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Year: 2018

Version:

Please cite the original version:

Mason, J., Frazer, A. K., Horvath, D. M., Pearce, A. J., Avela, J., Howatson, G., & Kidgell, D. J. (2018). Ipsilateral corticomotor responses are confined to the homologous muscle following cross-education of muscular strength. Applied Physiology, Nutrition and Metabolism, 43(1), 11-22. https://doi.org/10.1139/apnm-2017-0457

Ipsilateral corticomotor responses are confined to the homologous muscle following cross-education of muscular strength.

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ABSTRACT

Cross-education of strength occurs when strength-training one limb increases the strength of the untrained limb and is restricted to the untrained homologous muscle. Cortical circuits located ipsilateral to the trained limb might be involved. We used transcranial magnetic stimulation (TMS) to determine the corticomotor responses from the untrained homologous (biceps brachii) and non-homologous (flexor carpi radialis) muscle following strength-training of the right elbow flexors. Motor evoked potentials were recorded from the untrained left biceps brachii and flexor carpi radialis during a submaximal contraction from 20 individuals (10 women, 10 men, aged 18-35 years; training group; n = 10 and control group; n = 10) before and after 3-weeks of strength-training the right biceps brachii at 80% of 1-repetition maximum (1-RM). Recruitment-curves for corticomotor excitability and inhibition of the untrained homologous and non-homologous muscle were constructed and assessed by examining the area under the recruitment curve (AURC). Strength-training increased strength of the trained elbow flexors (29%), resulting in a 18% increase in contralateral strength of the untrained elbow flexors (P <0.0001). The trained wrist flexors increased by 19%, resulting in a 12% increase in strength of the untrained wrist flexors (P = 0.005). TMS showed increased corticomotor excitability and decreased corticomotor inhibition for the untrained homologous muscle (P < 0.05); however, there were no changes in the untrained non-homologous muscle (P > 0.05). These findings show that the cross-education of muscular strength is spatially distributed; however, the neural adaptations are confined to the motor pathway ipsilateral to the untrained homologous agonist.

Key Words: Agonist, excitability, inhibition, synergist, cross-activation.

INTRODUCTION

Cross-education of muscular strength is a type of motor learning, whereby unilateral strength training imparts an increase in strength of the contralateral, untrained homologous muscle (Scripture et al. 1894). The neural mechanism mediating cross-education, appear to be related to bilateral cortical activity, whereby, during unilateral strength training, there is concurrent activation in both cerebral hemispheres that are involved in motor output (Lee et al. 2010; Frazer et al. 2017; Hendy and Kidgell 2014). The primary motor cortex (iM1) ipsilateral to the training limb has been shown to play an important role in mediating the cross-education effect (Hortobágyi et al. 2011; Lee et al. 2010; Hendy et al. 2015; Frazer et al. 2017). Specifically, cross-education studies have reported increased corticomotor excitability (Kidgell et al. 2015), decreased corticomotor inhibition (Coombs et al. 2016; Zult et al. 2016; Hendy and Kidgell 2014; Hendy et al. 2015), reduced interhemispheric inhibition (IHI) (Hortobágyi et al. 2011; Zult et al. 2016) and increased voluntary activation (Lee et al. 2009) in the M1 ipsilateral to the training limb. In support of this, several imaging and transcranial magnetic stimulation (TMS) studies have revealed increased excitability of the ipsilateral M1 of a resting limb during unilateral voluntary contractions (increased cross-activation) (Hortobágyi et al. 2003; Liepert et al. 2001; Muellbacher et al. 2000; Frazer et al. 2017; Hendy and Kidgell 2014; Zult et al. 2016). Therefore, the elevated activity in the ipsilateral M1 that is detected during unilateral motor practice appears to be, in part a mediator for the cross-education of muscular strength (Kidgell et al. 2015; Leung et al. 2015; Hortobágyi et al. 2011; Hendy et al. 2015; Ruddy et al. 2017; Zult et al. 2016; Lee et al. 2010).

The cross-education of muscle strength has been demonstrated comprehensively using isometric, dynamic and imagined muscle contractions (Shaver 1975; Brown et al. 1990; Cannon and Cafarelli 1987; Hortobágyi et al. 1999). The cross-education effect is also related to specific parameters of the training load, for example, an at home-home resistance training program has shown cross-education effects, whilst exercises that employ novelty or are unfamiliar, such as ulnar deviation, also show cross-education effects (Farthing 2009; Magnus et al. 2014). Several studies have shown a strong association between the magnitude of cross-education and the degree of strength gained in the trained muscle (Kidgell et al. 2011; Hendy et al. 2015; Farthing et al. 2005; Hortobágyi et al. 1999; Zhou, 2000; Zult et al. 2014). Collectively, these lines of investigation provide convincing evidence of the existence of the cross-education phenomenon (Zhou 2000; Lee and Carroll 2007).

However, to date, only two studies have measured the effects of cross-education outside of the contralateral untrained homologous muscle (Hortobágyi et al. 1999; Sariyildiz et al. 2011), with one study showing no change and the other a small change in strength. Furthermore, there have been no studies that have examined the

corticospinal responses from a heterologous muscle following cross-education, resulting in a major gap in the literature.

The clinical efficacy of cross-education has been examined in studies of immobilization (Farthing et al. 2009; Magnus et al. 2010; Pearce et al. 2012), wrist fracture (Magnus et al. 2013) and more recently in stroke (Dragert and Zehr 2013). However, to optimise the clinical benefits of cross-education, exploring the spatial effects is important. Recently, we have shown that strength training alone, results in spatial adaptations in strength and corticomotor excitability and inhibition that is not just restricted to the agonist muscle, but also to synergist muscles, making the spatial effects of cross-education conceivable due to the dynamic and integrated nature of the M1, including its divergent corticospinal projections (Mason et al. 2017). Accordingly, it is therefore possible that cross-education is also not restricted to the agonist muscles, but also has spatial crossover effects to the synergist muscle. Identifying the activation and performance improvements of synergist muscles would provide valuable insight into the collective, functional and spatial effects of cross-education.

Therefore, the aim of this study was to examine the spatial effects of the cross-education of strength and to characterise the input-output properties of the corticomotor pathway for the untrained homologous agonist and non-homologous synergist muscle following three-weeks of unilateral strength training. It was hypothesised that unilateral strength training of the right biceps brachii muscle would induce changes in corticomotor excitability and inhibition for both the untrained homologous muscle (left biceps brachii) and the untrained homologous synergist (left flexor carpi radialis).

MATERIALS AND METHODS

Participants

Twenty participants (10 women, 10 men, and aged 18-35 years) volunteered to participate. All volunteers provided written informed consent prior to participation in the study, which was approved by La Trobe University Human Research Ethics Committee in accordance with the standards by the Declaration of Helsinki. All participants were right-hand dominant as determined by the Edinburgh Handedness Inventory (Oldfield 1971) with a Laterality Quotient Score greater than 40, had not participated in strength-training for at least 12 months (on a recreational basis), and were free from any known history of neuromuscular impairment. All participants were recreationally active in competitive sport during their teenage years, but none were currently participating in any formal competitive sport. Prior to the experiment, all participants completed the adult safety screening questionnaire to determine their suitability for TMS (Keel et al. 2001).

Experimental approach

A schematic representation of the study is presented in Figure 1. After obtaining consent, participants completed a familiarisation session one week prior to the study that involved performing a one-repetition maximum (1-RM) strength test of the right and left elbow flexors (to establish training load) and were then exposed to single-pulse TMS. Following the familiarisation session, all participants completed a 1-RM strength, and then were matched for gender and baseline strength, then randomly allocated into either the control (no training) or training group. A purpose made Excel macro was used to randomize participants based upon baseline strength and gender (Rantalainen et al. 2013). All participants underwent TMS and maximum strength testing of the right and left biceps brachii and flexor carpi radialis before and after a three week supervised strength-training program of the right elbow flexors with post-testing occurring within 48 hours of the final training session. Control participants undertook pre- and post-testing only. Specifically, control participants attended the laboratory at baseline and then again, three weeks later for post testing. All control participants were required to maintain their current levels of physical activity.

Voluntary strength testing

Participants in both groups performed a standard unilateral 1-RM test for both the right and left elbow flexors, specifically targeting the biceps brachii. Following previous work (Munn et al. 2005), participants were asked what they believed their 1-RM elbow flexion strength was and this load served as their initial starting weight. Participants performed the 1-RM test standing, holding a weighted dumbbell with one hand, with their elbow in full extension, forearm supinated, and the opposite arm placed behind their back while standing against a wall to prevent extraneous body movement. Participants were then asked to flex their arm and lift the dumbbell as if performing a standard biceps curl. If the trial was successful, the weight of the dumbbell was increased accordingly (0.5 kg increments) on each trial following a three-minute recovery to minimise the development of muscular fatigue (Kidgell et al. 2011). This procedure continued until the subject could no longer complete one repetition and their prior successful trial served as their 1-RM isotonic biceps brachii strength for both the right and left arm (Munn et al. 2005; Kidgell et al. 2011). Participants completed on average three trials to achieve their 1-RM strength.

Maximum voluntary isometric contraction force (MVC) of the right and left wrist flexors was determined on a custom-made force transducer (Futek Force Transducer LSB302, Melbourne). For the wrist flexors MVC, participants were seated in a chair, shoulders in a neutral position with their elbow flexed at 110

degrees (Frazer et al. 2016). With the hand supinated, the force transducer was positioned over the middle aspect of the palmar surface of the hand and was adjusted to ensure that the external moment arm was individually established for each participant. Once the external moment arm was established, participants were instructed to push up against the transducer as forcefully as possible for three seconds. Three trials were performed, separated by a three-minute rest to minimise fatigue. The greatest recorded output was used for data analysis.

Strength-training protocol

Using the same set-up as in the 1-RM testing, participants completed flexion-extension movements of the right elbow with the forearm supinated (biceps curl). Special attention was paid to the instructions to keep the opposite arm completely immobile and as relaxed as possible during the training. Participants completed four sets of 6-8 repetitions at 80% 1-RM with the right arm only (to contractile failure) with three-minute recovery between sets (Kidgell et al. 2010). A repetition timing of three seconds concentric and four seconds for the eccentric phase was maintained using an electronic metronome (Kidgell et al. 2010). The use of an automated timing device was selected as previous research has shown that controlled slow velocity strength training facilitates greater changes in TMS evoked MEP responses compared to self-paced training (Leung et al. 2015; Kidgell et al. 2015). Progressive overload was applied once participants could complete four sets of 8 repetitions by increasing the training weight by 2.5% (Kidgell et al. 2011).

Surface electromyography

The area of electrode placement was shaven to remove fine hair, rubbed with an abrasive skin gel to remove dead skin, and then cleaned with 70% isopropyl alcohol. Surface electromyography (sEMG) was recorded from the left biceps brachii and left flexor carpi radialis muscle using bipolar Ag-AgCl electrodes. For the biceps brachii, the site of measurement was determined by marking the skin two thirds of the distance between the acromion and the lateral epicondyle, while the participant stood relaxed in the anatomical position (Pearce et al. 2012). This mark was then extended to the most anterior point of the muscle bulk where the electrodes were placed 2 cm apart over the mid-belly of the biceps brachii, with a ground electrode secured on the lateral epicondyle of the humerus (Wilson et al. 1993). The exact sites were marked with a permanent marker by tracing around the electrode, and this was maintained for the entire three-week training period by both the researcher and participant to ensure consistency of electrode placement relative to the innervation zone.

sEMG was also recorded from the left flexor carpi radialis muscle using bipolar Ag-AgCl electrodes as described by Selvanayagam et al. (2012). The electrodes for the flexor carpi radialis were positioned 9 cm from the medial epicondyle of the humerus with an inter-electrode distance of 2 cm (Selvanayagam et al. 2012). A grounding strap was placed around the wrist as the common reference point for all electrodes. sEMG signals were amplified (x1000), band pass filtered (20 Hz - 1 kHz), digitized online at 2 kHz, recorded (1 sec) and analysed using Power Lab 4/35 (AD Instruments, Bella Vista, Australia).

Transcranial magnetic stimulation

TMS was delivered using a MagPro Compact (MagVenture A/S, Lucernemarken, Denmark) and a single C-B60 Butterfly Coil (external diameter of each loop 75 mm). The motor hotspots for the left biceps brachii and flexor carpi radialis (with posterior-to anterior-induced current flow in the cortex) was determined, and active motor threshold (AMT) was established as the stimulus intensity at which at least 5 of 10 stimuli produced motor evoked potential (MEP) amplitudes of greater than 200 μV (Rossini et al. 1999). Following the unilateral strength-training intervention, AMT was re-tested and adjusted if required. To ensure all stimuli were delivered to the optimal motor hotspots throughout testing, participants wore a tight-fitting cap marked with a latitude-longitude matrix, positioned with reference to the nasion-inion and interaural lines.

All stimuli were delivered during a low-level isometric contraction of the left biceps brachii and the left flexor carpi radialis. For the MEPs obtained from the left untrained biceps brachii, participants were required to maintain an elbow joint angle of 90 degrees' elbow flexion. Holding the lower arm in this joint position equated to 5 ± 1% of the maximal root-mean squared electromyography (*rms*EMG). Because this position resulted in a low level of muscle activity, and to ensure that background muscle activity was consistent between TMS stimuli, *rms*EMG was recorded 100 ms before the delivery of each TMS pulse. During the TMS trials, visual feedback was presented to the volunteer to display an upper limit of 5% *rms*EMG and participants were instructed to maintain their muscle activation levels below this upper limit. The stimulus delivery software (LabChart 8 software, ADInstruments, Bella Vista, NSW, Australia) was set, so that stimuli were not delivered if the *rms*EMG value, 100 ms immediately prior to the stimulus, exceeded 5 ± 1% (Table 1). The MEPs obtained from the flexor carpi radialis were collected during low-level isometric contractions of the wrist flexors. Low-level contractions equated to 5 ± 1% of *rms*EMG obtained during MVC and were performed by maintaining the wrist and fingers in a straight position (Hendy and Kidgell 2013). This level of background sEMG has been previously used to produce reliable MEPs amplitudes and silent period durations (Sale and

Semmler 2005; Kidgell et al. 2015) and represents 2% of MVC force. The order of testing for the construction of corticospinal excitability and inhibition (silent period duration) recruitment curves were randomized between the untrained biceps brachii and flexor carpi radialis.

Recruitment Curves

Recruitment curves for both corticomotor excitability and inhibition (silent period) were constructed for the untrained contralateral homologous agonist muscle (biceps brachii) and for the untrained contralateral synergist (flexor carpi radialis) muscle. The stimulus intensities used to establish the TMS recruitment curves were determined for each individual according to their AMT before the training intervention for both the untrained agonist and synergist. At each stimulus intensity, 10 stimuli were applied over the right M1, with the percentage of stimulator output delivered in a pseudo-randomized manner. Specifically, two sets of 5 stimuli were given at stimulus intensity from, 110% of each participant's AMT up to 170% AMT, in 20% increments. Each stimulus was delivered in random intervals every 10 to 12 s to avoid stimulus anticipation, and 30 sec rest was provided between each set of stimuli to reduce the possibility of muscle fatigue.

Cross-activation

To determine cross-activation, 10 stimuli were delivered to the right M1 at 130% AMT during maximal voluntary contraction (MVC) of the right biceps brachii and right flexor carpi radialis.

Maximum compound muscle action potential

Direct muscle responses were obtained from the left untrained biceps brachii muscle by supramaximal electrical stimulation (pulse width, 200 μs) of the brachial plexus at Erbs point (DS7A; Digitimer, Hertfordshire, United Kingdom). The stimuli were delivered while the participant sat in an upright position, with the elbow at 90 degrees elbow flexion holding 5 ± 1% of maximal *rms*EMG. This low level of muscle activity was used to match the conditions under which TMS was delivered (Frazer et al. 2016). An increase in current strength was applied to Erbs point until there was no further increase observed in the amplitude of the sEMG response (M_{MAX}).

Direct muscle responses were also obtained from the untrained left flexor carpi radialis muscle by supramaximal electrical stimulation (pulse width 200 μs) of the median nerve under active conditions (5 ± 1% *rms*EMG [DS7A, Digitimer, Hertfordshire, UK]). The site of stimulation that produced the largest M-wave was located by positioning the bipolar electrodes in the cubital fossa. An increase in current strength was applied to

the median nerve until there was no further increase observed in the amplitude of the sEMG response (M_{MAX}) (Kidgell et al. 2015). To ensure maximal responses from both the untrained biceps brachii and flexor carpi radialis, the current was increased an additional 20% and the average M_{MAX} was obtained from five stimuli, with a period of 6–9 seconds separating each stimulus. M_{MAX} was recorded at baseline and following the strength-training intervention to control for possible changes in peripheral muscle excitability that could influence MEP amplitude.

Contralateral Strength Transfer

The contralateral transfer of strength was calculated to determine the difference in change in the mean strength of the untrained non-dominant elbow flexors and wrist flexors in the control and trained groups following the training period (Kidgell et al. 2011). The calculation was performed as follows:

$$(EPost-EPre)/EPre)\times100)-(CPost-CPre)/CPre)\times100)$$

Where EPost refers to mean post-training maximum strength for the trained groups' untrained elbow flexors, EPre refers to mean pre-training maximum strength for the trained groups' untrained elbow flexors, CPost refers to mean post-training maximum strength for the controls' untrained (non-dominant) elbow flexors, and CPre refers to mean pre-training maximum strength for the control groups' untrained (non-dominant) elbow flexors. The same procedures were applied to calculate the cross-transfer of strength to the untrained wrist flexors.

Data analysis

Pre-stimulus *rms*EMG activity was determined in the untrained left biceps brachii and flexor carpi radialis 100 ms prior to each TMS stimulus during pre- and post-testing. Any trial in which pre-stimulus *rms*EMG exceeded 5 ± 1 % of maximal *rms*EMG were discarded, and the trial was repeated. The range of *rms*EMG was accepted at 3-5% of maximal *rms*EMG activity. The peak-to-peak amplitude of MEPs evoked as a result of stimulation was measured in the left biceps brachii and flexor carpi radialis contralateral to the cortex being stimulated in the period 10-50 ms after stimulation. MEP amplitudes were analyzed (LabChart 8 software, ADInstruments, Bella Vista, NSW, Australia) after each stimulus was automatically flagged with a cursor,

providing peak-to-peak values in μV, averaged and normalized to the M_{MAX}, and multiplied by 100, separately for the untrained biceps brachii and flexor carpi radialis.

Silent period durations were obtained from single-pulse stimuli delivered at 130-170% AMT during a light elbow flexor and wrist flexors contraction (5 ± 1 % of maximal *rms*EMG), separately from the biceps brachii and flexor carpi radialis. The start of the silent period was calculated from the onset of the MEP and the cessation of the silent period was measured at the return of consistent sEMG to pre-stimulus levels. In order to do this, a horizontal cursor was positioned on the maximum and minimum of the pre-stimulus sEMG level and determined the time when the sEMG crossed these threshold levels following the silent period. Importantly, the experimenter was blinded to each condition. The average from 10 stimuli was used for silent period duration (Wilson et al. 1993).

In addition, the total area under the recruitment curve (AURC) was calculated with the method of trapezoidal integration using the data collected during the construction of corticospinal excitability and inhibition recruitment curves for both the untrained left biceps brachii and flexor carpi radialis separately. The data obtained from the AURC is presented as arbitrary units (AU) (Carson et al. 2013).

Statistical analysis

All data were screened with the Shapiro-Wilk test and found to be normally distributed (all $P > 0.05$). Sphericity was confirmed using Mauchly's Test of Sphericity, specifically, looking at Greenhouse Geisser and Huynh-Feldt correction to test for equality of variance to ensure the assumptions of the ANOVA were not violated. To ensure that there were no significant differences between groups at baseline, a one-way analysis of variance (ANOVA) was used for all dependent variables. To test the hypothesis that unilateral strength training increases contralateral strength and corticomotor excitability and decreases corticomotor inhibition, a two-way ANOVA and Tukey HSD for *post hoc* testing, for the untrained limb was used to compare group interaction (trained vs. control) by testing session (pre vs. post) for each dependant variable (strength, pre-stimulus *rms*EMG, corticomotor excitability, silent period duration and AURC for the contralateral untrained homologous and non-homologous muscles). To determine if any changes in the AURC for corticomotor excitability and inhibition were different between the untrained contralateral agonist and untrained synergist muscle following the unilateral strength-training program, a One-Way ANOVA was used on the change score. Linear regression analysis was also used to examine any potential association between changes in muscle strength [(post strength/pre strength x 100) - 100], changes in MEP amplitude after training (pooled MEP

amplitude post/pre x 100) - 100], and changes in silent period duration after training (pooled silent period duration post/pre x 100) - 100] for the untrained homologous and untrained non-homologous muscle. Prism 7.1 for Windows (Graphpad Software Inc, CA, USA) was used for all statistical analyses with the level of significance set as $P < 0.05$ for all testing. All data are presented as mean ± SE.

RESULTS

*Pre-stimulus rms*EMG, *Maximal Compound Waves, and Motor Thresholds*

Table 1 presents the mean (± SE) for AMT stimulus intensity, M_{MAX} and single-pulse TMS pre-stimulus *rms*EMG amplitude prior to and following strength training for the contralateral untrained biceps brachii and the contralateral untrained flexor carpi radialis. At baseline, there were no differences in pre-stimulus *rms*EMG ($P = 0.51$), AMT stimulus intensity ($P = 0.77$) and M_{MAX} ($P = 0.78$) between groups for the left untrained biceps brachii and flexor carpi radialis (all $P > 0.05$). Following the training intervention, Pre-stimulus *rms*EMG did not vary for single-pulse TMS trials ($P = 0.53$), and there were no time or interactions effects ($P = 0.48$). Similarly, there was no time or interactions detected for AMT stimulus intensity or M_{MAX} ($P = 0.41$ and $P = 0.56$, respectively).

Maximal voluntary force

Biceps Brachii Strength

At baseline, there we no differences in 1-RM strength of the untrained left elbow flexors between groups ($F_{1,18} = 0.319$; $P = 0.585$). Following the intervention there was a main effect for time ($F_{1, 18} = 122.8$; $P < 0.0001$) and a group by time interaction ($F_{1, 18} = 154.8$; $P < 0.0001$). *Post hoc* analyses revealed there was a 23% increase absolute strength in left elbow flexor when compared with control ($P < 0.0001$; Fig. 2a).

There was a significant positive correlation between the percentage of strength gained in the trained right elbow flexors and the percentage of the contralateral transfer of strength to the untrained left elbow flexors (r^2 0.680; $P = 0.003$; Fig. 2b). Unilateral strength training of the right elbow flexors resulted in an 18% strength-transfer to the contralateral untrained left elbow flexor.

Wrist Flexor Strength

At baseline, no differences in 1-RM strength of the untrained left wrist flexors were present between groups ($F_{1, 18} = 0.106$; $P = 0.751$). Following the intervention there was a main effect for time ($F_{1, 18} = 14.8$; $P = 0.003$) and a group by time interaction ($F_{1, 18} = 13.1$; $P = 0.005$). *Post hoc* analyses revealed there was a 12%

increase in absolute strength of the left wrist flexors when compared with the control group ($P < 0.0001$; Fig. 2c).

There was a significant positive correlation between the percentage of strength gained in the trained right wrist flexors and the percentage of the contralateral transfer of strength to the untrained left wrist flexors (r^2 0.581; $P = 0.010$; Fig. 2d). Unilateral strength training of the right elbow flexors resulted in a 10% strength-transfer to the contralateral untrained left wrist flexors. Further, there was a positive correlation between the percentage of strength gain for the untrained biceps brachii and the untrained wrist flexors (r^2 0.831; $P = 0.002$).

Corticomotor excitability

Biceps Brachii

Figure 3a shows the AURC obtained prior to and following the strength training intervention for the left untrained biceps brachii for the control group (Figure 4a), and the trained group (Fig 3b). Total AURC were similar between groups at baseline ($F_{1, 18} = 0.248$; $P = 0.624$). Following the intervention there was a main effect for time ($F_{1, 18} = 10.68$; $P = 0.043$) and a group by time interaction ($F_{1, 18} = 9.144$; $P = 0.007$). *Post hoc* analyses revealed there was a 25% increase in total AURC for the untrained left biceps brachii (pre 1356 ± 144 arb.units; post 1705 ± 164 arb.units), compared to the 1% increase in the control group (pre 1429 ± 126 arb. units; post 1443 ± 117 arb. Units; $P = 0.006$; Fig. 3a-b).

Flexor Carpi Radialis

For the untrained left flexor carpi radialis, total AURC were similar between groups at baseline ($F_{1, 18} = 2.098$; $P = 0.164$). Following the intervention there was a main effect for time ($F_{1, 18} = 8.461$; $P = 0.001$) and a group by time interaction ($F_{1, 18} = 8.14$; $P = 0.012$). *Post hoc* analyses revealed there was a 20% increase in total AURC for the untrained left wrist flexors (pre 1733 ± 700 arb.units; post 2125 ± 528 arb.units, $P = 0.001$), however the magnitude of change was not different to the control group (pre 1729 ± 121 arb. units; post 1732 ± 140 arb. units; $P = 0.216$; Fig. 3c-d).

We also examined if the magnitude of change in the AURC for corticomotor excitability was different between the contralateral untrained agonist and the contralateral untrained synergist muscles following three weeks of unilateral strength training. One-Way ANOVA revealed that there was a significant difference in the AURC for corticomotor excitability for the agonist and synergist when compared to the control group ($F_{1, 18} = 6.18$; $P = 0.008$), however there were no within-group effects for muscle for the training group ($F_{1, 18} = 0.95$; $P = 0.543$). *Post hoc* analysis showed that AURC for both the agonist and the synergist was different to the control group (*P*

<0.0001), however, there was no difference in the magnitude of change in the AURC for corticomotor excitability between the trained agonist and the synergist muscle ($P = 0.542$).

Changes in corticomotor excitability and contralateral muscle strength

Using linear regression, there was no association between the change in biceps brachii MEP amplitude of the ipsilateral M1 and the change in maximum strength of the untrained contralateral biceps brachii ($r^2 = 0.317$, $P = 0.09$). In a similar manner, there was no association between the change in MEP amplitude of the flexor carpi radialis of the ipsilateral M1 and the change in maximum strength of the untrained contralateral wrist flexors ($r^2 = 0.138$, $P = 0.290$).

Corticomotor inhibition

Biceps Brachii

Figure 4a-b shows the total AURC obtained for corticomotor inhibition prior to and following the unilateral strength-training intervention for the untrained left biceps brachii. For the left untrained biceps brachii, total AURC were similar between groups at baseline ($F_{1, 18} = 0.419$; $P = 0.525$). Following the intervention, there was a main effect for time ($F_{1, 18} = 22.87$; $P < 0.0001$) and a group by time interaction detected ($F_{1, 18} = 28.09$; $P < 0.0001$). *Post hoc* analysis revealed that there was a 15.3% decrease (pre 6.5 ± 0.21 arb. units; post 5.47 ± 0.11arb. units) in the total AURC compared to a 1% decrease (pre 6.13 ± 0.23 arb. units; post 6.19 ± 0.23 arb. units, $P = 0.036$) in the control group.

Flexor Carpi Radialis

Figure 4c-d shows the total AURC obtained for corticomotor inhibition prior to and following the unilateral strength-training intervention for the flexor carpi radialis. The total AURC for corticomotor inhibition were similar between groups at baseline ($F_{1, 18} = 0.506$; $P = 0.486$). Following the intervention, there was a main effect for time ($F_{1, 18} = 51.62$; $P <0.0001$), and group by time interaction detected ($F_{1,18} = 70.98$; $P < 0.0001$). Following strength-training, there was a 9% decrease (pre 6.5± 0.2 arb. units; post 5.6 ± 0.2 arb. units) in the total AURC for the wrist flexors, however, this magnitude of change was not different when compared with the control group who had a 1% increase in the total AURC (pre 6.44± 0.25 arb. units; post 6.49 ± 0.23arb. units, $P = 0.200$).

We also examined if the magnitude of change in the AURC for corticomotor inhibition was different following three weeks of unilateral strength training between the contralateral untrained agonist and synergist muscles. One-Way ANOVA revealed that there was a significant difference in the AURC for corticomotor inhibition for the agonist and synergist when compared to the control group ($F_{1, 18}$ = 6.18; P < 0.0001) and a within-group effect for muscle ($F_{1, 18}$ = 107.5; P < 0.0001). *Post hoc* analysis revealed the decrease in the AURC for the untrained homologous muscle was greater when compared to the magnitude of change in the AURC for the untrained wrist flexors (P < 0.0001).

Changes in corticomotor inhibition and contralateral muscle strength

Using linear regression, there was no association between the change in biceps brachii silent period duration of the ipsilateral M1 and the change in maximum strength of untrained contralateral biceps brachii (r^2 = 0.064, P = 0.479). In a similar manner, there was no association between the change in silent period duration of the ipsilateral M1 of the flexor carpi radialis and the change in maximum strength of the contralateral untrained wrist flexors (r^2 = 0.005, P = 0.950).

Cross-Activation

Biceps Brachii

Mean changes in cross-activation following each condition are displayed in Figure 5a-b. There were no significant differences in MEP amplitude for the left biceps brachii during contralateral MVC at baseline ($F_{1, 18}$ = 0.2486; P = 0.6241). Following the intervention, there was a main effect for time ($F_{1, 18}$ = 10.68; P = 0.0043); however, there was no group by time interaction ($F_{1, 18}$ = 9.14; P = 0.730). *Post hoc* analysis revealed that there was a 41% increase in cross-activation of the left untrained biceps brachii following unilateral strength training of the right biceps brachii (Pre 31.6 ± 18.3% M_{MAX}; post 44.1 ± 22.5% M_{MAX}), however the magnitude of this change was not different to the 5% change in the control group (Pre 32.08 ± 5.9% M_{MAX}; post 35.3 ± 7.1% M_{MAX}; P = 0.571, Fig 5a).

Flexor Carpi Radialis

Mean changes in cross-activation following each condition are displayed in Figure 5b. There were no significant differences in MEP amplitude for the left flexor carpi radialis during contralateral MVC at baseline ($F_{1, 18}$ = 1.13; P = 0.500). Following the intervention, there was a main effect for time ($F_{1, 18}$ = 4.717; P = 0.043); and a group by time interaction ($F_{1, 18}$ = 8.692; P = 0.008). *Post hoc* analysis revealed that there was a 45%

increase in cross-activation of the left untrained flexor carpi radialis following unilateral strength training of the right biceps brachii (Pre 30.4 ± 4.5% M_{MAX}; post 41.0 ± 4.8% M_{MAX}), however the magnitude of this change was not different when compared to the 4% decrease in the control group (Pre 33.5 ± 4.6% M_{MAX}; post 32.1 ± 4.1% M_{MAX}; $P = 0.502$, Fig 5b).

DISCUSSION

This is the first study to examine the corticomotor responses of the ipsilateral motor pathway from an untrained contralateral synergist muscle following three weeks of unilateral strength training. The current findings, extend on existing evidence that supports the efficacy of contralateral strength and corticomotor changes following a period of unilateral strength training. The key findings from the current study demonstrate unilateral strength training of the right elbow flexors increased voluntary strength for both the right trained (29% increase) and left untrained (23% increase) biceps brachii; but critically, we demonstrate for the first time that an untrained synergist muscle also increased strength (12%). Thus it can be suggested, that the cross-education of strength is not spatially restricted to the contralateral homologous agonist muscle. Interestingly, the corticomotor responses only changed in the untrained homologous agonist muscle, with an increase in corticomotor excitability and a decrease in corticomotor inhibition of the ipsilateral motor pathway. These data suggest that the neural adaptations following the cross-education of strength are spatially confined to the cortical representation of the homologous agonist muscle.

Unilateral strength training improves strength of the untrained synergist muscle

Whilst the increase in maximal voluntary strength of the untrained synergist wrist flexors is an important new finding, this increase in strength is likely because of the changes in strength of the trained wrist flexors. Previously, it has been proposed, that the magnitude of the cross-education of strength is proportional to the amount of strength gained in the training limb (Munn et al. 2004). Given that the training task involved heavy-load strength training of the biceps brachii muscle, the wrist flexors of the trained limb acted as a synergist muscle, and thus were isometrically active. The submaximal and isometric contribution resulted in a 19% increase in the maximal voluntary strength of the wrist flexors of the trained limb. The increase in strength of the trained synergist muscle is consistent with previous research, which confirms that isometric strength training increases strength of the trained limb (Hortobágyi et al. 1997; Zhou et al. 2000; Kidgell and Pearce 2010), even at training intensities of 10-20% of MVC (Laidlaw et al. 1999; Kobayashi et al. 2014). Such an

increase in strength of the trained synergist, likely accounts for the increase in strength of the untrained synergist wrist flexors, which supports the strong correlation shown in this study. This finding is common for the trained agonist (Munn et al. 2004; Munn et al. 2005; Carrol et al. 2006; Kidgell et al. 2011; Goodwill et al. 2012; Zult et al. 2014), however we have shown that there is also a direct correlation between the gain in strength of a trained synergist muscle and the ensuing strength gains in the untrained synergist muscle.

Also consistent with previous cross-education of strength studies, was the 23% increase in strength for the untrained bicep brachii (Goodwill et al. 2012; Latella et al., 2012, Kidgell et al. 2011; Coombs et al. 2016, Farthing et al. 2005). Although current systematic reviews report lower magnitudes of cross-education (Carroll et al. 2006; Munn et al. 2004), these training paradigms typically employ only moderate intensity and untimed contractions, which seems to lessen the cross-education effect (Kidgell et al. 2011; Goodwill et al. 2012, Coombs et al. 2016).

Interestingly, the magnitude of strength increase for the untrained wrist flexors was less than the strength increase for the untrained bicep brachii. Firstly, it is important to establish that the trained biceps brachii improved more than the trained wrist flexors, suggesting that the type of muscle action is important. The trained synergist wrist flexors was contracting isometrically and sub-maximally, as opposed to the isotonic nature of the biceps brachii contractions. Dynamic strength training that involves both concentric and eccentric contractions has been shown to greatly increase strength (Kidgell et al. 2015, Hortobágyi et al. 1997), particularly when compared to isometric training alone, and this may contribute to the observed differences in the strength of the untrained synergist wrist flexors (Higbie et al. 1996; Uematsu et al. 2010). While involvement of the trained synergist wrist flexors in the biceps brachii training protocol allowed for the cross-education of strength, the isometric and sub-maximal nature of its contribution may have limited the magnitude of cross-education for the synergist muscle. Despite this, the submaximal isometric nature of the wrist flexors, still imparted a significant increase in strength to the untrained limb. This contribution, seems to be strongly related to the amount of strength gained in the trained wrist flexors, showing the important role that the wrist flexors play during a biceps curl exercise.

Corticomotor excitability is spatially confined to the untrained agonist

Although the improvements in strength following the cross-education of strength are commonly reported (Carroll et al. 2006), the neural mechanisms mediating these improvements are not completely

understood, but changes in cortical areas that are associated with motor planning have been suggested (Ruddy et al. 2017).

Consistent with previous cross-education of strength studies (Kidgell et al. 2011; Goodwill et al. 2012; Hendy et al. 2015), we have shown an increase in the AURC for corticomotor excitability of the contralateral untrained homologous agonist muscle. Strong voluntary contractions of the right elbow flexors increased the size (i.e., AURC) of the MEPs of the elbow flexors of the other arm. Given that there was only a within time effect for increased MEPs of the untrained left synergistic wrist flexors, it seems that the corticomotor responses are spatially confined to the cortical representation of the untrained contralateral homologous agonist muscle. These data indicate that unilateral strength training of one limb, has bilateral effects, that manifest as increased corticomotor excitability of the motor pathway ipsilateral to the training limb.

The increase in the AURC for corticomotor excitability of the untrained homologous elbow flexors, likely represents a general increase in excitability of the neurons in the M1 and the motor neuron pool (Rothwell et al. 1991), making it unclear whether such changes are of a cortical or sub-cortical origin. Regardless of this, there is evidence to show that during strong voluntary contractions of one limb, cervico-medullary MEPs remain unchanged and H-reflexes decrease (Hortobágyi et al. 2003), therefore, unilateral strength training that incorporates strong voluntary contractions seems to lead to an increase in M1 excitability, ipsilateral to the trained limb.

There was no difference in the magnitude of change for the untrained synergist muscle compared to the control group. On this basis, there was no alteration in the AURC for corticomotor excitability of the M1 ipsilateral to the trained synergist wrist flexors. Although we correctly hypothesised an increase in contralateral synergist muscle strength, we thought (incorrectly) this would be accompanied by increased corticomotor excitability projecting to the untrained synergist wrist flexors. This hypothesis was based on well-established evidence, that unilateral voluntary contractions not only activate the contralateral motor pathway, but also the ipsilateral motor pathway targeting the resting or untrained limb (Frazer et al. 2017; Hortobágyi et al. 2003; Ruddy et al. 2017; van Duinen et al. 2008; Perez and Cohen 2008; Verstynen and Ivry 2011; Carson and Ruddy 2013; Hendy and Kidgell 2014; Zult et al. 2016). On this basis, it was likely that the untrained synergist wrist flexors would experience increased excitability following training, because of its isometric role in the strength training protocol and because of the contribution of shared corticospinal inputs between agonists and synergists (Smith and Fetz 2009; Capaday et al. 2013). Despite this, several lines of evidence could explain why

corticomotor excitability was not different between groups for the untrained synergist wrist flexors. Firstly, during unilateral voluntary contractions, the ipsilateral motor pathway is active; but this response is highly dependent upon the intensity of the voluntary contraction, with greater activation of the ipsilateral motor pathway occurring during stronger voluntary contractions (Stedman et al. 1998; Muellbacher et al. 2000; Stinear et al. 2001; Hortobágyi et al. 2003; Perez and Cohen 2008). Secondly, there is evidence to suggest that agonists and synergists share common corticospinal inputs, implying that agonist and synergist muscles might share neural drive as an integrated unit (Porter and Lemon 1993; De Luca and Erim 2002; Smith and Fetz 2009). Thirdly, these shared inputs appear to be highly specialised with selective input (Smith and Fetz 2009; Capaday et al. 2013). Because of this selective input, it is possible that corticomotor excitability during the performance of voluntary contractions will only target the agonist muscle in a specialised manner. Certainly, given that there was an increase in the AURC for the motor pathway of the untrained contralateral homologous agonist muscle, the overall contribution of force production from the synergist wrist flexors has restricted the activation of the motor pathway of the synergist wrist flexors and has subsequently had a limited spatial effect on corticomotor excitability. This is important, because cross-activation appears to be the primary mechanism for increased excitability within the ipsilateral motor pathway following a unilateral strength training intervention (Hortobágyi et al. 2003; Ruddy and Carson 2013; Hendy and Kidgell 2014; Frazer et al. 2017) and it seems that cross-activation is limited to the homologous agonist muscle. This finding is consistent with the increases in cross-activation reported in this study (Figure 5a-b). Therefore, the cross-education of strength, only effects the excitability of one muscle representation, the untrained homologous agonist muscle. This likely explains the lack of interaction between the groups for increased corticomotor excitability of the untrained synergist wrist flexors.

Corticomotor inhibition is restricted to the untrained agonist muscle

Several lines of evidence support the view that GABA-mediated corticomotor inhibition contributes to motor cortical plasticity (Werhahn et al. 1995). Certainly, previous studies have reported that voluntary contractions practiced by one hand reduces inhibition in the other hand and that this reduction diminishes with increasing force production (Camus et al. 2009; Muellbacher et al. 2000; Zult et al. 2016). Based upon this evidence, it was hypothesised that a reduction in corticomotor inhibition in the untrained contralateral agonist and synergist would occur. Contrary to this expectation, we only observed a reduction in inhibition for the untrained contralateral agonist muscle and only a small within time effect for reduced corticomotor inhibition in the untrained synergist wrist flexors.

To our knowledge, this is only the third study to report a reduction in silent period following the cross-education of strength (Coombs et al. 2016; Zult et al. 2016). Because there was no interaction effect for reduced corticomotor inhibition in the untrained synergist wrist flexors, it seems that the motor cortical response to cross-education is spatially confined to the untrained homologous agonist muscle. The reduction in the AURC for corticomotor inhibition, suggest that an important neural adaptation to cross-education, is a reduction in silent period duration to the untrained homologous agonist muscle. Overall, it looks as if unilateral strength training targets specific populations of intracortical inhibitory neurons that collectively result in increased activation of the target motor neuron pool (Coombs et al. 2016; Kidgell et al. 2015; Hendy et al. 2015; Hortobágyi et al. 2011). On the other hand, critical to the purpose of this study, it only occurs in the untrained contralateral homologous agonist muscle.

It is unclear why there was no significant reduction in corticomotor inhibition of the untrained synergist wrist flexors between groups. Several lines of evidence showed that isometric contractions also reduced inhibition in a resting or in an untrained muscle (Camus et al. 2009; Muellbacher et al. 2000; Kidgell et al. 2015). Previous cross-education studies have reported significant reductions in silent period durations in the untrained limb following unilateral strength training, which directly trained the agonist with near-maximal contractions (Coombs et al. 2016; Kidgell et al. 2015; Zult et al. 2016), rather than the indirect and sub-maximal contribution of the synergist wrist flexors within this study. Again, it seems that the submaximal contribution of the synergist wrist flexors on the trained limb, has potentially limited the magnitude activation of the ipsilateral cortical representation of the untrained synergist wrist flexors compared to the untrained biceps brachii. This seems plausible, because inhibition diminishes with increased force production, and other forms of inhibition are recognized to experience greater reductions with increased voluntary force production (Perez et al. 2008). For example, interhemispheric inhibition (IHI) is reduced in untrained contralateral agonist muscles, but remains unchanged in an antagonist muscle not involved in the training task (Hortobágyi et al. 2011; Zult et al. 2016).

A major limitation to the current study was that only single-pulse TMS was used, thus we were unable to identify other forms of inhibition. For example, a number of studies have explored the relationship between strength gain in the untrained limb and modulation of IHI (Hortobágyi et al. 2011; Howatson et al. 2011; Zult et al. 2016); and short interval intracortical inhibition (SICI) has been reported as being modulated by the cross-education of strength (Hendy and Kidgell 2014; Kidgell et al. 2015; Goodwill et al. 2012). While the current study did not measure IHI and SICI, it is conceivable that these experimental techniques that test other specific neural networks are implicated in the strength gain of the untrained agonist and untrained synergist wrist flexors

(Zult et al. 2016). Consequently, we cannot discount that other cortical structures involved in motor learning are involved (Ruddy et al. 2017). Another limitation is that sEMG activity from the right trained wrist flexors muscle was not verified, thus it remains unclear what the contribution of the wrist flexors are, during an isotonic biceps curl exercise. Obtaining this information, in part would strengthen our understanding regarding the effects of cross-activation as a potential mediator for the cross-education of strength. Furthermore, given that we have only measure motor responses from one synergist muscle, it remains unclear if other wrist flexor muscles (i.e. flexor carpi ulnaris) also increased excitability and reduced inhibition.

In conclusion, this study has demonstrated that the cross-education of muscular strength is not confined just to the contralateral homologous agonist muscles, but rather it has a spatial affect. Intriguingly, the neural adaptations that occur following the cross-education of strength are not associated with the changes in contralateral strength of the homologous agonist and synergist, and the neural adaptations are spatially confined. Overall, these findings suggest that unilateral isotonic strength training provides a greater stimulus in cross-education paradigms due to their spatial effects on strength transfer and should be used in the rehabilitative process following unilateral injury to maximize the number of muscles that could receive a cross-education of strength benefit.

Conflicts of interest

None of the authors have potential conflicts of interest to be disclosed.

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Table 1. Mean (± SE) for AMT stimulus intensity, M_{MAX} and single-pulse TMS pre-stimulus *rms*EMG prior to and following three weeks of unilateral strength training for the untrained contralateral homologous biceps brachii and the synergist flexor carpi radialis for both control and trained group.

		AMT SI (%)		M_{MAX} (mV)		SP *rms*EMG (% *rms*EMGmax)	
		Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**
Control	***Biceps Brachii***	46.00 ± 2.08	45.30 ± 1.48	7.54 ± 0.58	7.71 ± 0.63	3.49 ± 0.95	3.83 ± 0.96
	Wrist Flexor	48.20 ± 2.56	49.70 ± 3.03	4.42 ± 0.42	4.71 ± 0.33	4.61 ± 0.86	4.72 ± 0.95
Trained	***Biceps Brachii***	47.60 ± 2.90	45.50 ± 2.63	7.45 ± 1.26	7.33 ± 1.29	4.33 ± 1.31	4.42 ± 1.06
	Wrist Flexor	50.00 ± 2.90	51.70 ± 3.10	4.72 ± 0.89	4.81 ± 0.89	48.20 ± 0.61	4.91 ± 0.42

AMT SI: active motor threshold stimulus intensity. Single pulse (SP) *rms*EMG was pooled across stimulus intensities.

FIGURE LEGENDS

Fig. 1 Schematic representation of the experimental design with measures obtained prior to and following three weeks of unilateral strength training of the right elbow flexors. Pre- and post-measures included assessment of peripheral muscle excitability (M_{MAX}), corticomotor excitability and inhibition recruitment curves and muscle strength of the left and right biceps brachii and wrist flexors.

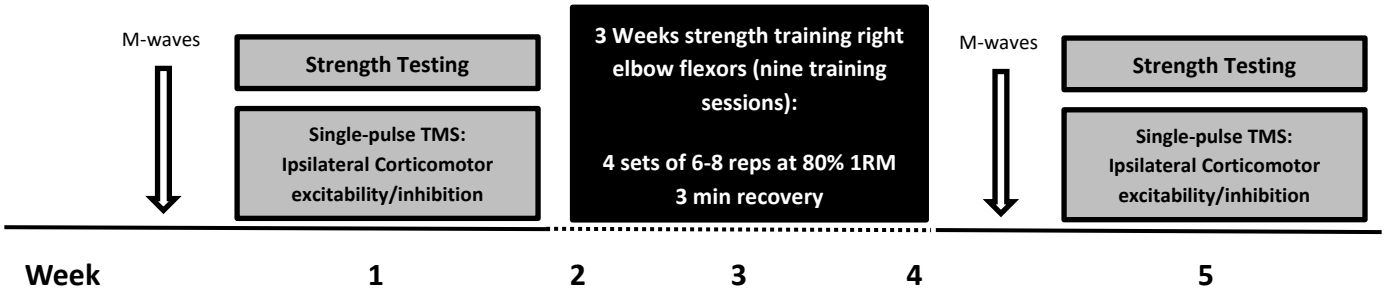
Fig. 2a-d: (a) Mean (± SE) changes in 1-RM strength of the untrained left biceps brachii muscle and **(b)** strength changes for the trained and untrained contralateral elbow flexors in trained participants following 3 weeks of unilateral strength training. Data are expressed as a percentage of pre-training strength (r^2 0.680; P = 0.003).* indicates significant to control. **: (c)** Mean (± SE) changes in 1-RM strength of the untrained left wrist flexor muscles and **(d)** strength changes for the trained and untrained contralateral wrist flexors in trained participants following 3 weeks of unilateral strength training. Data are expressed as a percentage of pre-training strength (r^2 0.581; P = 0.010).* indicates significant to control.

Fig. 3a-d: The AURC was calculated using the method of trapezoidal integration. The AURC obtained prior to the strength training intervention is shaded in grey. The additional area enclosed by the recruitment curve obtained following 3 weeks of strength training is patterned. **(a)** depicts the AURC calculated from corticomotor excitability recruitment curves of the untrained biceps brachii for the control group whereby MEP amplitude was plotted against stimulus intensity. **(b)** depicts the AURC calculated from corticomotor excitability recruitment curves of the untrained left biceps brachii for the strength training group whereby MEP amplitude was plotted against stimulus intensity. There was a significant increase in AURC compared to the control group (P = 0.007). **(c)** depicts the AURC calculated from

corticomotor excitability recruitment curves of the untrained wrist flexors for the control group whereby MEP amplitude was plotted against stimulus intensity. **(d)** depicts the AURC calculated from corticomotor excitability recruitment curves of the untrained wrist flexors for the strength training group whereby MEP amplitude was plotted against stimulus intensity. There was a significant within-group effect for increased AURC for the untrained wrist flexors ($P = 0.012$).

Fig. 4a-d: The AURC was calculated using the method of trapezoidal integration. The AURC obtained prior to the strength training intervention is shaded in grey. The additional area enclosed by the recruitment curve obtained following 3 weeks of strength training is patterned. **(a)** depicts the AURC calculated from corticomotor inhibition recruitment curves of the untrained left biceps brachii for the control group whereby silent period duration was plotted against stimulus intensity. **(b)** depicts the AURC calculated from corticomotor inhibition recruitment curves of the untrained left biceps brachii for the strength training group whereby silent period duration was plotted against stimulus intensity. Following the intervention, there was a 15% decrease in the total AURC for the elbow flexors compared to the control group ($P = 0.036$). **(c)** depicts the AURC calculated from corticomotor inhibition recruitment curves of the untrained wrist flexors for the control group whereby silent period duration was plotted against stimulus intensity. **(d)** depicts the AURC calculated from corticomotor inhibition recruitment curves of the untrained wrist flexors for the strength training group whereby silent period duration was plotted against stimulus intensity. Following the intervention, there was a significant within-group effect for decreased AURC for the untrained wrist flexors ($P = 0.012$), however the decrease was not different to the control group.

Fig. 5a-b: (a) Mean (±SE) changes in MEP amplitude at 130% AMT during a contralateral MVC of the right biceps brachii following unilateral strength training, **(b)** Mean (±SE) changes in MEP amplitude at 130% AMT during a contralateral MVC of the right wrist flexors following unilateral strength training. *Indicates significant to baseline.



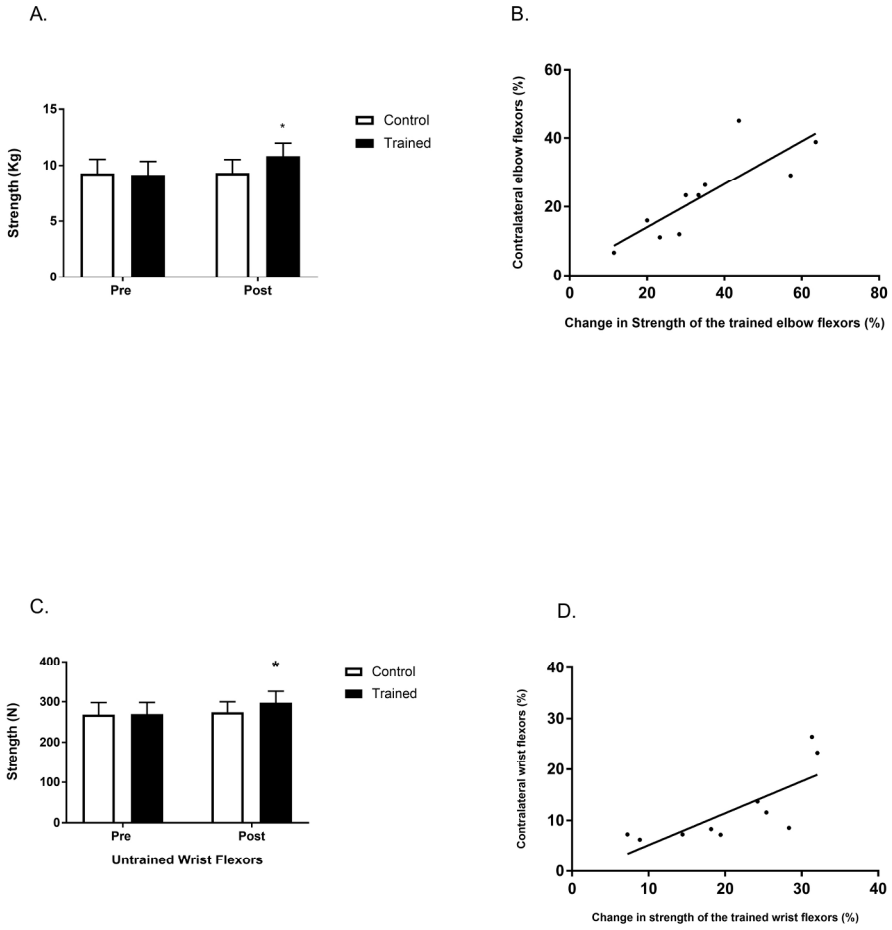


Figure 2a-d

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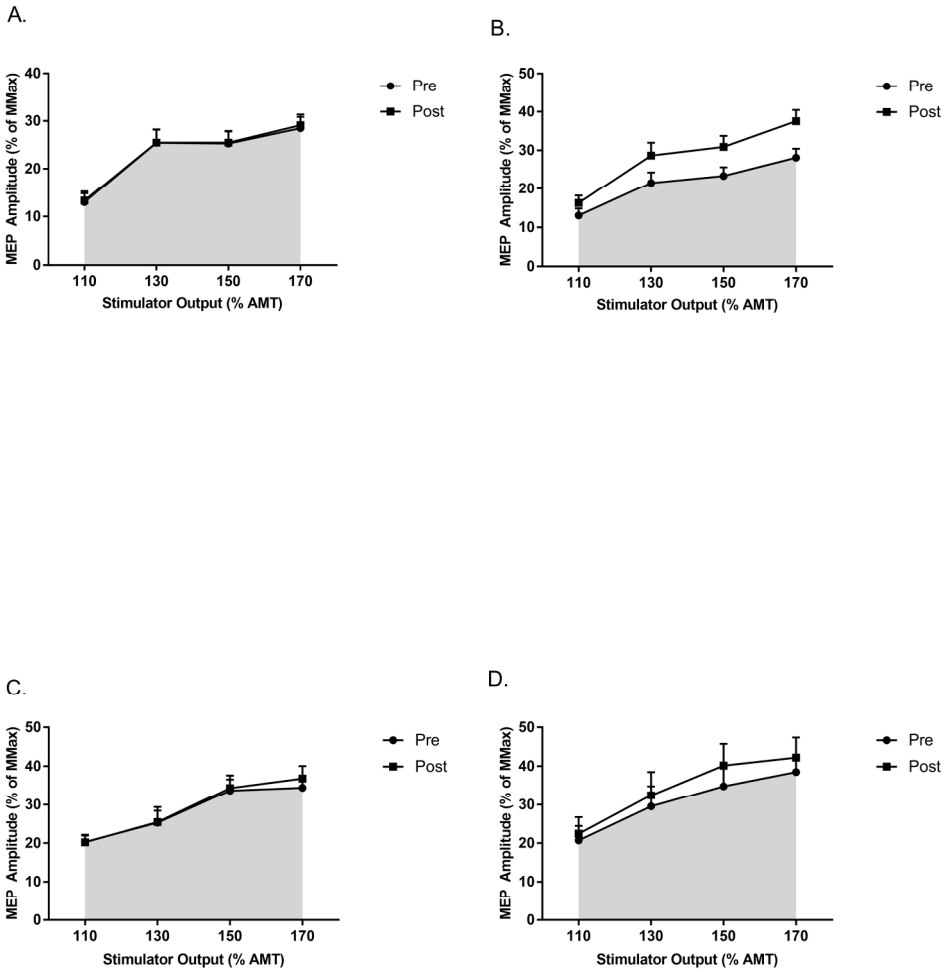

Figure 3a-d

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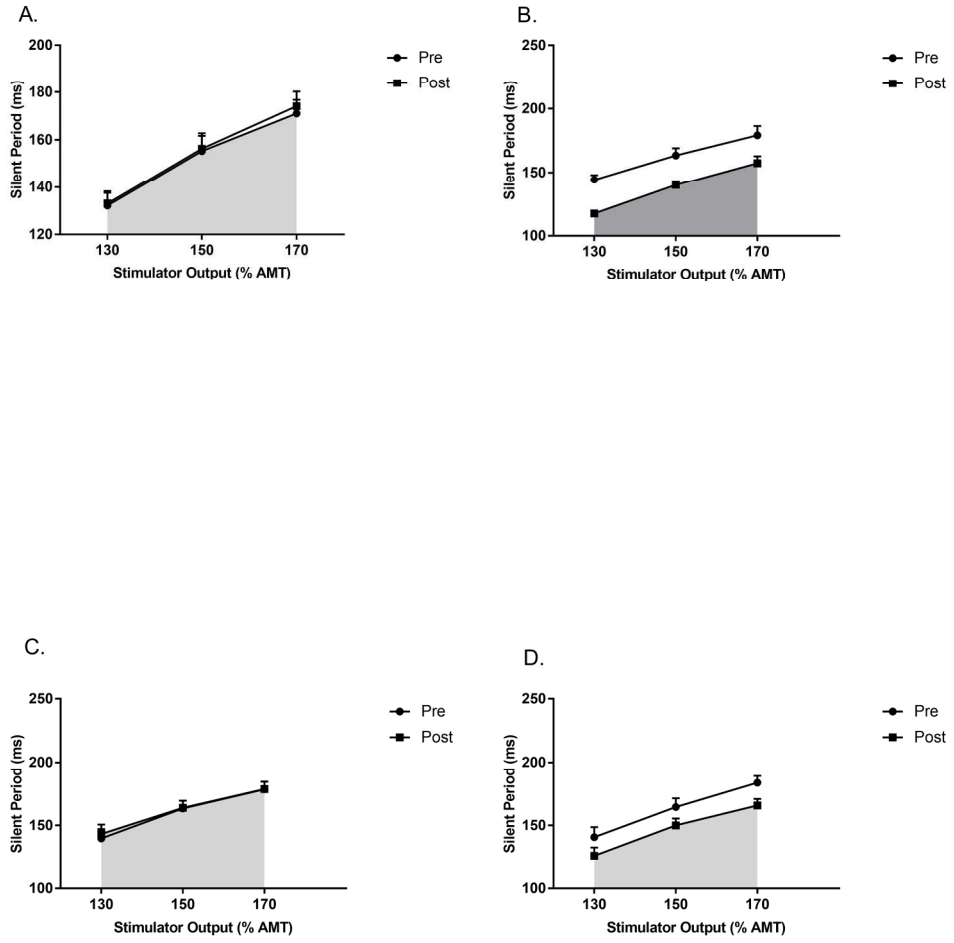

Figure 4a-d

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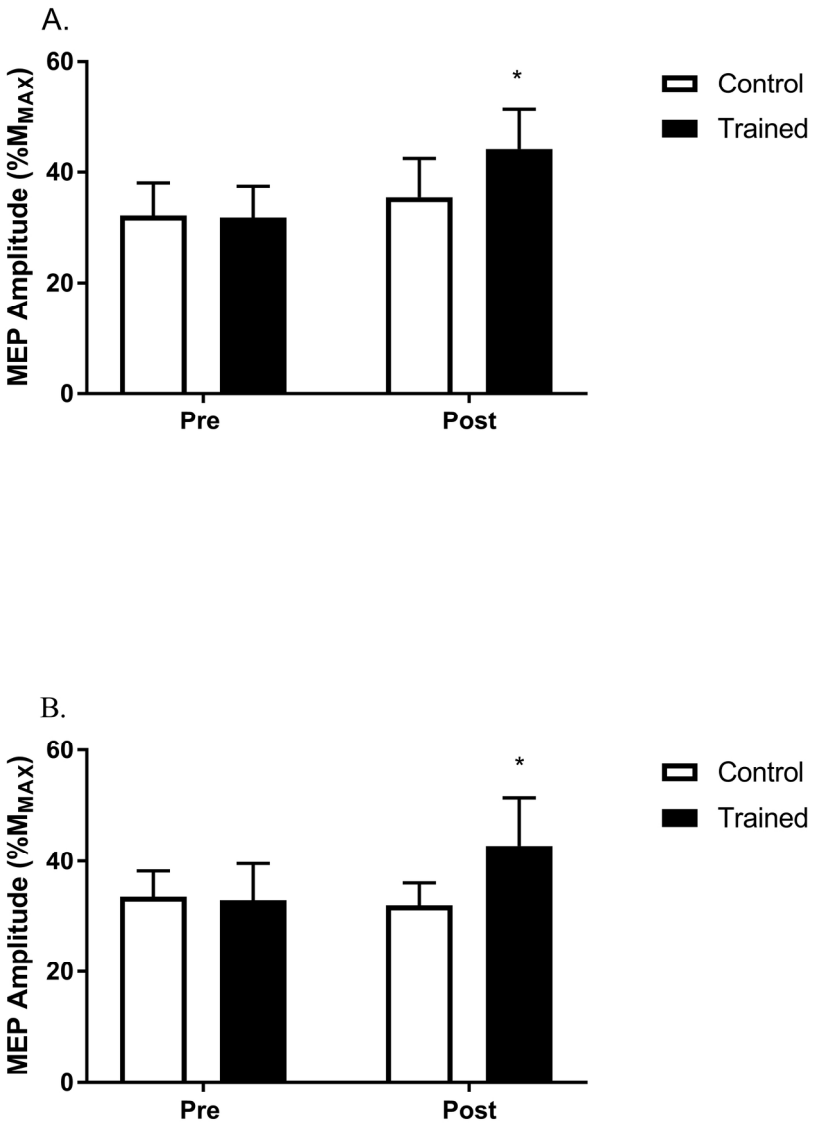

Figure 5

170x225mm (300 x 300 DPI)