMS measures of corticospinal or intracortical excitability.

Keywords: glucose; SICI; SAI; LAI

1. Introduction

Glucose is the brain's primary energy substrate and provides the main carbon source for de novo synthesis of large compounds required for essential ranging processes, from neurotransmission to the management of oxidative stress [1–4]. Although the substantial influence of glucose is evident in several clinical contexts [5–7], a comprehensive neurophysiological profile has yet to be compiled in healthy humans during periods of fasting versus high-circulating glucose following feeding.

A small number of studies have used non-invasive transcranial magnetic stimulation (TMS) to compare neurophysiological measures in hyperglycemic, normoglycemic or fasting conditions [8–10]. Specterman et al. [10] reported a 3-fold increase in the size of motor-evoked potentials (MEPs) 60 min after ingestion of 68 g of glucose, such that greater increases in MEPs were correlated with greater increases in blood glucose levels. Badawy and colleagues [9] observed greater long interval intracortical inhibition (LICI) in epileptic and healthy individuals when in a fed (i.e., two hours after a meal) compared to fasted state (i.e., 12 h overnight). However, not all studies have detected an effect of glucose on TMS measures. Andersen and colleagues [8] manipulated glucose levels in type 1 diabetics via an intravenous glucose pump and did not observe any change in cortical motor thresholds, a common measure of corticomotor excitability. TMS can also be used to probe the sensorimotor system with measures of short- and long-latency afferent (SAI, LAI). To date, no studies have investigated the

influence of glucose on afferent inhibition. SAI and LAI are impaired in populations with Alzheimer's and Parkinson's disease [11], both of which display altered central glucose metabolism [12,13].

Most research to date that directly tests the effects of glucose on the motor system provides information regarding a limited variety of neurophysiological measurements. For example, the study conducted by Specterman and colleagues [10] measured MEPs using a time-efficient protocol involving the delivery of only 15 suprathreshold TMS pulses to obtain a measure of average MEP size. This approach allows for relatively high temporal resolution but contrasts with more comprehensive tests which could probe underlying mechanisms. For example, acquisition of MEP recruitment curves, while more time consuming, provides information regarding cortical glutamate levels [14]. Investigating a proxy measure of glutamate levels is useful, since glutamatergic neurotransmission has been linked with glucose in in vitro [15,16] and in situ [17] neurobiological studies. It may also be worthwhile to probe gamma-aminobutyric acid (GABA)-mediated intracortical inhibition using paired-pulse TMS. Although Badawy and colleagues found no significant effect of glucose on short interval intracortical inhibition (SICI), all significant and non-significant differences between fasted and fed participants were in the direction of increased inhibition or decreased facilitation after feeding as opposed to after fasting [9]. This is counterintuitive, given the apparent increase in MEP size after glucose ingestion [10], and warrants further investigation.

The goal of this study was to test the effect of glucose ingestion on the healthy human brain using a variety of non-invasive neurophysiological measures which have relevant mechanistic underpinnings. This investigation also controlled for the influence of a sweet placebo and time-locked measurements to individually measured peak blood glucose latencies. It was hypothesized that glucose would increase corticospinal excitability and SICI. The primary observation was that glucose did not change SICI, SAI, LAI or corticospinal excitability. Furthermore, corticospinal excitability and blood pressure increased over time when data were averaged across all treatments.

2. Materials and Methods

2.1. Participants

Healthy, young (n = 18, 22.8 ± 2.4 years), right-handed, male non-smokers were recruited from the McMaster University student population. Participants passed a screening for TMS contraindications [18] and were identified as right-handed using a modified handedness questionnaire [19]. Inactive individuals were excluded using the International Physical Activity Questionnaire (IPAQ, <600 MET-minutes/week) to reduce the risk of influence from prediabetic impairment of glucose metabolism, which is inversely correlated with physical activity level [20]. This study was approved by the Hamilton Integrated Research Ethics Board (HiREB) and conformed to the declaration of Helsinki.

2.2. Electromyography

Motor-evoked potentials were recorded via surface electromyography (EMG) over the first dorsal interosseous (FDI) muscle. FDI was chosen because MEPs from this muscle have demonstrated good inter- and intra-session reliability [21]. Adhesive electrodes (9 mm diameter Ag-AgCl) were placed over the FDI muscle belly and the metacarpal head of the index finger. The EMG signal was amplified 1000x and sampled at 5 kHz with low and high pass signal filters of 2.5 kHz and 20 Hz, respectively. EMG data was recorded using an analog-to-digital interface (Power 1401; Cambridge Electronics Design, Cambridge, UK) in combination with Signal/CED analysis software (Signal version 6.02; Cambridge Electronics Design).

2.3. Transcranial Magnetic Stimulation

Participants sat upright in the testing chair with their palms resting supine and elbows at an approximate 45° angle. Single and paired-pulse TMS was delivered with a custom 50 mm figure-of-eight coil, connected to a Magstim Bistim stimulator (Magstim, Whitland, UK). The coil was held over the

left primary motor cortex (M1) and the optimal stimulation location or "motor hotspot" was targeted using Brainsight neuro-navigation software (Rogue Research, Canada). The motor hotspot for FDI muscle was determined by delivering pulses at 50% of the maximum stimulator output (%MSO) over the approximate location of M1 while adjusting coil placement until the TMS pulses reliably evoked large MEPs in the FDI muscle. The angle of the coil relative to the midsagittal plane was maintained at 45° to induce posterior-to-anterior current in cortical tissue.

2.4. Resting Motor Threshold

Resting motor threshold (RMT) was defined as the stimulus intensity (%MSO) that evokes an MEP (i.e., peak-to-peak amplitude >50 μV) 50% of the time. This value was determined using TMS_MTAT_2.0 freeware (http://clinicalresearcher.org/software.htm). The starting stimulus intensity was set to 37% MSO. Twenty TMS pulses were then delivered over M1, adjusting the intensity after each pulse, as determined by the MTAT software based on the MEP occurrence (or lack thereof) in the previous trial [22].

2.5. MEP Recruitment Curve

Corticospinal excitability was measured using single-pulse TMS to obtain MEP recruitment curves. Eight TMS pulses were delivered at 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, and 200% of RMT in a randomized order, with an inter-stimulus interval of 4 s. The MEP amplitude was plotted against stimulus intensity and data were fit with a Boltzmann sigmoidal curve. The regression line was segmented 1000 times and the area under the recruitment curve (AURC) was quantified by trapezoidal integration.

2.6. Short Interval Intracortical Inhibition

Short-interval intracortical inhibition (SICI) was measured using a paired-pulse TMS protocol. The suprathreshold test stimulus (TS) was adjusted to the intensity that evoked an MEP with a size of approximately 1 mV in peak–peak amplitude. The conditioning stimulus (CS) preceded the TS by 2 ms, with a stimulus intensity of 80% of RMT. Twelve unconditioned (MEPTS) and 12 conditioned (MEPCS-TS) pulses were delivered in a randomized order, with a 5 s inter-trial interval. The magnitude of SICI was quantified using the ratio of conditioned to unconditioned MEP size (MEPCS-TS/ MEPTS).

2.7. Afferent Inhibition

Electroencephalography (EEG) electrodes were positioned over C3′ (located 2 cm posterior to C3) and referenced to Fz (International 10–20 system). A bar electrode was positioned over the median nerve at the wrist (cathode proximal) to deliver square wave electrical pulses (0.2 s pulse width) using a constant current stimulator (DS7AH; Digitimer, Welwyn Garden City, UK). Nerve stimulation was delivered at the minimum intensity that evoked a visible twitch in the abductor pollicis brevis (APB) muscle. Time-locked averaging of five hundred stimuli delivered at 3 Hz was used to determine the latency of the N20 peak of the somatosensory-evoked potential (SEP).

To acquire afferent inhibition, the TMS intensity was set to evoke a MEP of ~1 mV in the right FDI muscle. Electrical stimulation was delivered to the median nerve at the wrist at the minimum intensity that evoked a visible twitch in the APB muscle. The average intensity of nerve stimulation was 11.1 ± 3.8 mA. For SAI, the interstimulus interval (ISI) between peripheral nerve stimulation and TMS was 4 ms longer than the N20 latency (i.e., N20 + 4 ms). An ISI of 200 ms was used to acquire LAI. Twelve unconditioned stimuli (MEPTS) were randomly presented among 36 conditioned stimuli (nerve stimulation followed by TMS, twelve stimuli per ISI), with a 5 s inter-trial interval. The magnitude of SAI/LAI was expressed as the ratio of the conditioned to the unconditioned MEP amplitude.

2.8. Blood Glucose and Blood Pressure

Capillary blood glucose measurements were performed via the glucose oxidase method using a hand-held diabetes monitoring device (Abbott MediSense FreeStyle Precision Neo Blood Glucose and Ketone Monitoring System, Abbott). Since previous research has indicated that blood pressure may be elevated by ingestion of a large glucose bolus [23,24], mean arterial blood pressure was measured using an automated blood pressure monitor (OMRON Blood Pressure Monitor, OMRON Healthcare). The mean arterial pressure (MAP) was calculated from the systolic (SBP) and diastolic blood pressure (DBP) as indicated below:

$$MAP = (2DBP + SBP)/3 \quad (1)$$

2.9. Experimental Design

This study implemented a double-blinded, three-way crossover design in which fasted participants were assessed before and after ingestion of water, a sucralose-flavored placebo or a 75 g glucose bolus. All solutions were 300 mL. Prior to the first experimental testing session, participants completed a preliminary testing session. A schematic of the study schedule for each participant is shown in Figure 1.

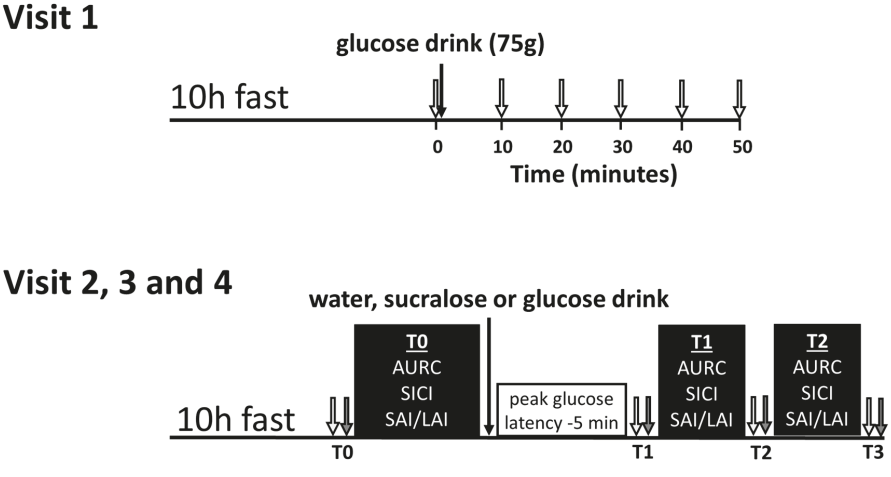

Figure 1. Timeline for the preliminary visit (visit 1) and the three experimental visits (visits 2, 3 and 4). For visit 1, baseline blood glucose was measured (0 min) and then participants drank 75 g of glucose. Next, blood glucose was measured every 10 min for 50 min or until peak was observed (open arrows). For visits 2, 3 and 4, the transcranial magnetic stimulation (TMS) testing bouts (black boxes) at T0 and T1 were separated by a rest period equal to the glucose latency minus 5 min. Mean arterial pressure (grey arrows) and blood glucose (open arrows) were measured before, after and in between the TMS testing bouts and are labeled T0, T1, T2 and T3. TMS—transcranial magnetic stimulation; AURC—area under the recruitment curve; SICI—short-interval intracortical inhibition; SAI/LAI—short-latency afferent inhibition/long-latency afferent inhibition.

Visit 1 was used to assess a time-course for glucose metabolism, allowing the subsequent TMS measures on visits 2, 3, and 4 to be individualized. Participants arrived in the lab having fasted for a minimum of 10 h, and then ingested a 75 g glucose bolus in 300 mL of solution. Finger-prick blood samples were collected and analyzed at 10 min intervals, as indicated in Figure 1 (top). The latency at which peak blood glucose occurred was used to ensure that TMS tests are conducted during a period of high-circulating glucose for each individual.

Visits 2, 3 and 4 were scheduled at least 48 h apart. On the day of each visit, participants arrived in the lab having fasted for 10 h and then ingested a 300 mL solution containing either plain water,

sucralose-sweetened placebo (5 g/300 mL Splenda® solution), or a 75 g oral glucose tolerance test bolus. TMS measures were acquired before ingestion (T0), 5 min before each participant's peak blood glucose latency (~30 min after drink ingestion) as determined in Visit 1 (T1), and ~1 h after ingestion, corresponding to the approximated peak of glucose levels in the cerebrospinal fluid (T2) occurring ~30 min after plasma glucose [25]. SAI, LAI, SICI and MEP recruitment curves were measured in a pseudorandomized order which was determined using an online Latin square generator (https://hamsterandwheel.com/grids/index2d.php). Capillary blood glucose and blood pressure were measured before and after each of the post-drink bouts, as denoted by the labels T1, T2 and T3 (see Figure 1, bottom).

The McMaster University Medical Centre (MUMC) research pharmacy provided a randomized treatment schedule. All treatment solutions were provided in uniform, shrouded bottles, with a letter code corresponding to the order of delivery. MUMC pharmacy held the drink randomization (i.e., drink identity) key until collection was complete to ensure that the experimenters were blind to the identity of the drink. Blood glucose and subjective ratings of sweetness were recorded by an unblinded researcher who did not otherwise take part in data collection or analysis. The sucralose-sweetened placebo was taste-matched with the 75 g glucose solution by MUMC pharmacy. Participants were explicitly asked not to comment on the taste of the drink to the researchers and it was made clear that this was very important to the integrity of the study. The participants were blind to the identity of the drink to the degree that they could not distinguish between the sucralose placebo and the glucose solutions (water was not masked with any taste).

2.10. Statistical Analyses

Trials were discarded if the EMG activity was >100 μV in the 100 ms preceding the stimulation artefacts, similar to previous work [26]. Normality was assessed with the Shapiro–Wilks test and a square-root or log transformation was applied in cases where data was not normally distributed.

First, to confirm that inhibition was observed for measures of SICI, LAI and SAI, two-way ANOVAs with the factors PATTERN (two levels: unconditioned MEP and conditioned MEP) and TIME (three levels: T0, T1, T2) were performed. Next, one-way ANOVAs using the within-subjects factor of TREATMENT (three levels: glucose, sucralose, water) were used to confirm that T0 data were not different between visits. Next, outlier analysis was performed using SPSS. Four outliers were removed from the SICI data and one was removed from the SAI data. Data were subsequently analyzed using repeated-measure ANOVAs with factors TREATMENT (three levels: glucose, sucralose, water) and TIME (three levels: T0, T1, T2). Post-hoc testing was performed with Bonferroni-corrected two-tailed paired t-tests. A Conover's ANOVA [27] was performed in lieu of a parametric ANOVA in cases where the data was not normally distributed (even after attempted transformations), with the Wilcoxon-signed rank test used for post-hoc testing. Supplementary measures of capillary blood glucose and MAP were assessed using two-way repeated measures ANOVA with four levels of TIME (T0, T1, T2, T3).

Wilcoxon signed-rank tests were used to assess the hypotheses that glucose would strengthen SICI and increase AURC, as well as all indicated post-hoc comparisons. Effect sizes were calculated using Cohen's d for paired t-tests and r for Wilcoxon's tests. Significance for all statistical tests was set to alpha <0.05. Data from TMS, blood glucose and blood pressure measurements are included in the supplementary materials.

3. Results

3.1. Blood Glucose

The peak plasma glucose concentration after ingestion of the glucose bolus on Visit 1 was 9.5 ± 1.0 mmol/L. This represents a 2-fold increase from the fasting glucose level of 4.8 ± 0.4 mmol/L, with a rise of 4.7 ± 0.9 mmol/L. The majority of participants had peak glucose latencies of 40 min

(n = 10), followed by six peaking at 30 min, and one participant peaking at 20 min and 50 min each. The observed peak glucose latency spread of 30 min emphasizes the importance of the individualized approach tested herein.

As expected, glucose levels for the experimental visits exhibited a significant effect of TREATMENT (F (2,17) = 108.261; $p < 0.001$), TIME (F (3,17) = 31.037; $p < 0.001$) and TREATMENT × TIME (F (6,17) = 29.722; $p < 0.001$). In the glucose delivery condition, plasma levels measured at T1 were 3.4 ± 1.9 mmol/L higher than fasting and remained elevated by 2.1 ± 1.2 mmol/L at T3. These data confirm that glucose levels were substantially increased during both post-drink TMS testing bouts in the glucose delivery condition. Notably, there was a slight increase in glucose level (<1 mmol/L) at T1 after the sucralose-sweetened placebo and a slight reduction at T1 following water. However, blood glucose returned to fasting levels at T2 and T3 in both cases. These data are displayed in Figure 2a and p-values and effect sizes for significant changes are reported in Table 1.

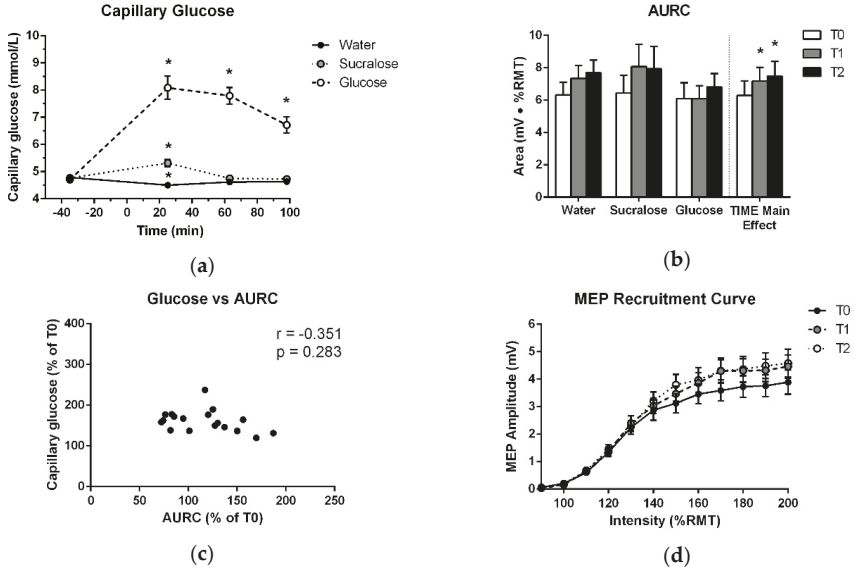

Figure 2. (**a**) Mean and standard error of capillary blood glucose levels over the course of each testing session. Measurements are plotted at each timepoint before and after ingestion of glucose, sucralose or water, which were consumed at 0 min. (**b**) Means and standard errors of AURC data entered into the ANOVA and treatment-averaged data, showing the significant effect of TIME. (**c**) Average relative increase in plasma glucose across T1, T2 and T3, plotted against the average relative increase in AURC across T1 and T2. (**d**) Treatment-averaged motor-evoked potentials (MEP) responses with mean and standard error at each TMS intensity of the recruitment curve for T0, T1 and T2. * indicates a significant difference from T0 ($p < 0.05$).

Table 1. Test statistics, effect sizes and *p*-values for all significant differences.

Measure	Timepoint	Test Statistic	Effect Size	*p*-Value
GLU_{glu}	T1–T0	7.781	1.834	<0.001
	T2–T0	9.370	2.209	<0.001
	T3–T0	6.963	1.641	<0.001
GLU_{water}	T1–T0	−3.044	−0.717	0.043
GLU_{suc}	T1–T0	7.434	1.752	<0.001
MAP_{all}	T1–T0	7.817	1.842	<0.001
	T2–T0	5.123	1.208	<0.001
	T3–T0	6.922	1.632	<0.001
$AURC_{all}$ *	T1–T0	2.940	0.490	0.003
	T2–T0	2.983	0.497	0.003

$AURC_{all}$: area under the recruitment curve averaged across all treatments, GLU: glucose data for each of the three drinks, MAP_{all}: mean arterial pressure averaged across all treatments * indicates that effect test statistics and effect size are derived from Wilcoxon's test (i.e., *z* and *r*, respectively).

3.2. MEP Recruitment Curve

As shown in Figure 2b, there was no increase in AURC after glucose at T1 or T2 ($p = 0.110$). There was only a significant effect of TIME, which was independent of treatment (Table 2, Figure 2b,d). No other effects in the ANOVA and no association between capillary blood glucose levels and the change in AURC at T1 or T2 were found (Table 3, Figure 2c). Further, no difference was detected between T0 data for the three treatments (Table 4).

Table 2. Results of two-way ANOVAs with factors TREATMENT and TIME, and the grand coefficient of variation (CV) of each TMS measurement.

Measure	Grand CV (%)	Factor	*df*	F	*p*-Value
AURC ¶	60.8	TREATMENT	2,32	1.555	0.226
		TIME	2,32	10.429	0.001
		TREATMENT × TIME	4,64	2.290	0.069
SICI #	80.8	TREATMENT	2,30	0.162	0.851
		TIME	2,30	2.640	0.088
		TREATMENT × TIME	4,60	1.712	0.200
SAI	44.6	TREATMENT	2,30	0.059	0.943
		TIME	2,30	0.118	0.889
		TREATMENT × TIME	4,60	0.268	0.897
LAI *	60.8	TREATMENT	2,32	2.663	0.085
		TIME	2,32	2.813	0.075
		TREATMENT × TIME	4,64	0.591	0.679

AURC: area under the recruitment curve, SICI: short-interval intracortical inhibition, SAI: short-latency afferent inhibition, LAI: long-latency afferent inhibition. * indicates data was square root transformed, # indicates log transformation, ¶ indicates data was ranked.

Table 3. Results from correlations between area under the recruitment curve (AURC) and capillary glucose (GLU) or mean arterial pressure (MAP).

Measure/Timepoint A	Measure/Timepoint B	Correlation Coefficient	*p*-Value
AURC $T1_{glu}$	GLU $T1_{glu}$	−0.432 *	0.261
AURC $T1_{glu}$	GLU $T2_{glu}$	−0.346	0.502
AURC $T1_{all}$	MAP $T1_{all}$	−0.127	0.978
AURC $T1_{all}$	MAP $T2_{all}$	0.078	0.996
AURC $T2_{glu}$	GLU $T2_{glu}$	−0.366	0.440
AURC $T2_{glu}$	GLU $T3_{glu}$	−0.492 *	0.144
AURC $T2_{all}$	MAP $T2_{all}$	0.017	1.000
AURC $T2_{all}$	MAP $T3_{all}$	−0.167	0.941
Ave AURC $T1_{glu}$ & $T2_{glu}$	Ave GLU $T1_{glu}$, $T2_{glu}$ & $T3_{glu}$	−0.351	0.283
Ave AURC $T1_{all}$ & $T2_{all}$	Ave MAP $T1_{all}$, $T2_{all}$ & $T3_{all}$	−0.089	0.924

Correlations were tested between relative change in AURC and GLU for the adjacent timepoint on the glucose visit only ($T1_{glu}$, $T2_{glu}$ and $T3_{glu}$). Mean arterial pressure correlations were calculated for treatment-averaged data at each timepoint ($T1_{all}$, $T2_{all}$ and $T3_{all}$). Correlations between the average change across all post-drink measures were also examined (bottom). All correlations were carried out using Pearson's r unless data were not normally distributed, in which case Spearman's rho (indicated by *) was used. Bonferroni correction was applied to all correlations.

Table 4. Results from preliminary ANOVAs confirming the presence of inhibition and no differences between T0 data for each measure.

Measure.	Factor	*df*	F	*p*-Value
AURC	TREATMENT (T0)	2,34	0.162	0.851
SICI	TREATMENT (T0)	2,32	0.206	0.732
Water	PATTERN	1,16	14.902	0.001
	TIME	2,32	0.463	0.560
	PATTERN × TIME	2,32	1.348	0.269
Sucralose	PATTERN	1,15	40.673	<0.001
	TIME	2,30	1.381	0.267
	PATTERN × TIME	2,30	3.372	0.069
Glucose	PATTERN	1,17	28.103	<0.001
	TIME	2,34	4.191	0.024
	PATTERN × TIME	2,34	1.384	0.264
SAI	TREATMENT (T0)	2,34	0.325	0.725
Water	PATTERN	1,16	49.518	<0.001
	TIME	2,32	0.002	0.998
	PATTERN × TIME	2,32	0.664	0.552
Sucralose	PATTERN	1,17	52.058	<0.001
	TIME	2,34	0.892	0.419
	PATTERN × TIME	2,34	0.345	0.710
Glucose	PATTERN	1,17	24.709	<0.001
	TIME	2,34	0.477	0.625
	PATTERN × TIME	2,34	0.100	0.905
LAI	TREATMENT (T0)	2,34	0.458	0.637
Water	PATTERN	1,16	16.100	0.001
	TIME	2,32	0.214	0.809
	PATTERN × TIME	2,32	2.256	0.121
Sucralose	PATTERN	1,17	28.422	<0.001
	TIME	2,34	0.858	0.414
	PATTERN × TIME	2,34	0.017	0.983
Glucose	PATTERN	1,17	8.101	0.011
	TIME	2,34	2.978	0.064
	PATTERN × TIME	2,34	1.024	0.370

AURC: area under the recruitment curve, SICI: short-interval intracortical inhibition, SAI: short-latency afferent inhibition, LAI: long-latency afferent inhibition.

3.3. SICI

The presence of SICI was confirmed by two-way ANOVAs which showed the main effects of PATTERN for each treatment (Table 4), indicating that inhibition was observed at all timepoints. There was no increase in SICI following glucose (Figure 3a) and there was no effect of TREATMENT, TIME or TREATMENT × TIME observed in the ANOVA (Table 2).

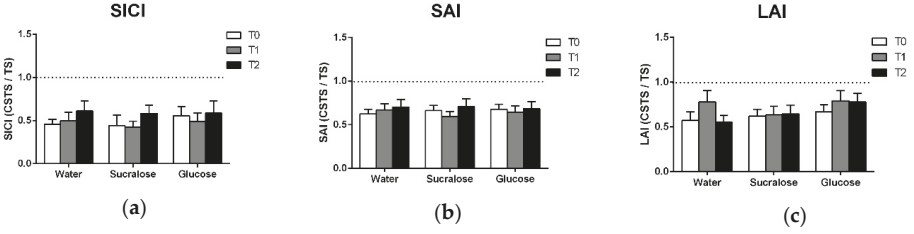

Figure 3. Means and standard errors of (**a**) SICI, (**b**) SAI and (**c**) LAI data. All data are expressed as the ratio of the conditioned response (CSTS) to the unconditioned response (TS) such that the degree to which the ratio falls below 1.0 (i.e., the dotted line) reflects the magnitude of inhibition observed.

3.4. Afferent Inhibition

For SAI and LAI, two-way ANOVAs confirmed the presence of significant inhibition (Table 4). There was no effect of TREATMENT, TIME or TREATMENT × TIME (Table 2, Figures 2c and 3b).

3.5. Mean Arterial Pressure

Figure 4 shows a main effect of TIME ($F_{(2,12}$ = 15.119; $p < 0.001$), with significant increases in mean arterial pressure at all post-drink pressure which were observed across all treatments. Post-hoc analysis of treatment-averaged MAP data indicates that this increase from baseline (T0) was evident at T1, T2 and T3 ($p < 0.001$) (Figure 4a). There were also no correlations between the increase in mean arterial pressure across treatments and changes in AURC at T1 or T2 (Table 3).

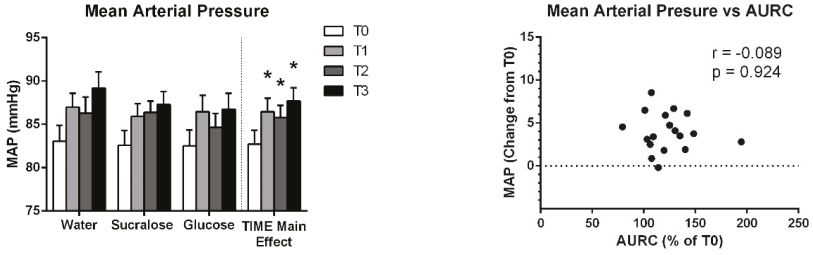

Figure 4. (**a**) Means and standard errors of mean arterial pressure for each treatment and for the treatment-averaged data showing the effect of TIME. (**b**) Average increase in mean arterial pressure across T1, T2 and T3 plotted against the average relative increase in AURC across the T1 and T2 TMS testing bouts. * indicates a significant difference from baseline ($p < 0.05$).

4. Discussion

The primary finding of this study is that glucose did not lead to an increase in corticospinal excitability. Further, there was no change in SICI, SAI or LAI. However, we did observe that all treatments contributed to an increase in corticospinal excitability and mean arterial pressure. This increase in excitability was not related to the increase in mean arterial blood pressure. We will

discuss below the lack of glucose effects on TMS measures and the possible role of hydration and prolonged brain stimulation on changes in mean arterial pressure.

The increase in AURC across all conditions contrasts with previous research reporting an increase in corticospinal excitability following glucose ingestion and not after a no-calorie placebo [10]. Notably, Specterman and colleagues [10] tested a small sample size (n = 4) compared to the present study. They also measured corticospinal excitability by quantifying the average size of MEPs evoked at 110% of RMT, while the present study did so across the multiple intensities of a recruitment curve. Therefore, it is possible that the contrasting results reflect a combination of differences in the study population and measurement protocol.

The finding that glucose did not alter SICI is in line with the results reported by Badawy and colleagues [9], who found that SICI was not significantly different after 12 h of fasting versus 2 h after a meal. It should be noted that the authors did report significantly greater LICI after feeding than after fasting. These findings suggest that glucose has a different effect on LICI versus SICI. Indeed, each measure is thought to reflect the activity of different neurotransmitter receptors, with $GABA_A$ receptors mediating SICI and $GABA_B$ receptors involved in LICI [28,29].

Intake of glucose, sucralose or water did not change measures of SAI or LAI. SAI and LAI are impaired in multiple neurodegenerative conditions, including Alzheimer's and Parkinson's disease [30], and have potential clinical utility as diagnostic tools or biomarkers of sensorimotor function. Therefore, it is important to establish whether factors of daily living influence the acquisition of these measures. The present study suggests that neither elevated glucose levels or hydration change the magnitude of SAI or LAI. The results also suggest that the weakening of SAI/LAI in these neurodegenerative populations [12,13] does not reflect the impairment in glucose metabolism.

Increased AURC at T1 and T2 coincided with a treatment-nonspecific rise in mean arterial pressure, suggesting influence from factors intrinsic to the testing protocol. These factors may include hydration, discomfort with the testing setup (i.e., a white-coat effect) or the delivery of a high number of TMS pulses over M1. Since participants were not asked to dehydrate themselves overnight, and the 300 mL drink is a relatively low volume of fluid, it seems unlikely that hydration was a major factor with respect to mean arterial pressure. It is more likely that changes in emotional state due to fatigue or level of interest, or the repetitive stimulation itself could have contributed to the observed TIME effects. The sensorimotor cortex has functional connections with brainstem structures involved in the regulation of vasomotor tone, which are modulated by cortical stimulation [31]. Transcranial direct-current stimulation of the sensorimotor area has been shown to acutely suppress blood pressure and adrenocorticotropic hormone levels [32]. If TMS changes blood pressure similarly to direct-current stimulation, it is possible that an association between changes in mean arterial pressure and AURC was masked by acute effects of TMS delivered at T1 and T2. In addition, Binkofski et al. [32] described longer-latency changes (1–2 h) in cerebral energy metabolism and glucose uptake following brain stimulation, suggesting that baseline TMS could have masked relationships between glucose and AURC at T1 and T2. This may provide an explanation for the contrasting results with Specterman et al. [10], who delivered only 45 TMS pulses at baseline and 15 per timepoint. They observed a positive association between glucose level and MEP size, while we delivered 180 pulses at T0, T1 and T2 and did not observe the same relationship.

In the present study, the timing of T1 was based on the latency of peak glucose levels obtained on Visit 1. The purpose of this protocol was to maximize the opportunity to observe a change in corticospinal excitability following glucose ingestion. However, the day-to-day variability in glucose metabolism was not assessed. Therefore, while we observed a significant rise in glucose levels following ingestion of the solution in the glucose session, the latency of peak glucose may have varied from day-to-day.

Future Considerations

It is important for future research to attempt to replicate previously observed effects of glucose on TMS measurements, using testing protocols which consider potential confounding factors such as the number of TMS pulses, session duration and hydration. The effect of TMS pulse load on changes in glucose levels and sympathetic tone should also be investigated to facilitate the interpretation and design of future TMS research. While glucose did not change our TMS measures, other dietary factors such as caffeine consumption [10,33], prolonged fasting [34] and ketogenic diets [35] merit further investigation as these may be important and easily modifiable factors in TMS research.

5. Conclusions

The present study found no explicit effect of glucose on corticospinal excitability, intracortical inhibition or afferent inhibition, but corticospinal excitability and mean arterial pressure increased across all treatments over the course of the experiment. These non-treatment-specific increases suggest that TMS measurements could be sensitive to various confounding factors related to repeated magnetic stimulation of the cortex, hydration, or fatigue. Further investigation of the influence of diet and acute carbohydrate consumption is warranted. However, studies should first directly examine the impact of the aforementioned confounding factors so that they can be effectively taken into account during the design and interpretation of research on this topic. Findings from such studies will work to reduce the likelihood that confounding effects arising from changing brain metabolism or autonomic modulation complicate the interpretation of TMS data.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-3425/9/12/339/s1, Table S1: AURC, Table S2: SICI MEP size and SICI ratio, Table S3: SAI MEP size and SAI ratio, Table S4: LAI MEP size and LAI ratio, Table S5: Glucose, Table S6: Mean Arterial Pressure.

Author Contributions: Conceptualization, S.L.T., C.V.T. and A.J.N.; Data curation, S.L.T. and A.J.N.; Formal analysis, S.L.T. and C.V.T.; Funding acquisition, A.J.N.; Investigation, S.L.T., C.V.T., M.B.L., C.N. and R.R.; Methodology, S.L.T., C.V.T. and A.J.N.; Project administration, S.L.T. and A.J.N.; Resources, A.J.N.; Software, A.J.N.; Supervision, S.L.T. and A.J.N.; Validation, A.J.N.; Visualization, S.L.T., C.V.T. and A.J.N.; Writing—original draft, S.L.T.; Writing—review and editing, S.L.T., C.V.T., M.B.L., C.N., R.R. and A.J.N.

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Exercise-Induced Fatigue in One Leg Does Not Impair the Neuromuscular Performance in the Contralateral Leg but Improves the Excitability of the Ipsilateral Corticospinal Pathway

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Abstract: To investigate the influence of pre-induced fatigue in one leg on neuromuscular performance and corticospinal responses of the contralateral homologous muscles, three experiments were conducted with different exercise protocols; A (n = 12): a 60 s rest vs. time-matched sustained left leg knee extension maximum voluntary contraction (MVC), B (n = 12): a 60 s rest vs. time-matched left leg MVC immediately followed by 60 s right leg MVC, and C (n = 9): a similar protocol to experiment B, but with blood flow occluded in the left leg while the right leg was performing the 60 s MVC. The neuromuscular assessment included 5 s knee extensions at 100%, 75%, and 50% of MVC. At each force level, transcranial magnetic and peripheral nerve stimuli were elicited to investigate the influence of different protocols on the right (tested) knee extensors' maximal force output, voluntary activation, corticospinal excitability, and inhibition. The pre-induced fatigue in the left leg did not alter the performance nor the neuromuscular responses recorded from the right leg in the three experiments (all $p > 0.3$). However, enhanced corticospinal pathway excitability was evident in the tested knee extensors ($p = 0.002$). These results suggest that the pre-induced fatigue and muscle ischemia in one leg did not compromise the central and peripheral components of the neuromuscular function in the tested contralateral leg.

Keywords: cross-over fatigue; isometric contraction; force; voluntary activation; transcranial magnetic stimulation

1. Introduction

Non-local muscle fatigue (NLMF) refers to a condition in which exercise-induced fatigue in a muscle group transiently impairs neuromuscular function in another limb [1]. In the absence of change in the contractile machinery of the non-exercised muscles (i.e., the index of peripheral fatigue), any attenuation in the neuromuscular function of these muscles is attributed to central mechanisms [2–4]. Presently, these central modulations are not well understood. However, it has been postulated that the fatiguing exercise-induced activation of group III and IV metabo- and mechano-receptors could compromise the motor control and execution of the non-fatigued muscles [5,6].

Transcranial magnetic stimulation (TMS) of the motor cortex in conjunction with electrical stimulation of the peripheral nerve (PNS) are non-invasive techniques that could be employed to investigate the central and peripheral mechanisms of fatigue [7]. Although several investigations have

explored the influence of lower limb exercise-induced fatigue on responsiveness of the corticospinal pathway innervating upper limb muscles (heterologous muscle groups) [3,5,8], currently, there is little empirical evidence pertaining to the influence of a fatiguing exercise performed by a lower limb muscle group on central voluntary activation and corticospinal responses of the contralateral homologous muscles.

A conventional research paradigm used to study the concept of NLMF includes a fatiguing exercise in one limb followed by the neuromuscular function assessment of the contralateral (non-fatigued) limb quantified during a single (4–6 s) maximum voluntary contraction (MVC) [9–13]. Given that in this approach the post-fatigue MVC is very brief, it has been postulated that participants might be able to overcome the sense of the pre-induced fatigue and maintain the MVC force output and central voluntary activation (VA) in the tested muscle group [4,10]. In an alternative paradigm, some investigators have recently used long-term sustained or intermittent MVCs performed by the tested muscle groups following the already fatigued contralateral limb [6,14,15]. The pre-induced fatigue in this approach has been shown to promote an augmented sense of fatigue and consequently, to attenuate the cycling performance in the tested contralateral limb [6]. However, there is little documented research that has studied this approach using sustained maximal isometric contractions. Halperin et al. [15] demonstrated a significant decline in the force output and VA (using the twitch interpolated technique) recorded during 12 (5 s) knee extension (KE) MVCs following a fatiguing exercise in the contralateral leg. On the contrary, Kennedy et al. [9] did not find any impairment in the MVC force, and VA recorded during 8 (2–3 s) KE MVCs. The controversy regarding these paradoxical findings was further fuelled by the fact that Kennedy et al. [9] maintained muscle ischemia in the fatigued leg to further activate the group III and IV muscle afferents, yet they did not find any change in MVC force and VA of the contralateral tested muscles. These findings argue against the idea that activation of group III and IV muscle afferents in one lower limb muscle could compromise the isometric performance in the contralateral limb. In addition to these controversies, none of the above-mentioned studies have explored the voluntary cortical activation (using TMS) nor the excitability and inhibition of the corticospinal pathway innervating the tested muscle groups following fatigue induced in the contralateral leg.

Thus, the purpose of the present study was threefold. In experiment A, we investigated whether fatiguing exercise in one leg (e.g., left) could alter the neuromuscular performance, corticospinal excitability, and inhibition of the tested (right) knee extensors recorded during brief maximal contraction performed immediately after the pre-induced fatigue. In experiment B, we explored whether pre-induced fatigue (in the left leg) combined with the subsequent sustained contraction (in the right leg) could alter the muscle performance and corticospinal responses in the tested (right) leg. In these two experiments, the group III and IV muscle afferents originating from the left knee extensors would stop firing after the termination of the sustained MVC in the leg [16]. Therefore, we hypothesized that the pre-induced left leg fatigue would not alter the neuromuscular performance recorded in the tested contralateral leg. In order to extend the input from group III and IV muscle afferents, we chose to maintain muscle ischemia in the fatigued (left) leg [16,17]. Thus, in experiment C, we investigated whether the prolonged activation of afferent receptors could modulate the neuromuscular performance and corticospinal responses in the tested (right) knee extensors. We hypothesized that maintaining blood ischemia in the fatigued leg could modulate the central motor drive and, consequently, the neuromuscular responses in the tested muscles.

2. Materials and Methods

2.1. Participants

Twelve healthy, recreationally active participants (six females: 22 ± 3 years, 168 ± 7 cm, 65 ± 1 kg; and six males: 25 ± 1 years, 175 ± 4 cm, 74 ± 9 kg) provided informed consent to participate in experiments A and B. Five participants from these two experiments and four additional participants

volunteered to complete experiment C (three females: 26 ± 3 years, 169 ± 9 cm, 66 ± 9 kg; and six males: 30 ± 4 years, 173 ± 6 cm, 76 ± 8 kg). Participants did not have any history of neurological, cardiovascular, or musculoskeletal injuries and were determined as right-leg dominant based on the preferred leg used to kick a ball [18]. They were instructed to refrain from rigorous physical activity, as well as ingesting caffeine and alcohol at least 24 hours prior to each testing session. Participants also completed the TMS safety checklist [19] and Physical Activity Readiness Questionnaire for Everyone PAR-Q+ form [20]. The procedures were conducted in accordance with the declaration of Helsinki and approved by the Health Research Ethics Authority of the University of Calgary (REB17-0147).

2.2. Experimental Setup and Procedures

The experimental protocol was performed on an isometric chair (Kin–Com, Chattecx Corporation, TN, USA) while the hips and knees were fixed at 90°. To ensure that the upper body did not contribute to the KE force, straps along the trunk and waist were secured, and participants crossed arms to shoulders during contractions. Left and right legs were each secured inside a padded ankle cuff attached to a strain gauge (LC101-2K; Omegadyne, Sunbury, OH, USA) with a non-extensible strap. Force data was sampled at 2000 Hz, digitally converted using the Power Lab acquisition hardware and Lab Chart software (ADInstruments, Bella Vista, Australia), and monitored on a computer. The electromyography (EMG) was recorded from the left and right knee-extensors, and both TMS and PNS were employed during the neuromuscular evaluations [21].

Bipolar self-adhesive Ag/AgCl surface EMG electrodes (Kendall MediTrace, MA) were positioned on the muscle belly of the right and left vastus lateralis (VL), rectus femoris (RF), and biceps femoris (BF) [22]. A reference electrode was placed on the patella of the right leg. Before the placement of electrodes, the area of skin was shaved and abraded to remove dead skin with sandpaper and cleaned with an isopropyl alcohol swab to decrease skin resistance. An inter-electrode impedance of <5 kΩ was obtained prior to recording to ensure an adequate signal-to-noise ratio. All EMG signals were digitized at a sampling rate of 2000 Hz by the PowerLab system (16/30-ML880/P, ADInstruments) and amplified with an octal bio-amplifier (ML138, ADInstruments). EMG signals were bandpass filtered (5–500 Hz) and all data were analyzed offline using the Labchart 8 software (ADInstruments).

Single-pulse TMS was manually delivered to the left motor cortex via a 110 mm concave double-cone coil (maximum output of 1.4 T) connected to a magnetic stimulator (Magstim 200^2 Company Ltd., Whitland, UK). The optimal location of the coil was determined on the scalp for every centimeter from 2 cm anterior and 1 cm to the left of the vertex (i.e., the midpoint from nasal–inion and tragi). Single pulses of TMS at 50% of maximum stimulator output (MSO) were delivered during brief (2–3 s) knee extensions at 20% of MVC and the location which evoked the highest twitch force and motor evoked potential (MEP) amplitudes for VL and RF (without an increase in BF MEP amplitude) was marked on a latex swim cap worn by participants. The optimal stimulator intensity was determined by delivering four stimuli at each intensity between 20 to 80% of the MSO (with 10% increments, in random order) and the intensity that elicited the highest twitch force, as well as VL and RF MEP amplitudes, was used for the rest of the session. The size of superimposed twitch (SIT), VL, or RF amplitude showed a plateau up to 80% of MSO for all participants, thus further increase in TMS intensities (90% or 100% MSO) was not necessary. The average stimulating intensity was 61 ± 8% (range: 40%–80%) of the maximal stimulator output for the six testing sessions.

Square-wave percutaneous electrical stimuli were delivered to the femoral nerve using a constant current stimulator (DS7A, Digitimer, Welwyn Garden City, UK). The cathode electrode (Kendall MediTrace, MA, USA) was secured on top of the inguinal triangle, and the anode (50 × 90 mm electrode, Durastick Plus, CA, USA) was placed in between greater trochanter and suprailiac projections. The stimuli intensity (pulse duration: 1000 µs, maximal voltage: 400 V) was increased in incremental steps until a maximal twitch force and compound muscle action potential (i.e., VL and RF Mmax) were observed. The supramaximal stimulation (i.e., 130% of stimulator output that achieved maximal twitch

and Mmax responses) was held constant throughout the protocol. The average stimulating intensity was 126 ± 52 mA (range: 65–185 mA) for the six testing sessions.

Experiment A (n = 12). Neurophysiological responses recorded immediately after a contralateral fatiguing exercise. In two randomly selected testing sessions, the fatigued (left) leg underwent either 60 s sustained KE MVC (Fatigue; Ftg-0) or 60 s rest (Rest-0) (Figure 1A). Before and immediately after these two protocols, the tested (right) leg performed a neuromuscular evaluation including a 5 s KE MVC superimposed with a PNS delivered on the force plateau and another PNS evoked 2 s after the MVC on the relaxed muscle (i.e., potentiated twitch, Pt). Three seconds after the Pt, subjects performed another 5 s MVC followed by contractions at 75% and 50% of MVC. Single-pulse TMS and PNS were delivered at 100%, 75%, and 50% of MVC with ~2 s interstimulus intervals, during which participants would reobtain the target force following each TMS interference to the voluntary force output. The resting interval between 100%, 75%, and 50% MVC was ~5 s. Two sets of neuromuscular evaluations were performed before and one set was performed immediately after each intervention. Additionally, immediately before and after each left leg protocol (either rest or 60 s sustained MVC), a PNS was evoked to the right leg at rest to account for any potential influence of contralateral protocols on contractile property and excitability of the tested muscles.

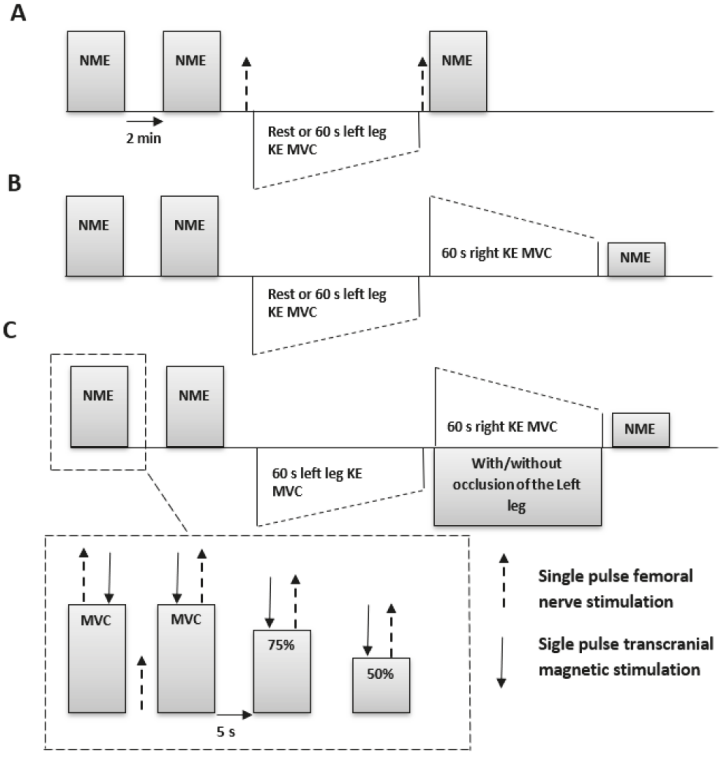

Figure 1. Details of experimental protocols. In experiment A (panel **A**) (n = 12), a 60 s rest or 60 s sustained left leg knee extension (KE) maximum voluntary contraction (MVC) were followed by a neuromuscular evaluation (NME) including 5 s sustained right leg KEs at 100%, 75%, and 50% of MVC. In experiment B (panel **B**) (n = 12), a 60 s rest or 60 s left leg MVC was immediately followed by a 60 s right leg MVC. In experiment C (panel **C**) (n = 9), a similar protocol as experiment B was performed while blood flow was occluded in the left leg while the right leg was performing a 60 s MVC.

Experiment B (n = 12). Neurophysiological responses recorded after a sustained MVC superimposed upon the pre-induced contralateral fatigue. In two randomly selected testing sessions (Figure 1B), participants undertook 60 s rest (Rest-60) or 60 s left leg sustained MVC (Ftg-60), followed immediately by 60 s sustained right leg MVC. Neuromuscular evaluations, as described in experiment A, were performed before and immediately after each experimental protocol.

Experiment C (n = 9). Influence of maintaining muscle ischemia in the fatigued contralateral leg on the neurophysiological responses of the tested muscles. In two randomly selected testing sessions, participants underwent the Ftg-60 protocol (see experiment B) with the exception of the "cuff session (Ftg-60-Occl), where blood flow to the left (fatigued) leg was occluded 2 s prior to the end of 60 s MVC (Figure 1C). A standard single-bladder adult thigh cuff connected to an automatic rapid inflation system (Hokanson E20 AG101, Bellevue, WA, USA) was located at the distal part of the thigh. The cuff was inflated to 300 mmHg using compressed air within 2 s.

2.3. Data Analysis

Performance. A force-time integral including the sum of the data points, multiplied by the sample interval during the 60 s sustained MVCs (performed by the tested leg) was calculated in experiments B and C.

Neuromuscular function. The maximal force output and the corresponding root mean square EMG [rmsEMG of the VL and RF muscles] was quantified during the first and last 2 s intervals of the sustained 60 s left and right leg MVCs, as well as over 500 ms prior to each TMS evoked during brief right leg MVCs. The VL and RF rmsEMG were normalized to the amplitude of the corresponding muscle compound action potential (Mmax) recorded during the same contraction to calculate the rmsEMG·Mmax^{-1} ratio (rmsEMG$_{100}$).

The central voluntary activation (VA$_{PNS}$) was measured using the twitch interpolation technique [23] with the following formula, where Fb is the voluntary force output just before the PNS, D is the difference between Fb and the maximum SIT force evoked by the stimulus, F_{MAX} is the highest force output during MVC, and FPt is the size of Pt force evoked at rest [24].

$$VA\ (\%) = 100 - D \times (Fb/F_{MAX})/FPt \times 100 \quad (1)$$

The voluntary cortical activation (VA$_{TMS}$) was assessed using the amplitude of SITs evoked by TMS during 5 s contractions at 100%, 75%, and 50% of MVC [25,26]. The y-intercept of the linear regression ($r^2 > 0.9$) between the SITs was computed to identify the estimated resting twitch (ERT). VA$_{TMS}$ was then calculated using the following equation:

$$VA_{TMS} = [1 - (SIT/ERT)] \times 100 \quad (2)$$

In order to explore the excitability of the corticospinal pathway, the areas under the MEP and the Mmax signals were measured for both VL and RF muscles. The onset of MEP and Mmax were defined as the point at which the voltage trace became tangential to baseline in either the positive or negative direction. The responses to VL and RF MEP were normalized to the subsequent Mmax recorded during the same contraction to calculate MEP·Mmax^{-1} ratios (MEP$_{100}$, MEP$_{75}$, MEP$_{50}$). The duration (ms) of the silent period (i.e., indicative of corticospinal inhibition) was assessed for VL and RF MEPs as the interval from the stimulus artifact to the return of the continuous EMG by visual inspection during 100%, 75%, and 50% of MVC contractions (SP$_{100}$, SP$_{75}$, SP$_{50}$). The end of the SP for two participants in experiments A and B was not distinguishable, so the values were removed from the data pool.

2.4. Statistical Analysis

Statistical analyses were computed using SPSS software (version 23.0; SPSS, Inc., Chicago, IL, USA). Shapiro–Wilk and Mauchly tests were used to ensure the assumptions of normality and sphericity for all dependent variables, respectively. All variables were normally distributed. Greenhouse–Geisser

correction factor was applied when the assumption of sphericity was violated. Unless otherwise notified, sphericity assumed data were reported. For each experiment separately, a paired sample *t*-test was used to compare baseline measures and then a two-way repeated measure analysis of variances (ANOVA) was run to explore the effect of 2times (baseline vs. post-intervention values) × 2 conditions for all outcome variables. When ANOVAs showed significant main effects of times or conditions, Bonferroni post hoc test was used to compare values. When an interaction effect was observed, paired *t*-tests with Holm–Bonferroni corrections were applied. In one case where baseline measures revealed a significant difference between conditions (i.e., MEP_{100} in experiment A), the post-intervention values were normalized to the baseline (to account for the day to day variations), and then the paired *t*-test was used to compare the relative values between different conditions. Paired *t*-test was used to compare the force-time integral values (calculated during the 60 s MVCs performed by the tested leg) between the two conditions in each experiment. The effect size was calculated for repeated measure ANOVAs by converting partial eta-squared to Cohen's d and for paired comparisons by using the mean ± SD values [27]. According to Cohen [27], the magnitude of effect size was classified as small ($0.2 \leq d < 0.5$), medium ($0.5 \leq d < 0.8$), and large ($d \geq 0.8$). The mean ± SD of variables are presented in the text, Figures 1–5, and the Table.

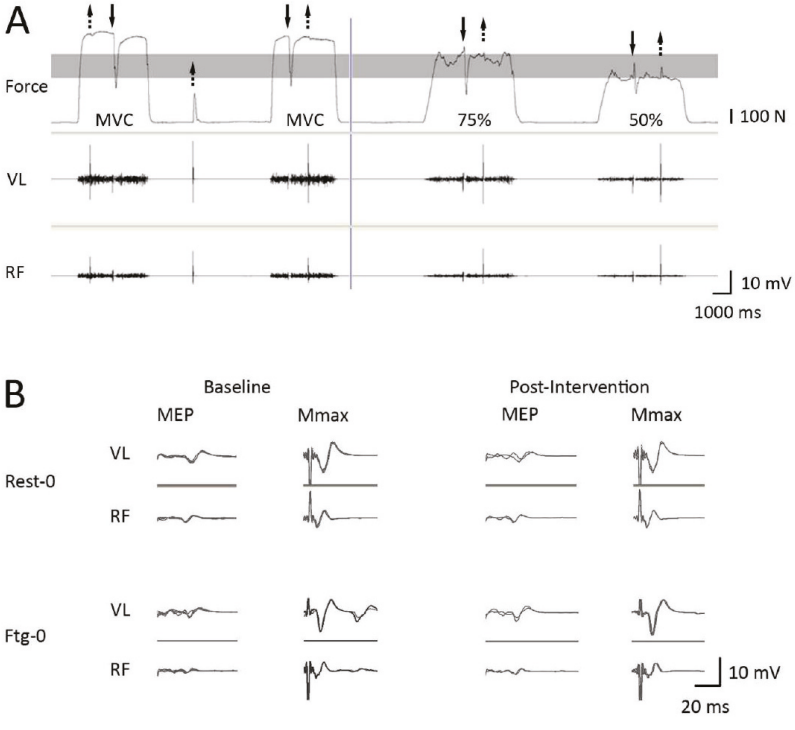

Figure 2. Representative traces from a single subject for the force output and VL and RF EMG signals at 100%, 75%, and 50% of MVC (panel **A**) and the MEPs and Mmaxs recorded from VL and RF at 100% of MVC at baseline and post-intervention levels following a 60 s rest (Rest-0) or 60 s knee extension MVC with the contralateral limb (Ftg-0) (panel **B**). The top and the bottom line of the shaded grey box (in panel **A**) represents 75% and 50% of MVC, respectively. In panel **B**, four MEPs and four Mmaxs recorded at baseline and two MEPs and two Mmaxs recorded at the post-intervention level are superimposed (see Figure 1 for more details).

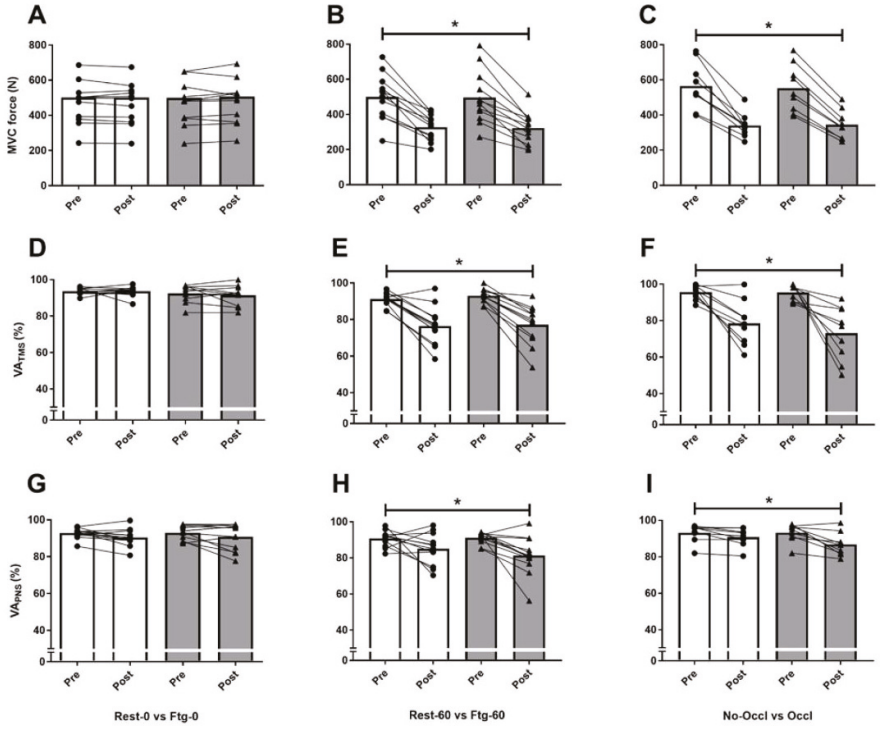

Figure 3. Mean and SD of knee extension (right leg) maximum voluntary contraction (MVC), voluntary activation (VA) using transcranial magnetic stimulation (VA_{TMS}), and peripheral nerve stimulation (VA_{PNS}) values normalized to the baseline. In experiments A (panels **A**,**D**,**G**), a 60 s rest (Rest-0) vs. a 60 s left leg MVC (Ftg-0) were compared. In experiment B (panels **B**,**E**,**H**), a 60 s rest (Rest-60) or a 60 s left leg MVC (Ftg-60) followed immediately by a 60 s right leg MVC were compared. In experiment C (panels **C**,**F**,**I**), Ftg-60 was performed at the absence or presence of a blood flow occlusion in the left leg (Ftg-60-Occl). * Significantly different from baseline (time effect).

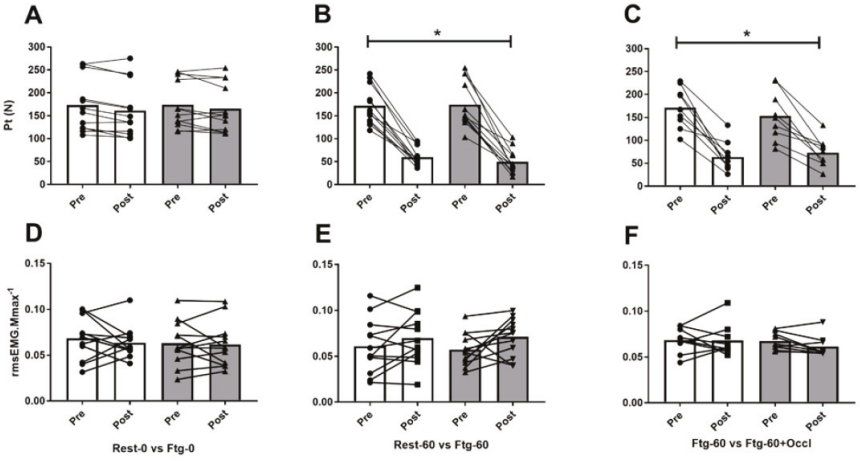

Figure 4. Mean and SD of potentiated twitch (Pt) and rectus femoris (RF) root mean square EMG (rmsEMG) values normalized to the baseline. In experiments A (panels **A**,**D**), a 60 s rest (Rest-0) or a 60 s left leg MVC (Ftg-0) were compared. In experiment B (panels **B**,**E**), a 60 s rest (Rest-60) or a 60 s left leg MVC (Ftg-60) followed immediately by a 60 s right leg MVC were compared. In experiment C (panels **C**,**F**), Ftg-60 was performed at the absence or presence of a blood flow occlusion in the left leg (Ftg-60-Occl). * Significantly different from baseline (time effect).

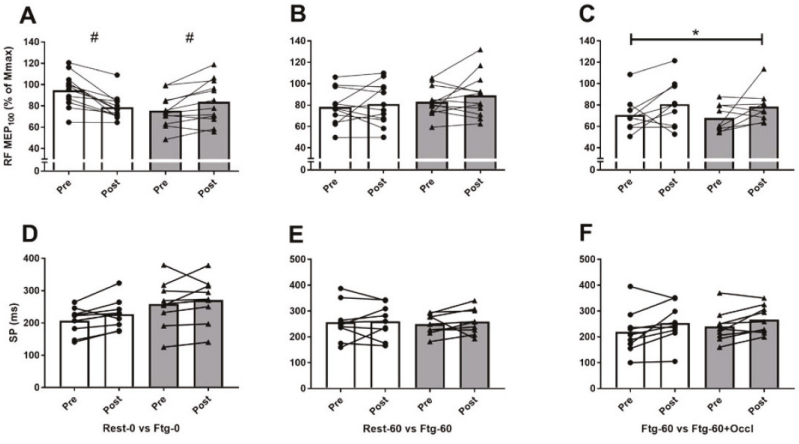

Figure 5. Mean and SD of motor evoked potential normalized to the subsequent muscle compound action potential (MEP·Mmax^{-1} [MEP]) and the duration of silent period (SP) recorded from the rectus femoris (RF) during MVC. The values are presented as a percentage of baseline. In experiments A (panels **A**,**D**), a 60 s rest (Rest-0) or a 60 s left leg MVC (Ftg-0) were compared. In experiment B (panels **B**,**E**), a 60s rest (Rest-60) or a 60 s left leg MVC (Ftg-60) followed immediately by a 60 s right leg MVC were compared. In experiment C (panels **C**,**F**), Ftg-60 was performed in the absence or presence of a blood flow occlusion in the left leg (Ftg-60-Occl). * Significantly different from baseline (time effect), # significantly different from baseline (interaction effect).

3. Results

Force changes during left (fatigued) leg sustained MVCs. The decline in the maximal force output recorded during the 60 s left KE MVC in three experiments was: −55% (mean ± SD: 471 ± 137 to 209 ± 67 N) following Ftg-0 in experiment A, −54% (477 ± 133 to 218 ± 54 N) following Ftg-60 in experiment

B, and −51% (529 ± 139 to 260 ± 35 N), and −56% (571 ± 170 to 251 ± 93 N) after Ftg-60 and Ftg-60-Occl in experiment C, respectively.

3.1. Experiment A

The pre-induced fatigue in the contralateral (left) leg did not alter MVC, $rmsEMG_{100}$, Pt, Mmax, VA_{PNS}, and VA_{TMS} recorded from the right (tested) leg in the Ftg-0 compared to Rest-0 condition (Figures 3A,D,G, and 4A,D). The resting twitch and corresponding Mmax evoked before and after 60 s left leg MVC (Figures 1A and 2B) also did not show any difference between the two conditions (data not shown). However, a significant interaction effect of time × group was observed for the corticospinal excitability ($MEP \cdot Mmax^{-1}$ ratio) recorded from VL ($F_{1,11} = 6.29$, $p = 0.031$, d = 1.58), and RF ($F_{1,11} = 13.33$, $p = 0.004$, d = 0.84) at 100% MVC (MEP_{100}). While, higher values were observed for VL ($p = 0.032$, d = 0.39) and RF ($p = 0.020$, d = 0.43) following the contralateral leg fatigue (in Ftg-0), a decline was observed for the resting condition (in Rest-0) (Figure 5A).

3.2. Experiment B

As expected, the MVC force ($F_{1,11} = 50.9$, $p < 0.001$, d = 4.29), Pt ($F_{1,11} = 109.3$, $p < 0.001$, d = 6.32), VA_{PNS} ($F_{1,11} = 7.21$, $p = 0.023$, d = 1.69) and VA_{TMS} ($F_{1,11} = 27.7$, $p = 0.001$, d = 3.71) significantly declined (all time effects) following 60 s right leg MVC. However, the pre-induced fatigue in the left leg did not result in any significant difference in the neuromuscular performance and corticospinal excitability and inhibition measures between Rest-60 and Ftg-60 conditions (Figures 3B,E,H, 4B,E and 5B,E).

3.3. Experiment C

The VA_{TMS} values from one participant were removed from the data pool because the linear regression line exhibited $r^2 < 0.9$. Similar to experiment B, the MVC force ($F_{1,8} = 84.3$, $p < 0.001$, d = 6.13), Pt ($F_{1,8} = 47.4$, $p < 0.001$, d = 4.87), VA_{PNS} ($F_{1,8} = 16.8$, $p = 0.005$, d = 3.09) and VA_{TMS} ($F_{1,7} = 16.8$, $p = 0.003$, d = 2.90) significantly declined and MEP_{100} ($F_{1,8} = 29.9$, $p = 0.001$, d =1.49) increased (all time effects) following 60 s right leg MVC. However, the pre-induced fatigue and the subsequent blood occlusion in the left leg (while the right leg was performing the 60 s MVC) did not result in any significant difference in the neuromuscular performance and corticospinal excitability and inhibition between Ftg-60 and Ftg-60-Occl conditions (Figures 3C,F,I, 4C,F and 5C,F).

The left leg pre-induced fatigue in experiments B and C did not alter the force-time integral values recorded across 60 s sustained right leg MVCs between Rest-60 (19,737 ± 6847 N·s) vs. Ftg-60 (18,264 ± 7413 Ns) as well as the Ftg-60 (22,422 ± 3645 Ns) vs. Ftg-60-Occl (22,744 ± 4197 Ns). In addition, the corticospinal excitability (MEP) and inhibition (SP) recorded from VL and RF during contractions at 100%, 75%, and 50% of MVC did not demonstrate any significance in the three experiments unless otherwise stated above. Since VL and RF rmsEMG, MEP, and SP demonstrated similar patterns, only the results of RF were presented in Figures 3–5.

4. Discussion

The most important findings of the present study are that (i) in line with our first hypothesis, the pre-induced left leg fatigue did not alter the right knee extensors neuromuscular function, while (ii) the VL and RF corticospinal excitability (MEP_{100}) significantly increased following the 60 s contralateral MVC (in Ftg-0) compared to a time-matched rest (in Rest-0), and (iii) contrary to our second hypothesis, the pre-induced fatigue, in combination with the subsequent left leg blood occlusion did not alter the neuromuscular function or performance (i.e., force-time integral during 60 s MVC) nor corticospinal responses between Ftg-60-Occl vs. Ftg-60 condition. These results confirm that 60 s sustained MVC-induced fatigue and subsequent muscle ischemia in one lower limb muscle, activating the group III and IV muscle afferents, does not compromise the neuromuscular performance in the contralateral limb when performed in isometric conditions.

4.1. MVC Force and Central Motor Drive

Multiple studies have found that exercise-induced fatigue in one leg may impair the neuromuscular function in the contralateral leg (for review see [1]). However, the results of the current study, in line with several other investigations [9–13], have failed to show the NLMF effect in the lower limb muscles. This lack of observable NLMF is apparent in a research paradigm where the post-intervention neuromuscular function is assessed during a single, brief (4–6 s) MVC [6,11,28,29]. It was hypothesized that in this paradigm, despite a general sensation of fatigue created by the pre-induced fatiguing task, participants would be able to maintain the level of central motor commands to the contralateral tested muscles to prevent a deterioration in maximal performance. In line with this hypothesis, the indices of central fatigue, including $rmsEMG_{100}$ as well as the central neural drive (measured by VA_{PNS}) and neural drive associated with circuits at or above the cortical motor cells (measured by VA_{TMS}), did not change following Ftg-0 and Rest-0 conditions. These findings confirm that a 60 s MVC in one leg does not result in any central constraint to the performance of the tested contralateral muscles when the post-fatigue assessment includes a brief MVC [4,30].

In an alternative research paradigm, Halperin et al. [4,15] demonstrated that MVC force and VA_{PNS} were compromised when 12 × 5 s MVCs (as opposed to a single MVC) were performed following the fatiguing contralateral limb. Contrary to their observation, however, Kennedy et al. [9] did not find any impairment in the MVC force and VA_{PNS} recorded during 8 × 3 s MVCs, despite the fact that these investigators maintained muscle ischemia in the fatigued leg to further activate the group III and IV muscle afferents. The results of our study (in experiments B and C) support those reported by Kennedy et al. [9] indicating that adding 60 s sustained MVC in combination with subsequent muscle ischemia to the contralateral leg does not alter the central ($rmsEMG_{100}$, VA_{PNS}, and VA_{TMS}) and peripheral (Pt and Mmax) indices of fatigue in the tested limb. Of note, in experiments B and C, while VA_{TMS} demonstrated 18 to 25% reduction (Table 1, Figure 3E,F), only 3% to 10% decrement was observed for VA_{PNS} (Table 1, Figure 3H,I). Although one may take these results to suggest that different sites within the central nervous system would play distinct roles in impaired maximal force output after the fatiguing task, limitations associated with the interpretation of the voluntary activation data [31] make it difficult to draw an explicit conclusion about the results. Nonetheless, pre-induced fatigue and muscle ischemia in the contralateral limb did not alter the contribution of cortical and motoneuronal circuits in the end-exercise central fatigue level.

Table 1. Group data (mean and SD) for variables recorded from the knee extensor muscles during 100%, 75%, and 50% of MVC in the three experiments.

Variables		Experiment A		Experiment B		Experiment C	
		Rest-0	Ftg-0	Rest-60	Ftg-60	Ftg-60	Ftg-60-Occl
MVC force (N)	Baseline	501.7 ± 159.9	499.9 ± 158.7	507.1 ± 148.2	495.3 ± 150.3	555.0 ± 128.8	573.9 ± 144.1
	Post-test	501.1 ± 170.7	506.3 ± 158.6	334.8 ± 88.9	322.2 ± 89.9	349.9 ± 71.1	363.6 ± 96.7
rmsEMG·Mmax^{-1} ratio (rmsEMG$_{100}$)	Baseline	0.074 ± 0.031	0.070 ± 0.037	0.067 ± 0.034	0.066 ± 0.026	0.063 ± 0.030	0.068 ± 0.026
	Post-test	0.069 ± 0.028	0.069 ± 0.044	0.066 ± 0.026	0.078 ± 0.029	0.066 ± 0.031	0.072 ± 0.038
PT (N)	Baseline	173.2 ± 54.4	173.7 ± 51.7	171.8 ± 41.7	174.5 ± 50.4	171.5 ± 44.3	153.4 ± 54.5
	Post-test	161.4 ± 59.1	165.6 ± 52.9	59.9 ± 20.2	49.8 ± 26.1	63.5 ± 33.2	72.8 ± 31.1
VA$_{PNS}$ (%)	Baseline	92.7 ± 2.9	93.1 ± 3.8	90.7 ± 4.5	91.1 ± 3.2	93.1 ± 5.1	93.2 ± 5.2
	Post-test	90.6 ± 4.9	90.7 ± 7.5	85.1 ± 9.1	81.2 ± 11.2	90.6 ± 5.2	86.7 ± 6.7
VA$_{TMS}$ (%)	Baseline	93.6 ± 1.9	92.6 ± 5.2	91.1 ± 4.2	93.1 ± 4.4	94.8 ± 5.5	85.4 ± 4.5
	Post-test	93.4 ± 3.1	91.2 ± 6.1	73.3 ± 18.3	76.6 ± 12.6	78.4 ± 12.2	70.1 ± 17.1
RF MEP·Mmax^{-1} (MEP$_{100}$)	Baseline	0.93 ± 0.18	0.80 ± 0.21	0.77 ± 0.18	0.83 ± 0.14	0.70 ± 0.16	0.67 ± 0.12
	Post-test	0.82 ± 0.18 #	0.90 ± 0.25 #	0.79 ± 0.21	0.88 ± 0.21	0.80 ± 0.22	0.78 ± 0.22
RF MEP·Mmax^{-1} (MEP$_{75}$)	Baseline	0.94 ± 0.19	0.84 ± 0.23	0.87 ± 0.20	0.92 ± 0.15	0.82 ± 0.15	0.81 ± 0.15
	Post-test	0.87 ± 0.22	0.82 ± 0.20	0.83 ± 0.22	0.89 ± 0.11	0.92 ± 0.24	0.84 ± 0.33
RF MEP·Mmax^{-1} (MEP$_{50}$)	Baseline	0.96 ± 0.15	0.90 ± 0.19	0.89 ± 0.18	0.97 ± 0.13	0.89 ± 0.14	0.81 ± 09
	Post-test	0.95 ± 0.16	0.88 ± 0.19	0.87 ± 0.14	0.88 ± 0.22	0.97 ± 0.34	0.79 ± 0.28
RF SP$_{100}$ (ms)	Baseline	206.7 ± 45.2	258.5 ± 78.3	257.5 ± 78.6	249.3 ± 41.6	219.1 ± 84.6	240.6 ± 60.5
	Post-test	227.3 ± 50.0	270.9 ± 74.7	260.1 ± 69.7	259.3 ± 52.0	253.4 ± 75.3	267.4 ± 53.7
RF SP$_{75}$ (ms)	Baseline	211.4 ± 48.3	256.8 ± 59.6	267.7 ± 64.0	252.0 ± 54.3	214.1 ± 77.7	208.5 ± 96.8
	Post-test	226.1 ± 67.3	270.3 ± 77.0	254.1 ± 70.3	254.9 ± 56.5	231.6 ± 67.5	250.2 ± 55.9
RF SP$_{50}$ (ms)	Baseline	231.6 ± 40.9	276.4 ± 61.4	270.0 ± 69.1	273.1 ± 45.9	207.3 ± 71.8	213.1 ± 47.4
	Post-test	248.9 ± 63.2	269.8 ± 61.2	256.9 ± 61.2	261.5 ± 55.6	248.5 ± 74.6	253.2 ± 77.3
BF MEP$_{100}$ (mV.s)	Baseline	0.009 ± 0.005	0.010 ± 0.008	0.007 ± 0.004	0.009 ± 0.005	0.008 ± 0.005	0.008 ± 0.004
	Post-test	0.007 ± 0.006	0.009 ± 0.006	0.007 ± 0.005	0.007 ± 0.005	0.008 ± 0.005	0.006 ± 0.005
BF MEP$_{75}$ (mV.s)	Baseline	0.007 ± 0.005	0.009 ± 0.008	0.007 ± 0.004	0.009 ± 0.006	0.007 ± 0.005	0.008 ± 0.005
	Post-test	0.007 ± 0.006	0.009 ± 0.007	0.005 ± 0.005	0.007 ± 0.007	0.006 ± 0.004	0.006 ± 0.005
BF MEP$_{50}$ (mV.s)	Baseline	0.007 ± 0.006	0.009 ± 0.008	0.007 ± 0.006	0.007 ± 0.003	0.007 ± 0.006	0.006 ± 0.004
	Post-test	0.006 ± 0.006	0.008 ± 0.007	0.004 ± 0.002	0.005 ± 0.003	0.005 ± 0.003	0.004 ± 0.002

Note: The shaded boxes indicate that there was a significant time effect between baseline and post-test measures; # means significantly different from baseline (interaction effect).

As opposed to previous experiments that used intermittent brief MVCs as the fatiguing task for the tested leg [9,15], we used 60 s sustained MVC to generate high intramuscular pressure and decreased oxygen delivery due to muscle ischemia induced by sustained MVC [32]. The 60 s MVC was chosen to match the total duration of intermittent MVCs (12 × 5 s MVCs) used by Halperin et al. [15]. However, the left leg pre-induced fatigue in combination with the blood ischemia did not alter the force-time integral values recorded during the 60 s right leg sustained MVCs. These results suggest that the activation of group III and IV muscle afferents induced by a 60 s sustained MVC in one leg does not modulate the motor performance in the contralateral leg during either sustained or brief MVCs.

A point of deliberation in the present study may be that the magnitude of the pre-induced fatigue generated by the left leg was not enough to constitute an NLMF. Indeed, two 100 s sustained KE MVCs used by Halperin et al. [15] resulted in ~70% force drop in the fatigue limb whereas one 60 s MVC resulted in ~55% reduction in MVC in our study. However, the neuromuscular performance was not altered in the study by Kennedy et al. [9] despite a 77% decline in MVC force. We also minimized the time delay between the fatiguing tasks and the post-intervention neuromuscular assessments to avoid recovery of central and peripheral fatigue, yet no evidence of NLMF was found. This time delay in previous studies was approximately 15–60 s [9,15]. It is also worth clarifying that the failure in observing NLMF phenomenon in the current study might be associated with the size of muscle mass involved (i.e., knee extensors in one leg) and the mode of exercise (i.e., sustained MVC) performed during the fatiguing task. Prior investigations that used bilateral dynamic knee extensions [8], leg cycling [5], and arm cranking exercises [32] found NLMF in the rested/remote muscle groups. The accelerated neuromuscular fatigue within the tested muscle groups in these studies have been attributed to central inhibitory factors mediated by afferent feedback from pre-fatigued muscles. Nonetheless, further studies using a longer duration of sustained MVC and larger muscle mass during whole-body dynamic exercises are required to explore the influence of group III and IV muscle afferents on the NLMF phenomenon.

4.2. Corticospinal Excitability and Inhibition

The corticospinal pathway excitability recorded from RF and VL muscles demonstrated relatively greater values (MEP_{100}) following 60 s contralateral MVC (in Ftg-0) compared to a time-matched rest (in Rest-0). Although the current study showed a small effect size, this so-called "cross-over facilitation" of the corticospinal pathway had previously been shown in the upper limb musculature [33] and was attributed to (i) transfer of excitatory signals from one hemisphere to another via the callosal commissure [34,35], (ii) increase in the excitability of motoneurons innervating the tested muscles via release of monoaminergic neuromodulators [36], and (iii) activation of additional brain regions including ipsilateral and contralateral prefrontal and sensorimotor areas as a compensatory mechanism to prevent reduction in maximal force output [37]. With the single-pulse TMS used in the present study, it is indeed difficult to confirm the contribution of the above-mentioned mechanisms; particularly because the MEP_{75} and MEP_{50} recorded from both VL and RF did not show an increase following contralateral fatiguing contraction. However, in support of these notions contributing to cross-over facilitation of MEP recorded during MVC (MEP_{100}), Tanaka and Watanabe [37] have suggested that additional brain regions are involved in a task if participants choose to mobilize all of their mental effort to execute a "maximal" rather than a "submaximal" contraction.

The failure in observing cross-over facilitation of MEP_{100} following Ftg-60 (experiment B) and Ftg-60-Occl (experiment C) is not clear; however, this could be attributed to the 60 s right leg MVC. More specifically, the corticospinal excitability (i.e., MEP_{100}) demonstrated a significant increase following the 60 s sustained right leg MVC (time effect) (Figure 5B,C). This MEP facilitation was observed regardless of the preceding conditions undertook by the left leg (i.e., 60 s MVC vs. 60 s MVC + occlusion in experiment C). Thus, our data suggest that the 60 s sustained MVC performed by the right (tested) knee extensors facilitated a strong excitatory effect to the corticospinal system while the contralateral fatiguing contraction did not enhance this facilitation any further.

Our data in all three experiments demonstrated no statistical difference in the duration of SP compared within or between conditions. This finding indicates that the pre-induced fatigue and the subsequent blood ischemia in one leg do not alter the rate of corticospinal inhibition in the contralateral homologous muscles recorded during 100%, 75%, and 50% of MVC contractions.

4.3. Limitations

Methodological considerations of the present study include: (i) The sequence of contractions in the neuromuscular assessment (i.e., 100%, 75%, and 50% of MVC) was kept consistent throughout the experiment whereas an early recruitment of high threshold motoneurons during the MVC could have offset the influence of contralateral contraction on MEP_{75} and MEP_{50}, (ii) the size of MEP monitors the excitability of the pyramidal tract neurons as well as the spinal motoneurons and spinal interneurons [38]; thus, further research is required to elucidate the influence of pre-induced fatiguing exercises on the cortical and spinal excitability responses, (iii) some may argue that the antagonist muscle excitability (M-wave amplitude) and contractile property (peak twitch) might have affected the slope of the regression line used to calculate the estimated resting twitch in VA_{TMS} measurement, however, the hamstring Mmax and peak twitch were not measured due to complexity of the sciatic nerve electrical stimulation; therefore, caution should be taken in interpretation of the VA_{TMS} data, as it is difficult to determine the contribution of hamstring coactivation on TMS-evoked knee extensors SITs, (iv) pre-induced fatiguing exercise in one limb may modulate central and peripheral homodynamic associates such as cardiorespiratory responses, neurotransmitters, neuromodulators, hormonal factors as well as autonomic responses, however, these parameters were not directly measured in the current study, thus they were not discussed, and finally, (v) more investigation should be directed towards the measurement of intracortical facilitation and inhibition using the paired-pulse TMS paradigm.

5. Conclusions

In conclusion, the results of the present study suggest that the pre-induced fatigue evoked by the 60 s sustained KE MVC, with or without subsequent blood ischemia, in the fatigued leg does not compromise the performance and neuromuscular function of the tested contralateral leg. However, the pre-induced fatigue may enhance the responsiveness of the ipsilateral corticospinal pathway innervating the tested limb, provided that the neuromuscular evaluation in the tested muscles is performed immediately after the pre-induced fatigue.

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Corticospinal-Evoked Responses from the Biceps Brachii during Arm Cycling across Multiple Power Outputs

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Abstract: *Background*: We examined corticospinal and spinal excitability across multiple power outputs during arm cycling using a weak and strong stimulus intensity. *Methods*: We elicited motor evoked potentials (MEPs) and cervicomedullary motor evoked potentials (CMEPs) in the biceps brachii using magnetic stimulation over the motor cortex and electrical stimulation of corticospinal axons during arm cycling at six different power outputs (i.e., 25, 50, 100, 150, 200 and 250 W) and two stimulation intensities (i.e., weak vs. strong). *Results*: In general, biceps brachii MEP and CMEP amplitudes (normalized to maximal M-wave (M_{max})) followed a similar pattern of modulation with increases in cycling intensity at both stimulation strengths. Specifically, MEP and CMEP amplitudes increased up until ~150 W and ~100 W when the weak and strong stimulations were used, respectively. Further increases in cycling intensity revealed no changes on MEP or CMEP amplitudes for either stimulation strength. *Conclusions*: In general, MEPs and CMEPs changed in a similar manner, suggesting that increases and subsequent plateaus in overall excitability are likely mediated by spinal factors. Interestingly, however, MEP amplitudes were disproportionately larger than CMEP amplitudes as power output increased, despite being initially matched in amplitude, particularly with strong stimulation. This suggests that supraspinal excitability is enhanced to a larger degree than spinal excitability as the power output of arm cycling increases.

Keywords: MEP; CMEP; arm cranking; motoneurone; exercise

1. Introduction

The influence of muscle contraction intensity on the excitability of the corticospinal pathway in humans has been well-studied during isometric contractions. Most of this research has involved the use of non-invasive stimulation techniques to assess corticospinal and/or spinal excitability to muscles of the upper [1–3] and, to a lesser extent, the lower limb [4] across a wide range of isometric contraction intensities. In general, the findings from these studies indicate that motor evoked potentials (MEPs) elicited by transcranial magnetic stimulation (TMS) increase in size as the strength of isometric muscle contractions increase up until a peak, after which they plateau and subsequently decrease as contraction strength approaches maximal ((i.e., 100% maximal voluntary contraction (MVC)) [1–4]. This modulation in MEP is accompanied by a similar change in the cervicomedullary MEP (CMEP) elicited by transmastoid electrical stimulation (TMES) of corticospinal axons, suggesting that the change in corticospinal excitability is largely mediated by spinal factors [1,4].

Using a strong stimulus intensity (set to evoke responses equal to 65–80% the maximal compound muscle action potential (M_{max})), Martin et al. (2006) showed that MEP and CMEP areas increased linearly in size during weak isometric contractions (i.e., <50% MVC) of the biceps brachii as muscle contraction intensity increased, whereas during strong contractions (i.e., >50% MVC) MEP and CMEP areas plateaued at ~75% MVC, and subsequently decreased as the contraction intensity approached 100% MVC [1]. When a lower stimulus intensity (set to evoke responses equal to 30–50% M_{max}) was used, MEP and CMEP areas followed a similar pattern of modulation with contraction intensity, however, peak responses were not observed until ~90% MVC, after which MEP and CMEP areas decreased. Moreover, the decline in MEP and CMEP area with the lower stimulus intensity was less marked than that observed when the stronger stimulus intensity was used [1]. Thus, the intensity of stimulation is an important factor to consider in assessing corticospinal excitability given how it can influence the primary measurement(s), and the associated interpretation of the data.

Substantially less information, however, is available regarding the influence of muscle contraction intensity on the modulation of corticospinal excitability during rhythmic motor outputs such as those observed during cycling [5–7]. This is an important topic to consider given that rhythmic motor outputs, such as arm cycling, are partially generated by spinally located networks of interneurons referred to as central pattern generators [8,9], and that corticospinal excitability is modulated differently during rhythmic locomotor outputs than during isometric contractions, indicating task-specificity [5,10,11]. In two separate studies from our lab, we have investigated changes in corticospinal and spinal excitability as arm cycling intensity (i.e., power output) was increased [6,7]. However, changes in excitability were assessed across a small range of power outputs, and thus may not have observed potential changes in excitability that occurred at higher cycling intensities. Thus, it remains unknown whether a similar peak, plateau and subsequent decline in corticospinal and spinal excitability are observed with increasing arm cycling intensity, as observed in isometric contractions.

Accordingly, the purpose of the present study was to: (1) characterize the influence of muscle contraction intensity on changes in corticospinal and spinal excitability projecting to the biceps brachii over a wide range of arm cycling intensities, and (2) assess the influence of stimulation intensity on corticospinal and spinal outputs as cycling intensity increased. Specifically, we sought to examine the effects of using a weak and a strong stimulus intensity on corticospinal and spinal excitability as power output was increased during cycling. We hypothesized that: (1) using the weak stimulus, corticospinal and spinal excitability would increase similarly across all arm cycling power outputs, and (2) using the *strong* stimulus, corticospinal and spinal excitability would increase but experience a plateau, and subsequent decrease as cycling intensity increased towards the maximum power output examined.

2. Materials and Methods

2.1. Participants

This study consisted of a familiarization session and two experimental sessions; (1) a transcranial magnetic stimulation (TMS) session and (2) a transmastoid electrical stimulation (TMES) session (see *Protocol* below). A total of nine healthy, male volunteers (24.2 ± 5.9 years, 180.7 ± 7.8 cm, 82.2 ± 8.3 kg, 1 left-hand dominant) with no known neurological impairment participated in session one, and eight of those volunteers (1 left-hand dominant) returned on a separate day (>24 h) to complete session two. In accordance with the Tri-Council guidelines in Canada, all participants gave written, informed consent prior to participating in the study, and potential risks were fully disclosed. Prior to TMS, all participants were screened for contraindications to magnetic stimulation using a safety checklist [12]. To determine limb dominance, the Edinburgh handedness inventory [13] was used. This information was gathered because all evoked responses elicited by TMS and TMES (see *Stimulation Conditions* below) were taken from the dominant arm. Additionally, all participants filled out a Physical Activity Readiness Questionnaire for Everyone (PAR-Q+, Canadian Society for Exercise Physiology (CSEP)) to screen for any contraindications to physical activity. Participants also refrained from caffeine for 12 h

and alcohol for 24 h prior to each experimental session. All procedures were performed in compliance with the Declaration of Helsinki and were approved by the Interdisciplinary Committee on Ethics in Human Research (ICEHR no. 20181196-HK) at Memorial University of Newfoundland.

2.2. Experimental Setup

Many of the experimental procedures and recording techniques herein are similar to those described previously [6,7,14]. All sessions were conducted with participants seated upright on an arm cycle ergometer (SCIFIT ergometer, model PRO2 Total Body, Tulsa, OK, USA). The seat height of the ergometer was adjusted so that participants' shoulders were approximately in line with the axis of rotation of the arm cranks, and the seat distance was manipulated to a position in which participants were at a comfortable distance (i.e., no reaching or trunk variation during cycling) from the hand pedals. The seat height and distance were recorded for each participant during the familiarization session and were used for the subsequent sessions. Arm cycling trials were performed in an asynchronous cranking pattern with the forearms fixed in a pronated position. Wrist braces were worn to limit the amount of wrist flexion and extension during cycling as a means to diminish the influence of short- and long-latency reflex connections that have been shown to exist between the wrist flexors and the biceps brachii (see Figure 1) [15].

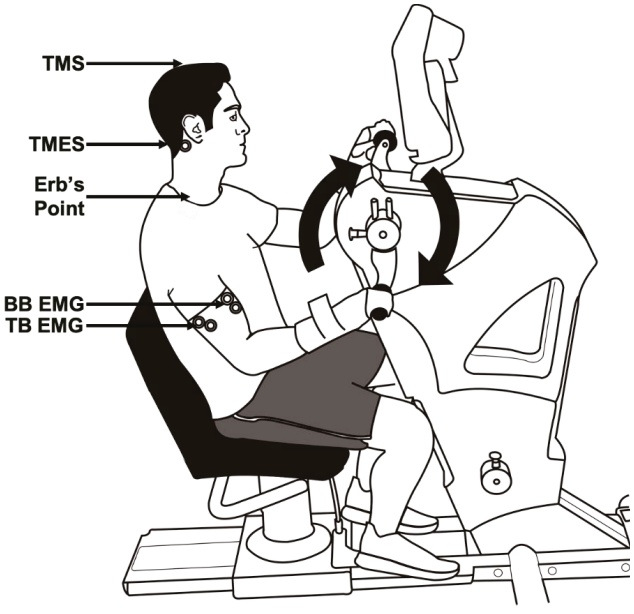

Figure 1. Experimental setup for arm cycling trials showing participant seated on the ergometer instrumented with surface EMG electrodes on the biceps and triceps brachii. Arrows point to the site of each stimulation technique. All arm cycling trials were conducted in the forward direction. Abbreviations: TMS, transcranial magnetic stimulation; TMES, transmastoid electrical stimulation; BB, biceps brachii; TB, triceps brachii; EMG, electromyography.

For this study, participants were required to cycle at 6 different power outputs: 25, 50, 100, 150, 200, and 250 Watts (W) all at a constant cadence of 60 revolutions per minute (rpm). These cycling conditions were repeated at two different stimulation intensities (see *Stimulation Conditions* below), for a total of 12 cycling trials.

2.3. Electromyography Recordings

Surface electromyography (EMG) was recorded from the biceps brachii of the dominant arm using pairs of disposable Ag-AgCl surface electrodes (MediTrace^{TM} 130 Foam Electrodes with conductive adhesive hydrogel, Covidien IIC, MA, USA). Electrodes were positioned approximately 2 cm apart (center to center) over the midline of the biceps brachii and on the lateral head of the triceps brachii in a bipolar configuration. A ground electrode was positioned on the lateral epicondyle of the dominant arm. To reduce the impedance for EMG recordings, the skin was thoroughly prepared by removing hair (via a handheld razor), abraded to remove dead skin cells (via abrasive paper), and cleaned using isopropyl alcohol swabs prior to electrode placement. The EMG signals were amplified (×300, CED 1902 amplifier; Cambridge Electronic Design Ltd., Cambridge, UK), and bandpass filtered using a 3-pole Butterworth filter with cut-off frequencies of 10–1000 Hz. All analog signals were digitized at a sampling rate of 5000 Hz and stored on a laboratory computer for off-line analysis (CED 1401 interface and Signal 5.11 software; Cambridge Electronic Design Ltd., Cambridge, UK).

2.4. Stimulation Conditions

Recordings were made of the motor responses in the biceps brachii to three different stimulation techniques: (1) brachial plexus stimulation at Erb's point, (2) magnetic stimulation of the motor cortex (i.e., TMS), and (3) electrical stimulation between the mastoids at the cervicomedullary junction (i.e., TMES). Motor responses were evoked during arm cycling at the 6 o'clock position, which corresponds to the mid-elbow flexion phase of arm cycling and when biceps brachii activity is relatively the largest (for a more detailed explanation of the phases of arm cycling see review by [16]). Stimulations were triggered automatically when the right hand passed a magnetic sensor on the ergometer, at either the 6 o'clock or 12 o'clock position for right-handed and left-handed participants, respectively. The intensities for all three stimulation techniques were set during arm cycling at a constant cadence of 60 rpm and power output of 25 W. For TMS and TMES, two different stimulation intensities were used: (1) a weak stimulation intensity (set to evoke responses equal to ~10% M_{max}), and (2) a strong stimulation intensity (set to evoke responses equal to ~40% M_{max}). These response amplitudes were chosen to provide insight into potential differences in excitability at different portions of the motoneurone pool as cycling intensity increased. All participants had prior experience with each of the stimulation procedures before participating.

2.5. Brachial Plexus Stimulation

For both sessions, single rectangular pulses (200-μs duration, 90–275 mA) were delivered via a DS7AH constant current stimulator (Digitimer Ltd., Welwyn Garden City, Hertfordshire, UK) to the brachial plexus at Erb's point to elicit maximal compound muscle action potentials (maximal M-wave (M_{max})) in the biceps brachii. The cathode was placed in the supraclavicular fossa and the anode over the acromion process. Stimulus intensity was initially set at 25 mA and was gradually increased until the size of the M-wave plateaued (i.e., M_{max}). At this point, the stimulation intensity was increased by 10% (supramaximal) to ensure that M_{max} was elicited throughout the remainder of the study.

2.6. TMS

TMS was delivered over the vertex of the motor cortex to elicit MEPs in the biceps brachii using a Magstim 200^2 magnetic stimulator (Magstim, Whitland, Dyfed, UK) and circular coil (13.5 cm outside diameter). The vertex was measured and marked on the participant's scalp with a felt-tip permanent marker. One investigator ensured proper and consistent coil placement directly over vertex throughout the experiment. The coil was held firmly against the participant's skull, parallel to the floor with the direction of current flow-oriented to preferentially activate either the left or right motor cortex, depending on hand dominance (i.e., "A" side up for right-handed participants, "B" side up for left-handed participants). Initially, TMS intensity was set at 25% of maximal stimulator output (MSO)

and was increased until MEPs were observed in the biceps brachii equal in amplitude to ~10% M_{max}. Once found, a trial consisting of 8 TMS was performed to ensure that the average MEPs were ~10% M_{max}. This stimulation intensity was recorded as the weak stimulation intensity, and was then used for the remainder of the experiment. For the strong stimulation intensity, the same procedures were performed except the %MSO was increased until MEPs from the biceps brachii were equal in amplitude to ~40% M_{max}. Once again, a trial consisting of 8 TMS was performed to ensure that the intensity of TMS would evoke MEPs equal to ~40% M_{max}. Once determined, this intensity was recorded and then used as the strong intensity for the rest of the experiment.

2.7. TMES

TMES was delivered (200 μs pulse-width duration, DS7AH, Digitimer Ltd., Welwyn Garden City, Hertfordshire, UK) to the corticospinal axons at the cervicomedullary junction to elicit CMEPs in the dominant arm biceps brachii. Self-adhesive Ag-AgCl surface electrodes were placed on the skin at the grooves between the mastoid processes and the occipital bone, with the anode and cathode on the side corresponding to each participant's dominant and non-dominant arm, respectively. Similar to the procedures for setting the stimulation intensities for TMS (see *TMS* above), the intensity of electrical stimulation was gradually increased (initially from 25 mA) until the amplitudes of the CMEPs were equal in amplitude to ~10% M_{max} (for the weak stimulation intensity) and ~40% M_{max} (for the strong stimulation intensity). Trials of 8 CMEPs were evoked at each stimulation intensity and the average was calculated. These stimulation intensities were recorded, and were then used for the remainder of the experiment. The latency of responses was monitored carefully to ensure that stimulation did not activate the corticospinal axons at or near the ventral roots, which would be indicated by a reduction in latency by ~2 ms [17,18].

2.8. Protocol

Following familiarization, participants were randomly assigned to complete either session one (TMS) or session two (TMES) first. For both sessions, the procedures were identical with the exception of the stimulation type. Following EMG preparation and ergometer modifications, stimulation intensities were determined (see above). In both sessions, M_{max} was determined first followed by the setting of stimulation intensities for the weak and strong stimulations for either TMS (session one) or TMES (session two). Once stimulation intensities were determined, participants began the 12 cycling trials consisting of six power outputs (25, 50, 100, 150, 200, and 250 W) performed at a constant cadence of 60 rpm with either the weak or strong stimulation intensity (i.e., six cycling trials at each stimulation intensity). The order of the cycling trials was randomized for each participant. While cycling, as the dominant hand passed the 6 o'clock position, one M_{max} and either six MEPs or six CMEPs (depending on the session) were evoked in a randomized order. The time between stimulations was 5–6 s. The total length of each trial was approximately 30 s. To reduce the potential influence of fatigue, one-minute rest periods were given following completion of the lower power output trials (i.e., 25, 50, 100 W), and two-minute rest periods were given after the higher power output trials (i.e., 150, 200, 250 W). Additionally, half-way through the 12 trials (i.e., after trial six), a 5-min rest period was given before the remainder of the trials were completed.

2.9. Data Analysis

For analysis of M_{max}, MEP, and CMEP, the averaged peak-to-peak amplitudes from each cycling trial were measured from the biceps brachii of the dominant arm. Since M_{max} is thought to represent the maximal response of the motor system [4], averaged MEPs (n = 6) and CMEPs (n = 6) from each trial were normalized to the M_{max} within each cycling trial. Response latencies of all evoked responses were carefully monitored throughout all cycling trials as well. The latency for each response was classified as the duration from the stimulus artifact to the initial deflection in the voltage trace from baseline and was averaged across the total number of stimulation trials. Additionally, since the level

of voluntary muscle contraction could potentially have an influence on changes in MEP and CMEP amplitudes, pre-stimulus EMG was measured from the rectified virtual channel created for the biceps and triceps brachii as the mean of a 50 ms window immediately prior to the stimulation artifact [14]. For two participants who completed CMEPs (n = 8), pre-stimulus EMG from the triceps brachii was not available due to a technical error during data collection. Therefore, the final sample size for CMEP pre-stimulus EMG data from the triceps brachii was n = 6.

2.10. Statistical Analysis

Group data are presented as means ± SD in the text and means ± SE in the figures (with n in the legends). All statistics were performed using IBM's SPSS Statistics (IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY, USA: IBM Corp.). Mauchly's test was employed to assess the assumption of sphericity for repeated measures analysis. In cases where sphericity was violated, the appropriate correction was applied (i.e., Greenhouse Geisser or Huynh-Feldt) and the degrees of freedom were adjusted. Separate two-way repeated-measures ANOVAs were used to assess the effects of stimulation intensity and cycling intensity (and any interaction) on the M_{max}, MEP, and CMEP amplitudes (both normalized to M_{max}), the average pre-stimulus EMG, and the MEP/CMEP ratios. Post hoc pairwise comparisons were made between means using the Bonferroni correction. Additionally, because one of our aims was to examine the effects of cycling intensity on corticospinal excitability measures within each stimulation intensity (weak and strong), separate one-way repeated-measures ANOVAs were conducted for both the weak and strong stimulus on M_{max}, MEP, and CMEP amplitudes (normalized to M_{max}), pre-stimulus EMG, and MEP/CMEP ratios as cycling intensity increased. If a main effect was identified, post hoc pairwise comparisons were made between means using the Bonferroni correction. Independent samples t-tests were conducted to compare whether MEPs and CMEPs (normalized to M_{max}) at both stimulation intensities were matched appropriately. To compare between MEP and CMEP amplitudes (normalized to M_{max}) at each power output, independent sample t-tests were used with a Bonferroni correction. Paired samples t-tests were conducted on MEP/CMEP ratios between stimulation strengths (weak vs. strong) at each power output. All statistics were performed on group data and statistical significance was set at $p < 0.05$.

3. Results

Evoked responses (i.e., M_{max}, MEPs, and CMEPs) were recorded from the dominant arm biceps brachii at two different stimulation intensities while participants performed arm cycling bouts over a range of contraction strengths. MEPs and CMEPs (normalized to M_{max}) were evoked on separate days but were initially matched to equal 10% (weak stimulus) and 40% (strong stimulus) of the M_{max} on each day. MEPs and CMEPs were not significantly different when either the weak or the strong stimulation intensity were examined ($p > 0.05$ for both conditions), suggesting that the responses were indeed matched initially between days.

3.1. Biceps Brachii Evoked Responses

3.1.1. MEP Amplitude

Figure 2 (top panel) and Figure 3A show representative and grouped data, respectively for MEP amplitudes from the biceps brachii during arm cycling across the various contraction intensities. Figure 2 shows evoked potential traces from one participant during arm cycling with the weak stimulation intensity. In this example, the amplitudes of the MEPs show a progressive and generally consistent increase from the lowest (25 W) to the highest (250 W) arm cycling/muscle contraction intensity. Results from the two-way ANOVA on MEP amplitudes showed a significant main effect for both stimulation intensity (strong > weak, $F_{5,40} = 96.81$, $p < 0.001$) and cycling intensity ($F_{1,8} = 65.30$, $p < 0.001$). Bonferroni post hoc tests revealed that MEP amplitudes at 25 W and 50 W were not different from one another ($p = 0.187$) but were significantly smaller than MEP amplitudes evoked

during the 100, 150, 200, and 250 W trials ($p < 0.05$ for all comparisons). Additionally, there was a significant interaction between the intensity of stimulation and the intensity of cycling on MEP amplitudes ($F_{5,40} = 65.30$, $p < 0.001$). Further analysis, through use of one-way ANOVAs for each stimulation intensity, showed a significant main effect for cycling intensity on MEP amplitudes at both the weak ($F_{5,40} = 55.61$, $p < 0.001$) and strong ($F_{5,40} = 41.28$, $p < 0.001$) stimulation conditions. Using the weak stimulation, Bonferroni post hoc tests revealed that MEP amplitudes increased as cycling intensity increased up until 200 W (200 W > 150 W >100 W > 50 W > 25 W, $p < 0.05$ for all comparisons) after which MEPs plateaued ($p > 0.05$). Using the strong stimulation, MEP amplitudes similarly increased with cycling intensity, however, a peak was observed at 100 W (100 W > 50 W > 25 W, $p < 0.05$ for all comparisons), at a lower power output than that observed using the weaker stimulation condition (i.e., 200 W). Beyond 100 W, there were no further increases in MEP amplitudes ($p > 0.05$).

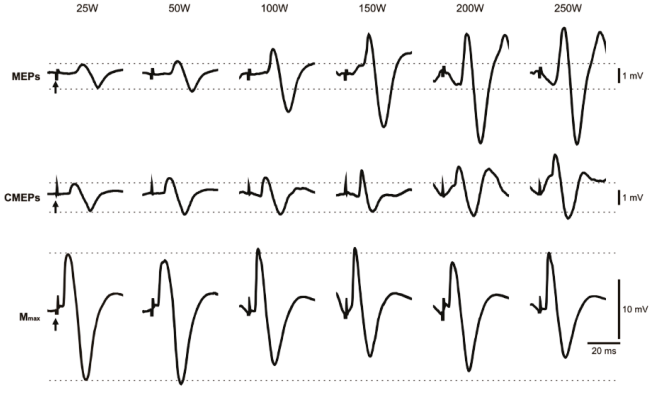

Figure 2. Raw traces for MEPs (top row), CMEPs (middle row), and M_{max} (bottom row) from the biceps brachii of a single participant ($n = 1$) across arm cycling power outputs using the weak stimulation intensity. Each MEP and CMEP waveform represent the average of six evoked potentials. Arrows indicate the stimulus artifact, and dashed lines portray the initial amplitudes of evoked potentials with the *weak* stimulation (~10% M_{max}). In this example, MEP and CMEP amplitudes show a general progressive increase as power output increases towards 250 W, while M_{max} gradually decreases.

3.1.2. Biceps Brachii Pre-stimulus EMG

Figure 3C shows group data for biceps brachii pre-stimulus EMG prior to MEPs during arm cycling. Results from the two-way ANOVA showed that mean biceps brachii pre-stimulus EMG in the 50 ms preceding an MEP was not different between the weak and strong stimulation intensity ($F_{1,8} = 1.42$, $p = 0.267$). Therefore, the average pre-stimulus EMG was pooled between the weak and strong stimulation conditions, which are represented in Figure 3C. There was a significant main effect on biceps brachii pre-stimulus EMG for cycling intensity ($F_{1.76,14.12} = 29.33$, $p < 0.001$), but, there was no interaction between stimulation intensity and cycling intensity ($F_{1.96,27.35} = 1.96$, $p = 0.137$). To further examine changes in pre-stimulus EMG with cycling intensity, one-way ANOVAs were performed. Pre-stimulus EMG increased as cycling intensity increased up until 200 W (Figure 3C, $p < 0.05$), and no differences were observed between the 200 W and 250 W conditions ($p = 1.00$).

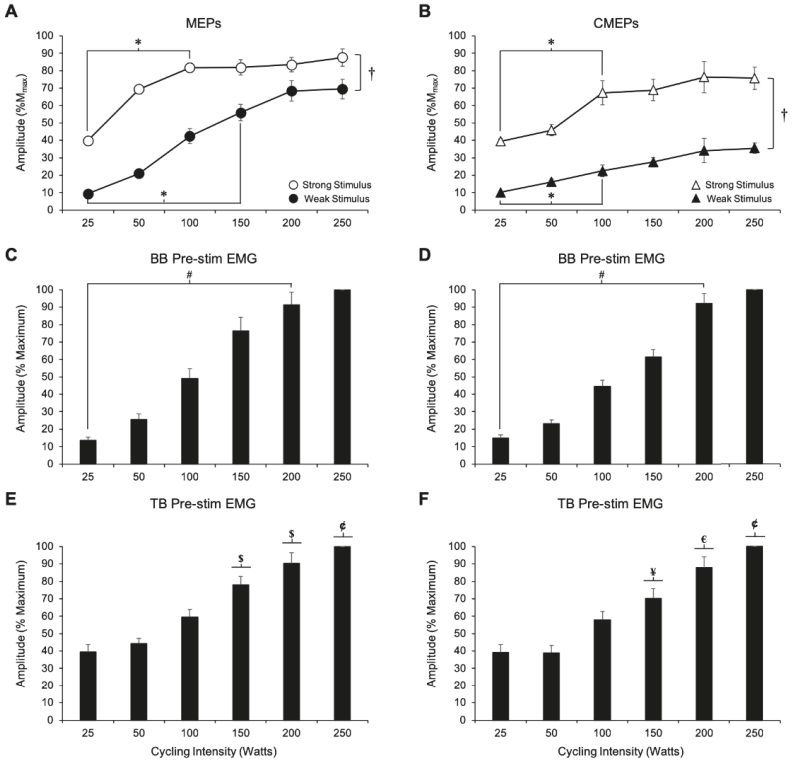

Figure 3. (**A**,**B**) Normalized grouped data (means ± SE) of the peak-to-peak amplitudes for MEPs (**A**) and CMEPs (**B**) obtained from the biceps brachii at each power output examined. MEPs and CMEPs were normalized to M_{max} at each corresponding cycling intensity. In both A and B, filled data points represent when the weak stimulus was used, while unfilled points represent data from the strong stimulus. For clarity, circles were used for MEPs, while triangles were used for CMEPs. In some cases, data points are bigger than SE bars. * Significant difference between illustrated data points. † Significant main effect for stimulation strength ($p < 0.05$). (**C**,**D**) Pre-stimulus EMG (means ± SE) from the biceps brachii which has been pooled and averaged between both stimulation intensities for the TMS session (**C**) and TMES session (**D**), respectively. # Significant difference between all data points. (**E**,**F**) Pre-stimulus EMG (means ± SE) from the triceps brachii which has been pooled and averaged between both stimulation intensities for the TMS session (**E**) and TMES session (**F**), respectively. $ denotes significant difference from all previous power outputs. ¥ denotes significant difference from the 25 W condition. € denotes significant difference from the 25, 50, and 100 W conditions. ¢ denotes significant difference from the 25, 50, 100, and 150 W conditions.

3.1.3. Triceps Brachii Pre-Stimulus EMG

Figure 3E shows group data for triceps brachii pre-stimulus EMG prior to MEPs. Similar to the biceps, results from the ANOVA showed no effect of stimulation intensity on triceps brachii EMG activity prior to a MEP (Figure 3E, $F_{1,8} = 0.100$, $p = 0.760$), but there was a significant main effect of cycling intensity ($F_{1.62,12.94} = 19.32$, $p < 0.001$). Also, there was no significant interaction between cycling intensity and stimulation intensity ($F_{5,40} = 0.803$, $p = 0.554$). To further examine the effect of cycling intensity on triceps brachii pre-stimulus EMG, one-way ANOVAs were performed using the pooled data. Results from these tests indicated that as cycling intensity increased, triceps brachii pre-stimulus EMG values were only significantly different at 150 W and 200 W. Specifically, triceps

brachii pre-stimulus EMG was larger at 150 W than at 100 W ($p = 0.006$) and was larger at 200 W than 150 W ($p = 0.044$).

3.1.4. CMEP Amplitude

Figure 2 (middle panel) and Figure 3B show representative and grouped data, respectively for CMEP amplitudes during the arm cycling bouts. Figure 2 portrays data from one participant from the weak stimulation intensity condition. Similar to the MEP amplitudes, in this example, CMEP amplitudes increase in a relatively consistent and progressive manner. The results from the two-way ANOVA on CMEP amplitudes showed significant main effects for both stimulation intensity (strong > weak, $F_{1,7} = 91.50$, $p < 0.001$) and cycling intensity ($F_{3.81,26.65} = 20.16$, $p < 0.001$), however, there was no significant interaction between the two factors ($F_{5,35} = 1.34$, $p = 0.271$). For cycling intensity, Bonferroni post hoc analysis revealed that CMEPs at 25 and 50 W are smaller than those at all other cycling intensities (i.e., 100, 150, 200, and 250 W) ($p < 0.05$ for all comparisons). To decipher specific effects of cycling intensity within each stimulation condition, separate one-way ANOVAs for the weak and strong stimulation conditions were performed on CMEP amplitudes. The results from the one-way ANOVAs showed a significant main effect for cycling intensity on CMEP amplitudes at both the weak ($F_{5,35} = 21.11$, $p < 0.001$) and strong ($F_{5,35} = 9.95$, $p < 0.001$) stimulation conditions. For the weak stimulation condition, Bonferroni post hoc analyses revealed that CMEP amplitudes increased up until 150 W (150 W > 100 W > 50 W > 25 W; $p < 0.05$ for all comparisons), after which CMEP amplitudes did not change ($p > 0.05$). When the strong stimulation intensity was used, post hoc analyses revealed that CMEP amplitudes increased up until 100 W (100 W > 50 W > 25 W, $p < 0.05$ for all comparisons), after which CMEPs plateaued ($p > 0.05$).

3.1.5. Biceps Brachii Pre-Stimulus EMG

Figure 3D shows group data for biceps brachii pre-stimulus EMG prior to CMEPs during arm cycling. Results from the two-way ANOVA showed that mean biceps brachii pre-stimulus EMG in the 50 ms preceding CMEPs was not influenced by stimulation intensity ($F_{1,7} = 0.02$, $p = 0.906$), thus the data was pooled between the weak and strong stimulation conditions as shown in Figure 3D. There was a significant main effect on biceps brachii pre-stimulus EMG for cycling intensity ($F_{1.49,10.41} = 43.08$, $p < 0.001$), but, there was no interaction between stimulation intensity and cycling intensity ($F_{5,35} = 1.22$, $p = 0.320$). To further examine changes in pre-stimulus EMG with cycling intensity, one-way ANOVAs were performed using the pooled data. Similar to MEPs, pre-stimulus EMG for CMEPs increased as cycling intensity increased up until 200 W (Figure 3D, $p < 0.05$), and there was no difference between the 200 W and 250 W conditions ($p = 0.885$).

3.1.6. Triceps Brachii Pre-Stimulus EMG

Figure 3F shows group data for triceps brachii pre-stimulus EMG prior to CMEPs. Similar to above, results from the two-way ANOVA showed no effect of stimulation intensity ($F_{1,5} = 0.761$, $p = 0.423$) and thus, the data was pooled between the week and strong stimulation intensities (Figure 3F). There was, however, a significant main effect of cycling intensity ($F_{1.31,6.55} = 14.04$, $p = 0.006$) on triceps brachii pre-stimulus EMG, but no significant interaction ($F_{5,25} = 0.961$, $p = 0.460$). To further examine the effect of cycling intensity on triceps brachii pre-stimulus EMG, one-way ANOVAs were performed using the pooled data. Results from these tests indicated that triceps brachii pre-stimulus EMG values for CMEPs were only increased at 150 W, 200 W and 250 W compared to the 25 W condition ($p < 0.05$ for all comparisons). However, triceps brachii pre-stimulus EMG was not significantly different with increased cycling intensity from 150 W to 250 W ($p > 0.05$ for all comparisons).

3.1.7. MEP/CMEP Ratios

Although MEPs and CMEPs were evoked on separate days, the responses were initially matched in amplitude to approximately 10% or 40% M_{max} for the weak and strong stimulation conditions,

respectively ($p > 0.05$ for both stimulation conditions). Thus, MEP amplitudes were expressed relative to CMEP amplitudes and multiplied by 100% to obtain MEP/CMEP percentages for each participant (Figure 4). This was done in an attempt to isolate whether changes in overall excitability could be attributed to changes in supraspinal and/or spinal excitability. Values greater than 100% indicate that MEP amplitudes are larger than CMEP amplitudes, suggesting that supraspinal excitability may be increased. Similarly, values less than 100% indicate that MEP amplitudes are less than CMEP amplitudes, suggesting that changes in spinal excitability are important factors in maintaining excitability of the corticospinal pathway. Results from the two-way ANOVA revealed a significant main effect for stimulation intensity (weak > strong, $F_{1,7} = 6.94$, $p = 0.034$) and cycling intensity ($F_{5,35} = 9.71$, $p < 0.001$). Bonferroni post hoc tests revealed that MEP/CMEP at 25 W and 50 W were not different from one another ($p = 0.413$) but were significantly smaller than MEP/CMEP at 100, 150, 200, and 250 W trials ($p < 0.05$ for all comparisons). As well, there was a significant interaction effect ($F_{5,35} = 8.18$, $p < 0.001$) between stimulation intensity and cycling intensity on MEP/CMEP ratios. To examine changes in MEP/CMEP with increased power output, one-way ANOVAs were conducted within each stimulation intensity. Results from the one-way ANOVAs showed a significant main effect for cycling intensity on MEP/CMEP ratios at both the weak ($F_{5,35} = 9.44$, $p < 0.001$) and strong ($F_{5,35} = 4.60$, $p = 0.003$) stimulation conditions. When the weak stimulation was used, Bonferroni post hoc analysis revealed that MEP/CMEP were only significantly larger than that at 25 W at 150 W ($p = 0.037$), and 200 W ($p = 0.05$). When the strong stimulation intensity was used, MEP/CMEP were significantly larger at 50 W than at 25 W ($p = 0.026$) but were not different for any other comparison. To compare changes in MEP/CMEP between the weak and strong stimulation intensities, paired samples *t*-tests were performed at each power output. Thus, a total of six comparisons were made. The *t*-tests revealed that the MEP/CMEP ratios were not significantly different at 25 W ($t_{(7)} = 1.22$, $p = 0.261$) or 50 W ($t_{(7)} = 0.52$, $p = 0.622$) when either the weak or strong stimulus was used. However, MEP/CMEP ratios were significantly larger at 100 W ($t_{(7)} = 2.51$, $p = 0.041$), 150 W ($t_{(7)} = 3.24$, $p = 0.014$), 200 W ($t_{(7)} = 3.03$, $p = 0.019$), and 250 W ($t_{(7)} = 2.41$, $p = 0.047$) when the weak stimulation was used.

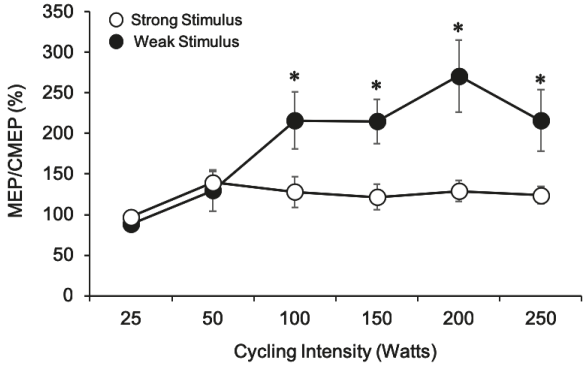

Figure 4. Comparison of MEP/CMEP ratios for the weak (filled circles) and strong (unfilled circles) stimulation intensities as power output increased from 25 W to 250 W. * represents significant difference between stimulation intensities at each given power output ($p < 0.05$). In some cases, SE bars were smaller than the symbols for the data points.

3.1.8. M_{max} Amplitude

For both the TMS and TMES sessions, the results from the two-way ANOVA revealed similar effects on biceps brachii M_{max} amplitudes. For both sessions, there was no effect of stimulation intensity (TMS: $F_{1,8} = 0.093$, $p = 0.769$, TMES: $F_{1,7} = 1.06$, $p = 0.337$), but there was a significant main effect for cycling intensity (TMS: $F_{5,40} = 15.66$, $p < 0.001$; TMES: $F_{1,7} = 8.89$, $p < 0.001$) on M_{max} amplitudes (Figure 5). As cycling intensity increased M_{max} amplitudes decreased (Figure 5A,B). Additionally,

there was no interaction observed between factors on either day (TMS: $F_{5,40} = 0.836$, $p = 0.532$, TMES: $F_{5,35} = 0.430$, $p = 0.825$). Since there was no effect of stimulation intensity on M_{max} values, the averages from each stimulation condition (weak and strong) were pooled across the cycling intensities for each session (as shown in Figure 5). For cycling intensity, Bonferroni post hoc analyses indicated that M_{max} values decreased for the TMS and TMES session as cycling intensity increased from 25 to 250 W.

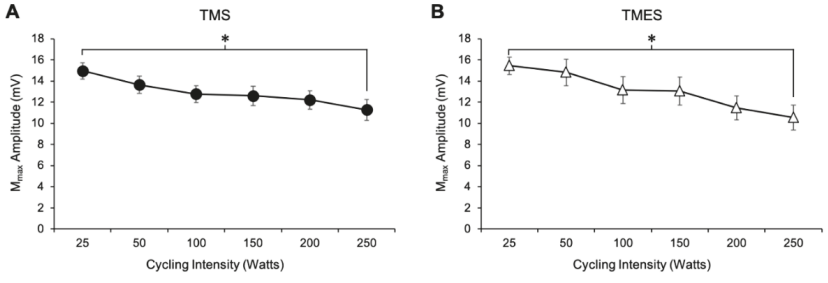

Figure 5. Changes in M_{max} amplitudes with increasing power output pooled between stimulation intensities for the TMS (**A**) and TMES (**B**) session. * denotes significant main effect of power output on M_{max} amplitude. M_{max} decreased by approximately 24.9 and 31.7% as power output increased from 25 to 250 W for the TMS and TMES sessions, respectively.

4. Discussion

This study shows that the amplitudes of TMS-evoked MEPs and TMES-evoked CMEPs increase with power output and plateau, but do not decrease in amplitude as has been previously shown by others during intense tonic contractions [1,4]. MEP amplitudes were much larger than CMEP amplitudes as power output increased regardless of stimulation strength, despite being initially matched in amplitude (Figures 3A,B and 4). This finding suggests that supraspinal factors mediate the change in overall corticospinal excitability observed during arm cycling as intensity increases. Importantly, stimulus strength had a substantial effect on MEP and CMEP amplitudes as cycling power output increased. Responses evoked by the weak stimulation (10% M_{max}) increased up to approximately 200 W for MEPs (Figures 3A and 4) and 150 W for CMEPs (Figures 3B and 4), whereas with the strong stimulation (40% M_{max}), responses reached a peak at 100 W for both MEPs and CMEPs and did not change afterward. Thus, the MEP/CMEP ratio used as a measure of supraspinal excitability was influenced by stimulation strength, which would lead to different conclusions on mechanisms of enhanced corticospinal excitability during arm cycling as power output increases.

4.1. Modulation of Corticospinal and Spinal Excitability with Cycling Intensity

Past research involving isometric contractions has shown that biceps brachii MEPs and CMEPs increase up until a peak at ~75–90% MVC [1–3], a finding which has been attributed to the motor unit firing and recruitment characteristics of the biceps brachii during progressively stronger isometric contractions [19,20]. Following the peak, there is a subsequent decline in responses as contraction intensity approaches 100% MVC [1] which is thought to reflect the inability for some motoneurones to fire in response to artificial excitatory input at strong contraction strengths, given the high degree of voluntary input to the motoneurone pool and the associated changes in their intrinsic properties [1]. In the present study, we did not observe a decline in corticospinal excitability as arm cycling intensity increased to the maximum intensity employed. Instead, we observed a plateauing of responses for both MEPs and CMEPs at intensities below 250 W, which were differentially influenced by stimulus strength (Figure 3A,B). Our results, however, do coincide with findings from the only other study to examine corticospinal excitability changes during a locomotor-like output over a wide range of contraction intensities [5]. In that study, MEPs and CMEPs from the knee extensors during leg cycling increased in amplitude up to 300 W, after which there was a plateauing, but no decline as cycling

intensity increased to 400 W [5]. Taken together, these studies suggest task-dependent changes in corticospinal and spinal excitability may be present, a finding we have previously reported [10,16,21].

In the current study, MEP and CMEP amplitudes increased at the lower, but not higher power outputs (Figure 3A,B), suggesting that the increase in overall corticospinal excitability at the low intensities (i.e., 25 to 100 W) is partially generated by increased spinal excitability. These finding are partially supported by biceps brachii pre-stimulus EMG values which increase for both stimulation types (Figure 3C,D) at the low cycling intensities, but are not significantly different between the highest cycling intensities (200 and 250 W). While this may explain the enhanced spinal excitability at the low power outputs, it does not explain why we observed a plateau in CMEP amplitudes beyond 150 W for the weak stimulus and 100 W for the strong stimulus in the present study, since EMG was still increasing beyond these power outputs. It is noted, however, that Weavil and colleagues showed increased EMG and workloads without changes in MEP and CMEP amplitudes. During isometric contractions, the biceps brachii is capable of recruiting additional motor units during contractions up to and beyond 90% MVC [19,20], which help to explain why CMEPs continue to increase beyond 90% MVC [1]. Corticospinal excitability to the biceps brachii is also task- [10,16] and forearm position-dependent [21] which is an important consideration when a comparison to tonic contractions is made. However, the lack of increase in CMEP amplitudes beyond 150 W and 100 W during arm cycling in the current study, while MEPs and background EMG are still increasing is unlikely to be explained by reaching the maximum motor unit recruitment of the biceps, given that these cycling intensities are not maximal, at least relative to a sprint test [7]. It is possible, however, that motoneurone recruitment strategies during a rhythmic motor output such as arm cycling may be different from those observed during isometric contractions (Power et al., 2018), and therefore could cause motoneurones to be maximally recruited sooner than 90% of maximal cycling power. Work in adult decerebrate cats and rats, for example, demonstrated that spinal motoneurones are characterized by changes in their electrical properties during locomotor outputs that would act to enhance their recruitment and firing [22–24]. These same changes in motoneurone excitability do not occur during tonic motor output [23].

4.2. Modulation of Supraspinal Excitability with Cycling Intensity

In the current study, MEP/CMEP ratios increased with power output, in particular when the weak stimulation intensity was used (Figure 4) suggesting that supraspinal excitability was enhanced to a larger degree than spinal excitability. It is plausible that changes in the excitability of interneuronal circuits and/or interhemispheric connections may be involved. During tonic contractions, short-interval intracortical inhibition (SICI) is reduced as muscle contraction intensity increases [25–27], a finding that is thought to downregulate the activity of the inhibitory neurons which project onto corticospinal cells involved in producing the movement. We recently showed that SICI was present during arm cycling, albeit not different than a tonic contraction [28]. Thus, it is possible that reductions in SICI during arm cycling as power output increases may underlay increases in MEP amplitudes as has been shown during tonic contractions.

Another potential mechanism involves cortical spread from the non-dominant to the dominant motor cortex as we have previously hypothesized [6,7,14]. Since arm cycling is a bilateral motor output it is possible that cortical excitation arising from the active, non-dominant motor cortex could facilitate excitability in the dominant motor cortex, which could reduce the input required to induce an MEP by a given TMS pulse. However, when the strong stimulation intensity was used, the changes in MEP/CMEP ratios were less marked and did not increase as cycling intensity increased suggesting a ceiling effect in the MEP amplitudes had been reached.

4.3. Differences between Stimulation Intensities

This study highlights the importance of stimulation intensity selection for experimental design during locomotor outputs. Notably, MEPs continued to increase with cycling intensity up until approximately 200 W when elicited with weak stimulation intensity (10% M_{max}), while they plateaued

at approximately 100 W under strong (40% M_{max}) stimulation. This led us to conclude that supraspinal excitability increases with increased power output, an effect only observed when weak stimulus intensity was used. In contrast, using the strong stimulation intensity leads one to believe, perhaps falsely, that spinal factors were driving the change in overall corticospinal excitability as a function of power output, a conclusion also reached by Weavil and colleagues (2015) who used a strong stimulation intensity (MEPs and CMEPs were ~50% M_{max}). The use of a weak stimulation intensity yielded a more precise measure of corticospinal excitability in this specific study as MEPs were less susceptible to ceiling effects than at the strong stimulation.

4.4. Methodological Considerations

An important methodological consideration in interpreting the current data is that we did not make the power outputs relative to each individual as we have recently done in two separate studies during arm cycling [6,7]. In Spence et al. (2016) we used 5 and 15% of peak power output determined by a sprint test (modified Wingate) while in Lockyer et al. (2018) we used 20, 40, and 60% of peak power output determined via a standard incremental aerobic test (20 W increases every two minutes) [29]. These methods were not without limitations, however. The former used a sprint test to prescribe aerobic cycling intensity at 60 RPM and the latter incremental test resulted in most of the participants reaching a similar peak power output of ~120 W. In the present study we used absolute power outputs as has been used by others [5,30] and all participants were able to cycle well above the aerobic test maximum power output of 120 W obtained in our prior work. We were thus able to have participants cycle at supramaximal intensities, albeit we did not quantify exertion levels. Additionally, the sample size of (n = 9) for MEPs and (n = 8) for CMEPs was not determined by a power analysis and therefore, it is unclear whether a larger sample size would have influenced the present results.

5. Conclusions

The present study describes the influence of stimulation strength over a wide range of cycling intensities on corticospinal and spinal excitability during arm cycling. We have demonstrated that corticospinal excitability to the biceps brachii is increased with cycling intensity during low power outputs, a finding that is partially mediated by spinal factors. As cycling intensity increases, however, it appears as though supraspinal factors may play more of a role in modulating overall corticospinal excitability. Additionally, this study highlights the importance of stimulation intensity selection to assess corticospinal excitability during motor output. It is concluded that the use of a weaker stimulation intensity provides a more precise measure of corticospinal excitability during locomotor outputs at high intensities as they are less susceptible to potential ceiling effects.

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Communication

Modulating Observation-Execution-Related Motor Cortex Activity by Cathodal Transcranial Direct Current Stimulation

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Abstract: The aim of this randomized sham-controlled study was to examine the impact of cathodal transcranial direct current stimulation (ctDCS) of the primary motor cortex (M1) during movement observation on subsequent execution-related motor cortex activity. Thirty healthy participants received sham or real ctDCS (1 mA) over the left M1 for 10 minutes, respectively. The participants observed a video showing repeated button pressing tasks of the right hand during the sham or real ctDCS, followed by performance of these tasks by the right hand. Motor-evoked potentials (MEP) were recorded from the resting right first dorsal interosseous muscle before movement observation during the sham or real ctDCS, immediately after observation of actions, and after subsequent movement execution. The results of the ANOVA showed a significant main effect on the group ($F_{1,28} = 4.60$, $p = 0.041$) and a significant interaction between time and the group ($F_{2,56} = 5.34$, $p = 0.008$). As revealed by respective post hoc tests, ctDCS induced a significant reduction of MEP amplitudes in connection with movement observation ($p = 0.026$, Cohen's $d = 0.861$) and after subsequent movement execution ($p = 0.018$, Cohen's $d = 0.914$) in comparison with the sham stimulation. It is concluded that ctDCS during movement observation was effective in terms of modulating motor cortex excitability. Moreover, it subsequently influenced execution-related motor cortex activity. This indicates a possible application for rehabilitative treatment in syndromes with pathologically enhanced cortical activity.

Keywords: movement observation; movement execution; transcranial direct current stimulation; motor cortex activity

1. Introduction

Movement observation or execution-related cortical networks are activated when individuals are conducting an action or observe the identical movement performed by another individual. These networks include the primary somatosensory cortex, primary motor cortex (M1), ventral premotor cortex and inferior frontal gyrus [1,2]. Alpha and beta frequency oscillations desynchronize within these networks during action observation and execution [3–5]. These alpha (8–13 Hz) and beta (14–30 Hz) oscillations in the electroencephalogram (EEG) are referred to as mu and beta rhythms and reflect mirror neuron activity [5–7].

Modulation of the activity of respective networks via non-invasive brain stimulation might alter respective task-related physiological processes and ultimately affect performance. Transcranial electrical stimulation is suitable to modulate cortical excitability and activity by a weak electrical current, which modulates synaptic activity and/or neuronal resting membrane potentials [8,9]. Cathodal transcranial direct current stimulation (ctDCS) results in long-term depression-like effects [8,10]. The effects of transcranial direct current stimulation (tDCS) on motor cortex activity are dependent on the state of the stimulated cortical region before and/or during the application [9]. With respect to treatment of brain diseases associated with pathological enhancement of cortical activity or excitability via cathodal tDCS, some studies have reported that ctDCS applied over an epileptogenic focus reduces epileptic EEG abnormalities [11,12] and seizure frequency in epilepsy [13]. Clinical studies moreover showed that cathodal tDCS over the contralesional M1 combined with motor training rebalances bi-hemispheric activity and facilitates motor performance in stroke patients [14,15] and in children with cerebral palsy [16,17]. Furuya et al. describe that ctDCS applied over the affected motor cortex in musicians cramp, combined with anodal tDCS over the contralateral motor cortex and bimanual finger movements, reduces dystonic symptoms in these patients, most probably due to a reduction of pathologically enhanced activity of the affected M1 [18].

In this study, we contrasted the effects of ctDCS and sham stimulation application to M1 during movement observation on subsequent execution-related motor cortex activity. It was expected that cathodal tDCS would reduce movement observation-related motor cortex activity, and that it subsequently would reduce execution-related motor cortex activity, as compared to a sham stimulation.

2. Materials and Methods

2.1. Participants

Thirty healthy adults (mean age, 25.93 ± 4.75 years; 12 females) participated in this study and were randomized to receive sham or real cathodal tDCS. They gave written informed consent. The ethics committee of the University Medicine Rostock approved this study and the study meets the standards of the Declaration of Helsinki. None of the participants had pregnancy, family history of epilepsy, the presence of neurological, psychiatric or musculoskeletal disorders, central nervous system acting medications, major medical diseases, cardiac pacemakers, skin lesions near the region of stimulation, or metal implants. They had normal or corrected-to-normal vision. The Oldfield's Edinburgh Handedness Inventory [19] was used to evaluate handedness, and all participants were right-handed. Each stimulation condition was performed in 15 participants.

2.2. Monitoring of Motor Cortex Excitability

A single-pulse transcranial magnetic stimulation (TMS) over the left M1 representation of the relaxed right resting first dorsal interosseus (FDI) muscle induced motor-evoked potentials (MEP) which were recorded to monitor motor cortex excitability changes. TMS with a biphasic pulse was conducted by a MagPro R100 magnetic stimulator (Medtronic, Skovlunde, Denmark) with a D-B80 coil. In order to minimize head to coil movement, a chin-forehead rest was used to stabilize the head of the participants. The magnetic coil was held tangentially to the skull over the left M1. A mechanical arm (Manfrotto Feltre, Italy) fixed the handle pointing backwards and laterally 45° from the midline. The optimal magnetic coil position (hotspot) was determined by a moderate suprathreshold stimulation intensity to constantly elicit the largest MEP in the right resting FDI muscle. The intensity of TMS was determined as the percentage of maximal stimulator output (% MSO) which elicited peak-to-peak MEP amplitudes of approximately 1 mV (SI1mV) at baseline before the intervention. This intensity was kept constant for the remaining experiment. Twenty MEPs were obtained from the right FDI muscle for each time bin using a pair of Ag-AgCl cup electrodes with a surface area of 3 mm^2 (GE Medical Systems, Milwaukee, USA) in a belly-tendon montage. The ground electrode was positioned over the right lateral biceps brachii muscle. The electromyographic (EMG) signals were amplified (input

resistance of 10 GΩ, bandwidth of 1–1000 Hz, Biovision, Wehrheim, Germany) with an amplification rate of 1000. DIAdem software was used to process EMG signals.

2.3. tDCS

Cathodal tDCS (1 mA) was delivered for 10 minutes (5 s of ramp-up and ramp-down) by a battery-driven electrical stimulator (BrainSTIM, EMS, Bologna, Italy) and applied through a pair of 25 cm^2 surface saline-soaked sponge electrodes. The cathode electrode was placed over the defined left M1 'hotspot' and the anode electrode was positioned over the right supra-orbital area. The direct current was switched on for 30 s and then turned off during sham stimulation.

2.4. Movement Observation and Execution

The participants kept their hands in a relaxed position. They comfortably seated in front of a computer screen (24-inch) to watch one movement observation video, which displayed the right hand pressing buttons. The video was 10 minutes long and incorporated 20 short clips. Twenty-second long clips in natural speed were presented 10 times. At half of the natural speed, 40 s long clips were presented 10 times. At the beginning, the 20 s long clip was displayed, followed by a 40 s long clip. Low-speed movements were included because these result in a more prominent modulation of motor cortex activity [20]. The clips showed that whenever a black spot changed into a red spot in a regular sequence, a human hand reached for the appropriate round button immediately, pressed it with the index finger only, and quickly got back to the original position afterwards. Participants were instructed to concentrate on the performing finger and the button press task. They were instructed to count the number of button presses, because attention is an important mediator of performance, and performance-related physiological effects [21].

After movement observation, participants performed a movement execution task (160 s) which was identical to the observed video. A 24-inch computer screen and a custom-made button-box with 4 red round buttons were positioned on the table in front of the participant. The distance was 20 cm from the right hand to the button-box. The four buttons corresponded to the black spots shown on the computer screen. When a black spot changed into a red spot in a modeled response sequence on the computer screen (each 3 s), participants pressed the button with the right index finger immediately, and quickly moved back to the initial hand position afterwards. The left hand always remained in the resting position.

2.5. Experimental Procedures

The participants received either ctDCS or a sham stimulation, while watching the movement observation video. Movement execution was conducted after the termination of action observation and tDCS. MEPs were recorded before (T0) and after (T1) movement observation combined with the sham or real ctDCS, and then after movement execution (T2). For an overview of the experimental procedure, refer to Figure 1.

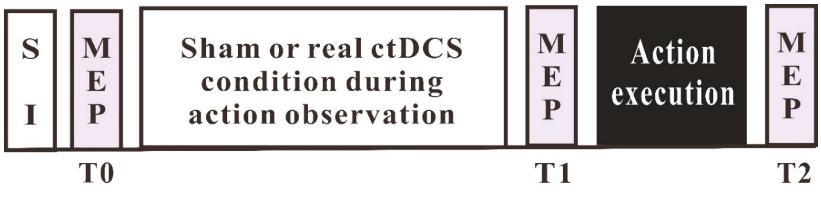

Figure 1. Timeline of the experimental protocol.

2.6. Statistical Analysis

SPSS (version 22.0; IBM, Armonk, NY, USA) and Prism (Version 8; GraphPad Software Inc., San Diego, CA, USA) were used to perform statistical analyses. Normal distribution of the data was confirmed by Kolmogorov-Smirnov tests. Independent-samples t-tests were used to examine differences of demographic and physiological data between experimental conditions respectively, including age, SI1mV, and baseline measurements of MEP amplitudes. Gender distribution differences between groups were examined via a χ^2 test.

For MEP analysis, first, the mean values of the peak-to-peak amplitudes of 20 MEPs for each time bin were calculated individually. If EMG activity larger than 50 µV was present in the 300 ms time-window before TMS, the respective MEP was excluded. A repeated measures ANOVA was performed to analyze MEP amplitude data with the between-subject factors tDCS (sham and real ctDCS condition), and Time course. The assumption of sphericity was examined by Mauchly's test and the Greenhouse-Geisser correction was applied if necessary. Fisher's post hoc tests were performed to determine differences between groups. Cohen's d was used to calculate effect sizes. A significance level of $p < 0.05$ was used for all statistical tests.

3. Results

All participants tolerated ctDCS well. There were not significant differences with respect to age, gender, SI1mV, and baseline MEP amplitudes between groups (all values of $p \geq 0.136$; Table 1). The results revealed a significant main effect for the group ($F_{1,28} = 4.60$, $p = 0.041$) and interaction between time and the group ($F_{2,56} = 5.34$, $p = 0.008$), but the main effect of time was not significant ($F_{2,56} = 2.76$, $p = 0.072$). The post hoc tests showed no significant differences between all time points in the sham stimulation condition. MEP amplitudes were however significantly decreased following ctDCS in comparison with the sham stimulation at time point T1 (after movement observation) ($p = 0.026$, Cohen's $d = 0.861$) and T2 (after movement execution) ($p = 0.018$, Cohen's $d = 0.914$, Figure 2A). In comparison to the respective baseline values, there were significant differences of MEP amplitudes from T0 to T1 ($p = 0.001$, Cohen's $d = 1.156$) and T0 to T2 ($p = 0.005$, Cohen's $d = 0.772$) in the real ctDCS group. In the real ctDCS group, 13 of 15 (86.67 %) participants at T1, and 11 of 15 (73.33 %) participants at T2 had reduced MEP amplitudes in contrast to the baseline (T0) (see Figure 2C).

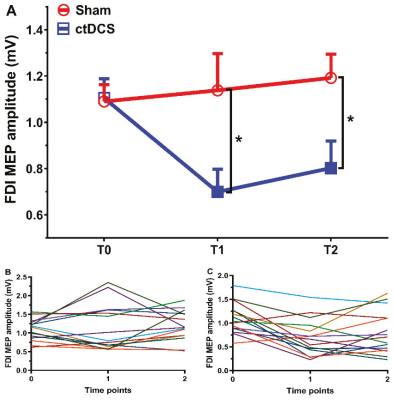

Figure 2. Results of the study. For time points T0, T1, and T2, averaged MEP amplitudes of the first dorsal interosseus (FDI) muscle for all participants (**A**) and individual MEP amplitude in the sham (**B**) and cathodal transcranial direct current stimulation (ctDCS) (**C**) groups are depicted. Filled symbols indicate significant differences of MEP amplitudes after movement observation and execution in comparison to baseline values. Error bars represent standard error of means. * denotes significant differences between groups at $p < 0.05$.

Table 1. Demographic information, SI1mV, and baseline measurements of motor-evoked potentials (MEP) amplitudes (mV) of the intervention groups.

Group	*n*	Gender (F/M)	Age (Years)	SI1mV	MEP (Baseline)
Sham	15	4/11	26.13 ± 3.98	44.13 ± 10.45	1.09 ± 0.28
Real	15	8/7	25.73 ± 5.55	45.67 ± 11.25	1.11 ± 0.32
Between group	-	$p = 0.136$	$p = 0.822$	$p = 0.702$	$p = 0.888$

SI1mV (% MSO) refers to the intensity of TMS required to elicit an average motor evoked potential (MEP) of approximately 1 mV. '-' refers to no data available. The mean values ± standard deviations are shown for the sham and real cathodal tDCS sessions.

4. Discussion

In the sham stimulation condition, there were not significant changes in MEP amplitudes at all time points. Thus, in accordance with another study with the same task, movement observation followed by movement execution did not promote MEP amplitude alterations when no effective stimulation was applied [22]. This result is also compatible with other studies in the field. Movement observation alone did not enhance contralateral motor cortex excitability immediately after movement observation [23,24]. Cortical activity returned to baseline levels within a few seconds [25], as shown by direct recordings of mirror neurons in monkeys [26,27] and indirect measures of neural activity in human motor regions [28].

Cathodal tDCS applied during movement observation induced a reduction of MEP amplitudes, and this effect remained after movement execution in comparison to the sham stimulation condition. Thus ctDCS had antagonistic effects on the modulation of motor cortex activity which was not abolished by movement observation or execution. This might have clinical implications, namely when symptoms are caused by task-related over-activity or lesions of respective cortical regions. Recent clinical studies have shown that ctDCS over the left sensorimotor cortex combined with action observation and electromyographic biofeedback training improved dystonic posture and writing movements of the right upper limb in a patient with writer's cramp [29]. Re-training of finger movements in musician's cramp [18] was improved by cathodal tDCS over the respective motor cortex. Cathodal tDCS combined with movement observation and/ or training thus might improve abnormal cortical excitability and enable a re-arrangement of motor representations. It was also reported that cathodal tDCS over the non-lesional M1 combined with movement execution facilitates motor performance in stroke patients [14,15] and in children with unilateral cerebral palsy [16,17]. The suggested mechanism is that ctDCS coupled with motor training downregulates activity of the non-lesioned hemisphere, while it upregulates activity of the lesioned hemisphere. The latter is via movement execution and reduced transcallosal inhibition, and thus re-balances bi-hemispheric activity and improves sensorimotor representations. In most motor performance studies, anodal tDCS enhanced cortical excitability and improved motor performance [30,31]. This is thought to be caused by improved induction of task-related LTP [32,33]. It was however also shown that cathodal tDCS can improve motor performance under certain conditions [34,35]. This could be due to a signal-to-noise-enhancing effect of the stimulation, which might be beneficial for noisy tasks [36], or when non-selective hyperactivity of a task-relevant area otherwise would reduce performance. This is assumed to be the case in occupation-related dystonia [18].

In the ctDCS group, 86.67 % of the participants at T1 and 73.33 % of the participants at T2 had reduced MEP amplitudes in comparison with the baseline. Given former descriptions of high variability of cathodal tDCS effects [37,38], which might be partially driven by relatively complex non-linear effects of this stimulation protocol [39,40], these effects are remarkably stable. One reason for this stability in the present study might be the known state-dependent effect of tDCS [9]. Brain states might be more variable under resting conditions, which is the context of most studies showing

high variability. In the present study, brain states with respect to the target area might have been relatively stable because of task performance during stimulation.

The limitations of this study include the lack of electroencephalographic recordings and motor performance data (technical limitation of the custom-made button-box), which would have improved mechanistical understanding, and evaluation of behavioral consequences. In addition, this pilot study did not include a ctDCS condition alone, and thus information on how ctDCS interacted specifically with movement observation is not available. The aim of this study was not to induce effects of maximum size, but to deliver proof of principle data. Future studies might show if recently developed optimized ctDCS protocols deliver larger effects [40].

5. Conclusions

It was demonstrated that ctDCS during movement observation was effective in terms of modulating motor cortex activity. Moreover, it subsequently influenced execution-related motor cortex activity. This evidence might guide the implementation of ctDCS combined with task performance to enhance physical therapy in clinical practice in syndromes with pathologically enhanced cortical activity.

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