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Neurophysiological adaptations associated ~~to~~ with the cross-education of muscle strength following chronic unilateral training: a systematic review and meta-analysis

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Abstract

This study reviewed the available evidence from randomized controlled trials (RCTs) focusing on the neurophysiological adaptations associated ~~to~~ with cross-education of strength (CE) and pooled data into definite effect estimates for neurophysiological variables assessed in chronic CE studies. Furthermore, scoping directions for future research were provided to enhance the homogeneity and comparability of ~~the~~ studies investigating the neural responses to CE.

A significant 21.1±18.2% increase in contralateral strength (p<0.0001) was detected from 22 RCTs (467 subjects) that measured at least one neurophysiological variable in the untrained side. Neurophysiological parameters measured were: EMG (n=14), MEP (n=8), SICI, RC and M-wave (n=6), cSP (n=5), IHI, ICF and H-reflex (n=2), V-wave, SICF, SAI and LAI (n=1). Only EMG, MEP, ICF, cSP and SICI entered the meta-analysis (18 studies, 387 subjects). No significant changes in EMG (p=0.26; 235 subjects) and MEP amplitude (p=0.11; 145 subjects) in the untrained limb were found. A significant decrease in cSP duration (p=0.02; 114 subjects) and SICI (p=0.001; 95 subjects) of the untrained hemisphere was detected depending on the body region, type and intensity of training. No correlation between changes in CE and changes in these TMS measures ~~was~~ were found. The paucity of data available ~~prevented the ability~~did not allow us to draw any conclusion on the utility of the remaining parameters.

Based on the data available for pooling, the use of TMS to assess the ipsilateral neurophysiological responses to unilateral training confirms the central neural origin hypothesis of chronic CE. However, how these neural adaptations may contribute to CE remains unclear.

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Introduction

It is now well established that unilateral strength training improves strength of the untrained side, producing the phenomenon commonly termed “cross-education” (CE) or also "contralateral strength training effect", "interlimb transfer", "cross-transfer" and "cross-training" (7, 34, 55, 81, 98). The CE effect was conventionally regarded as a small contralateral increase in strength by approximately 8% of the initial level (7, 70). However, in a recent meta-analysis (65) of 31 randomized controlled trials (RCTs), data pooling from 785 subjects revealed a significant 11.9% CE, with an effect of 9.4% in the upper limb and 16.4% in the lower limb, following chronic unilateral strength training.

Several studies have attempted to elucidate the physiological underpinnings of the CE phenomenon. Early contralateral increases in muscle strength are usually not associated with increased limb girth or enzymatic activity (32, 68). Consequently, neural mechanisms are likely to underlie the CE effect (7, 15, 82). Acute studies that employed transcranial magnetic stimulation (TMS) protocols, reported consistently increased corticospinal excitability not only in the contralateral (M1) but also in the ipsilateral primary motor cortex (iM1) not directly involved in the motor task (24, 37, 40, 59, 69, 75, 100). Decreased short-interval intracortical inhibition (SICI) (59, 75) and decreased interhemispheric inhibition (IHI) from the trained to the untrained M1 (35, 40, 75) have been observed in response to acute unilateral strength training.

However, there is still much debate over the presence and nature of neurophysiological adaptations to chronic unilateral strength training, ~~also considering that~~and whether measures made at rest ~~are~~ can be ~~mostly~~ employed to probe neural adaptations ~~occurring~~ that occur in active states (*i.e*, during contraction).

Overall, current evidence is controversial, with reports ranging from persistent and bilateral changes in resting excitability to no substantial change (10, 35, 46, 57, 61). These discrepancies have been partly linked to the heterogeneity of the exercise programs delivered and to the wide range of neurophysiological techniques and protocols employed, which provide a large number of outcomes that can be considered as indicators of cortical and spinal excitability (7, 21, 24). Moreover, very few investigations have evaluated the time course of the neural adaptations to chronic CE (i.e., week by week), whilst the majority of the studies generally assess participants before and after a chronic intervention. This common Pre/Post assessment practice may ~~result in~~mean that ~~missing~~ some ~~of the~~ adaptations are missed if they occur~~(i.e., early acute and subacute responses to training), which could be detected at~~ only at certain time points but not at others (e.g. early acute and subacute responses to training).

Although a mechanistic explanation of the neural adaptations associated ~~to~~ with the CE remains elusive (80), experimental findings on chronic unilateral training seem to suggest a role for a combination of increased excitability and decreased inhibition in the neural structures innervating the contralateral untrained limb may act as relevant neurophysiological correlates of the strength gain detected in the untrained limb (29).

Despite the considerable amount of data accumulated so far, which have been summarized in a number of narrative and systematic reviews (7, 34, 48, 55, 70, 81, 98), no meta-analysis of the neural changes induced by chronic unilateral strength training has ever been carried out. Such aggregated quantification seems necessary in light of the high heterogeneity of the neurophysiological outcomes chosen by the individual studies for explaining the CE effect, thus making it difficult for researchers to reach an informed decision on which parameters to be selected.

Therefore, this study was planned to *i)* systematically appraise the available evidence from RCTs that focused on the neurophysiological underpinnings of the CE effect induced by chronic unilateral strength training; *ii)* meta-analytically pool data into a defined estimate of effect for a range of neurophysiological variables commonly assessed in CE studies and determine if body region, type of training, and exercise intensity affect the magnitude of the CE effect, and *iii)* provide scoping lines for future research to establish a common methodological platform to enhance the homogeneity and comparability of the studies investigating the neural response to unilateral strength training.

Materials and Methods

The review was registered in the International Prospective Register of Systematic Review (PROSPERO), registry number: CRD42017070939.

Literature Search Strategy

The literature search was based on 5 databases (MEDLINE, PubMed, Scopus, the Cochrane library, and Web of Science). Two public registers of clinical trials (ClinicalTrials.gov; Cochrane Central Register of Controlled Trials) were also inspected for further ongoing or completed trials investigating the CE effect. A search strategy was conducted combining "cross-education" and its synonyms ("cross-transfer", "cross-training", "interlimb transfer", "strength transfer", "contralateral strength training", "unilateral strength training", “contralateral resistance training”), with “neural adaptations” and “neurophysiology” keywords. The keywords “resistance training” and “strength training” were meant as synonyms and combined in all the strategies by the *OR* Boolean. Moreover, the following specific terms referring to the neurophysiological variables commonly employed in strength literature, were searched in combination with the abovementioned keywords: electromyography (EMG); maximal wave (M-wave), Hoffmann reflex (H-reflex), volitional wave (V-wave), motor evoked potential (MEP), recruitment curve (RC), cortical silent period (cSP), short-interval intracortical facilitation (SICF), SICI, intracortical facilitation, (ICF), short-latency afferent inhibition (SAI), long-latency afferent inhibition (LAI) and IHI.

Each database was searched from inception up to August 31, 2017. Only RCTs published in English were selected and the reference lists of all included articles were checked for further relevant publications.

Eligibility Criteria

Studies were considered for this review if they met the following criteria: 1) intervention consisting of a resistance training programme (duration ≥2 weeks); 2) at least one neurophysiological variable investigated as a measure of the CE effect; 3) healthy participants randomly assigned to chronic unilateral training (minimal duration of 3 weeks) or to a control group undergoing no intervention; 4) for multi-arm trials, at least one group of the study undergoing a unilateral resistance training.

Studies were excluded if: 1) neurophysiological variables were used only acutely (*i.e.*, during or immediately after one single training session of unilateral contractions) or not in the perspective of measuring chronic CE (*i.e.*, EMG to control for the presence of muscle activation in the unexercised muscles during single contralateral training sessions); 2) a non-active control group was absent; 3) focused on mixed exercise interventions other than resistance training, or if a combined approach was delivered, or if both the upper and lower limbs were trained within the same protocol; 3) the CE effect was investigated during non-conventional CE approaches (*i.e* unilateral limb immobilization, mirror training, electrical muscle stimulation) since these were considered peculiar conditions, thus requiring a tailored investigation; 4) participants with pathological conditions (*i.e.*, orthopaedic and neurological populations) were enrolled.

Selection of Studies

The initial search was undertaken by two of the authors. Titles and abstracts of all the retrieved studies were screened. Items that were clearly outside the purposes of the present meta-analysis were removed. After title/abstract screening, two authors independently selected the articles for inclusion. Duplicates were removed at this stage. The full text of any paper potentially satisfying the inclusion criteria was carefully read as the results of CE investigations are frequently presented only as secondary findings in strength training reports, with no mention in titles, abstracts and/or among the paper's keywords. Eligible studies were included in the meta-analysis. In case of disagreement, a comparison between different views to reach a final decision was performed and, if necessary, a third author contributed to the final decision.

Data Extraction and Management

Main methodological features of the included studies (design, interventions' description, sample size) and outcome measures were extracted and summarized by two of the authors.

Contralateral transfer of strength

To calculated the magnitude of strength transfer "between-groups" we employed the equation ~~by~~ used by Carroll et al. (7) $\left[\frac{E_{POST} - E_{PRE}}{E_{PRE}} - \frac{C_{POST} - C_{PRE}}{C_{PRE}}\right] * 100$ where E_{POST} refers to mean POST-training strength for the trained group's untrained limb, E_{PRE} refers to mean PRE-training strength for the trained group's untrained limb; C_{POST} refers to mean POST-training strength for the controls' untrained limb while C_{PRE} refers to the mean PRE-training strength for the control group's untrained limb.

Risk of Bias

The Cochrane Collaboration risk-of-bias tool was employed by two of the authors independently to rate the methodological quality of the included studies. A rating of "low" or "high" was assigned if criteria for a low or high risk of bias were met, respectively. The risk of bias was judged "unclear" for a domain if inadequate details were reported. In case of disagreement between different views a third author was consulted. ~~To specifically~~Visual inspection of a funnel plot was used to assess the risk of publication bias~~, a funnel plot was built and visually inspected~~.

Statistical analysis

All meta-analyses were conducted using RevMan 5.3 (Review Manager, The Cochrane Collaboration). Changes from baseline in the dynamometric and neurophysiological outcome measures related to the untrained limb/hemisphere were extracted from each study. Raw data (means and standard deviations, SD) were derived or calculated from standard errors, 95% confidence intervals (CI), *p* values, *t* values, or *F* values. When only graphs were available, data was possible like in a previous meta-analysis (92). To this aim the exact mean scores and SDs were obtained from the graphs using GetData Graph Digitizer 2.26. In case of missing data, a written request was mailed to the authors of the article. In order to account for the heterogeneity that may derive from pooling data obtained by different testing approaches (*i.e.* isokinetic or isometric; maximal or submaximal MEPs), a random-effects model was chosen. The Standardized Mean Difference (SMD), which expresses the intervention effect in standard units rather than the original units of measurement, was calculated to allow the interpretation of the effect sizes of the Pre-Post changes: an SMD of 0.2 was considered as low, 0.5 moderate and 0.8 large (11). For those outcome measures for which studies were found to be highly homogeneous and to employ the same unit of measurement (*i.e.*, milliseconds, millivolts) as well as consistent methodological procedures for the neurophysiological recordings, the mean difference (MD) of the changes along with its SD was used to obtain an absolute estimate of effect.

The Chi-square test and the inconsistency (I^2) statistic were employed to assess the heterogeneity between the studies within each meta-analysis carried out (31). A value I^2 >50% along with a p<0.05 was considered indicative of high heterogeneity. In case of heterogeneity beyond such threshold, sensitivity analyses were conducted to identify those studies carrying the excess of heterogeneity. When necessary, a *leave-one-out* approach was performed by removing one study or one arm of a study which mean difference from baseline lie outside the overall pattern of the distribution. To this aim, box and whisker plots were constructed to verify whether data of the study or of the single arm of the study carrying the excess of heterogeneity were 1.5 times the interquartile range (1.5 x IQR) below the first quartile or above the third quartile (90). Estimates of the effect of contralateral training for the maximal strength outcome were calculated by body region (pooled "upper + lower limb", "upper limb" subgroup, "lower limb" subgroup). For the neurophysiological outcomes, sub-group analyses were conducted by body region (upper *versus* lower limb), type of exercise (static *versus* dynamic training) and by training intensity (maximal *versus* sub-maximal). Pairwise comparisons between the different subgroups were conducted to determine which of the above-mentioned factors significantly influenced the neurophysiological variables.

Results

Study selection

The comprehensive flow chart showing the process of identification, screening and evaluation of the eligibility for inclusion of the studies is displayed in Fig. 1. The initial search identified 1421 studies, of which 55 RCTs were recognized as pertinent to the topic. Of these, 33 studies were removed for the reasons detailed in Table 1.

Twenty-two studies (467 healthy participants including 261 experimental subjects and 206 controls), conducted between 1987 and Aug 31 2017, reported at least one neurophysiological measure obtained from the untrained side and were therefore included in the qualitative analysis. Table 2 reports the demographic features of the participants, muscle groups trained, intervention, magnitude of the CE effect on strength, and neurophysiological measures recorded along with their changes, as reported by the authors in the full-text of the article. In summary, 11 studies focused on CE in the upper limb and 11 on the lower limb. Training periods lasted between 3-12 (5.5 ± 2.6) weeks with 4.5-weeks being the most common. Interventions were generally carried out with a frequency of 3 sessions/week. On average the studies consisted of 21 ± 6 participants. Eight out of 22 studies (36%) disclosed in the manuscript that the unilateral strength training program was supervised.

Risk of bias in individual studies

There was a high risk of bias across all studies (Fig. 2). In particular, the majority of the reports were exposed to high risk for selection, performance, detection, attrition and reporting biases. The analysis of publication bias in the largest meta-analysis (contralateral strength changes) revealed a potential for publication bias for 4 (27, 33, 35, 54) out of 22 studies (18%), that can be found as scattered observations (standard error >0.6) at the bottom and on the right side (SMD >1.7) of the funnel plot (Fig. 3).

Strength gains in the untrained muscles

Table 2 reports for each study the magnitude of the CE effect as reported by the authors in the full-text article of the individual studies for the intervention group (within-subjects results). The meta-analyses of the 22 CE RCTs that measured at least one neurophysiological variable in the untrained side, resulted in a significant 21.1 ± 18.2% increase in contralateral strength (p<0.0001) with a large effect size (SMD 0.78; CI 0.48-1.07). The sub-group analysis by body region revealed significant increases both in the upper (12.9 ± 12.2%; p=0.006; SMD 0.56; 95% CI 0.17 to 0.95) and lower (27.1 ± 19.8%; p<0.0001; SMD 0.99; CI 0.58 to 1.11) limbs.

Neurophysiological changes in the untrained side following contralateral training

Complete neurophysiological data were extracted from 18 of the 22 RCTs, since these could not be retrieved from 4 of them (33, 36, 56, 57). In 6 studies (5, 6, 12, 25, 35, 54) data for at least one neurophysiological measure were extracted from the graph, since they were not reported in the tables or running text of the manuscript. Five of the 18 studies that entered the meta-analysis did not report raw data in the full-text for some of the variables measured (Ref. 12: MEP, SICI; Ref. 35: EMG, ICF; Ref. 54: M-wave; Ref. 58: SIB; Ref. 66: RC slope or peak height).

EMG

Data pooling from 11 studies (n = 235) revealed a non-significant increase in the EMG burst activity during maximal voluntary isometric contraction (MVIC) of the untrained limb (p=0.26, SMD 0.20, Fig 4a). The sub-group analysis by body region revealed no significant changes in EMG activity in the upper (p=0.31, SMD 0.19; n = 5 studies) and lower limbs (p=0.38, SMD 0.31; n = 6 studies). Sub-group analyses by type of training and exercise intensity revealed no significant changes in EMG activity after static maximal (p=0.56, SMD 0.1; n = 7 studies) and dynamic submaximal (p=0.24, SMD 0.67; n = 4 studies) training.

M-wave, H-reflex and V-wave

No differences were observed in the amplitudes of the M-wave (p=0.68; SMD -0.1; n = 7 studies, 112 subjects) and H-reflex (p=0.48; SMD 0.2; 42 subjects) evoked from the untrained muscles (Figs. 4b and 4c). No meta-analysis was conducted for the V-wave, since only one study employed this variable as a measure of CE (23). Sub-group analyses could be carried out only for the M-wave, which was found unchanged in the upper (p=0.68, SMD 0.1; n = 5 studies; 70 subjects) and lower limbs (p=0.85, SMD 0.1; n = 2 studies; 42 subjects).

MEP

Pooled data from 7 studies (159 subjects) showed a significant increase in MEP amplitude in the untrained hemisphere (p=0.04) with only a moderate effect size (SMD 0.50). However, the sensitivity analyses revealed that the pooled estimate of the effect of unilateral training on MEPs elicited in the untrained homologous muscles was highly influenced by the study of Goodwill et al. (27). This was therefore removed, resulting in acceptable heterogeneity across the remaining 6 studies (I^2=28%; 145 subjects) and a non-significant increase in MEP (p=0.11) and a small effect size (SMD 0.33) (Fig 5a). The sub-group analysis by body region could not be done for the lower limb (n = 2 studies) due to the excess heterogeneity in the study of Goodwill et al. (27). For the upper limb, no significant MEP changes were detected (p=0.12, SMD 0.38; n = 5 studies; 107 subjects). Similarly, MEP amplitude was not significantly influenced by the training type (static: p=0.35, SMD 0.82; n = 2 studies; 54 subjects; dynamic: *p*=0.11, SMD 0.39; n = 5 studies; 106 subjects) and intensity (maximal: *p*=0.57, SMD 0.15; n = 2 studies; 54 subjects; submaximal: *p*=0.06, SMD 0.73; n = 5 studies; 106 subjects).

RC

Recruitment curves were presented in 6 studies (27, 35, 46, 50, 62, 66), but only 3 studies performed analyses to quantify any changes in the peak height and/or in the slope and/or in the area under the curve, following the intervention (27, 50, 66). No meta-analysis was conducted for the RC variable, since these studies reported complete data on different parameters: one for the peak height, which was found significantly increased (27), one for the slope of the curve, which was found unchanged (50) and the other for the area under the RC, which was found significantly increased (66).

cSP

Data from 5 studies (114 subjects) showed a significant decrease in the duration of the cSP of the untrained hemisphere (p=0.02) with a moderate effect size (SMD 0.46) (Fig 4b). Since the included studies were highly homogeneous (I^2 = 0%; p=0.66) and consistently reported cSP changes in milliseconds (ms), the estimate of effect was additionally calculated by pooling the MD (±SD), which revealed a significant reduction of the cSP duration by 16.7 ms (CI: 4.97 to 28.42; *p*=0.005) (Fig. 5c). The sub-group analysis by body region could be carried out only for the upper limb (only one lower limb study available), which showed a significant decrease in cSP duration of 12.8 ms (CI: 0.48 to 25.2 ms, *p*=0.04; n = 4 studies, 96 subjects). All the 5 studies that assessed the cSP delivered dynamic (isokinetic or isotonic) training. Of these, 4 employed a submaximal exercise intensity obtaining a significant decrease in cSP duration of 18.2 ms (CI: 5.2 to 31.2 ms, p=0.006; 69 subjects). No significant correlation was detected between the reduction in the cSP duration in the untrained hemisphere and the change in strength in the untrained limb (r <0.1).

SICF

No meta-analyses were conducted for SICF, since only one study (34 subjects) employed this variable as a measure of CE and found no changes (62).

SICI

Figure 5d details the meta-analysis carried out for SICI. A significant decrease of SICI in the untrained hemisphere was detected (p=0.001, SMD = 1.1, n = 4 studies, n = 95 subjects). The sub-group analysis by body region was performed only for the upper limb (only one lower limb study available) and revealed a significant decrease in SICI (p=0.01, SMD 1.1; n = 3 studies, 67 subjects). A sub-group analysis by type of training found a significant reduction in SICI after both dynamic (p=0.0006, SMD 1.7; n = 2 studies, 41 subjects) and static (p=0.005, SMD 1.2; n = 2 studies, 54 subjects) training, with no superiority of one type of training over the other (T=0.77, p=0.45). SICI appeared significantly reduced after training at both submaximal (p=0.0002, SMD 1.5; n = 2 studies, 34 subjects) and maximal (p=0.047, SMD 1.4; n = 2 studies, 47subjects) intensities, with no significant differences between them (T=0.18, p=0.86). The significant reduction in SICI within the untrained hemisphere did not correlate with the change in strength in the untrained limb (r <0.1).

ICF

Only 2 studies (35, 62) measured ICF (Fig 5e). The meta-analysis revealed no significant changes following the intervention (p=0.08; SMD 0.50; 54 subjects).

IHI

Only 2 studies (35, 62) entered this meta-analysis (54 subjects) but an excess of heterogeneity (I^2=97%) prevented us from performing further analysis (Fig 5f).

SAI and LAI

One single study (34 subjects) assessed the effects of a unilateral chronic training on SAI and LAI (62) and results showed no significant changes.

Discussion

The present study is the first to provide a meta-analytic quantification of changes in neurophysiological variables employed as measures of the extent of the CE effect by RCTs. The main finding was that only SICI and cSP measured in iM1 were found consistently changed across the included studies following chronic unilateral training.

Changes in contralateral strength produced during a maximal voluntary contraction in the untrained muscles

In line with a recent meta-analysis (65) which pooled data from 31 RCTs revealing a significant 11.9% CE (upper limb: 9.4%; lower limb: 16.4%), strength gains in the untrained muscles across the 22 chronic CE studies included in the present meta-analysis showed a significant 21.1% increase in contralateral strength (upper limb: 12.9%; lower limb: 27.1%). These results confirm the effectiveness of unilateral training in inducing significant contralateral gains in strength. However, unlike the previous available meta-analyses which found only modest effects (Refs. 7, 69: +7.8%), they portray CE protocols as capable of inducing moderate to large contralateral gains in strength, which may have potential clinical relevance.

Neurophysiological changes in the untrained side following contralateral training

In CE literature, EMG is the most commonly employed neurophysiological variable (14 studies), followed by MEP (8 studies), RC and M-wave (6 studies), then SICI and cSP (5 studies), IHI, ICF and H-reflex (2 studies) with the V-wave, SICF, SAI and LAI being the least measured (1 study). Not all of these variables entered the quantitative meta-analysis due to the absence of raw data in the full-text of the studies or because data were not provided upon formal request sent to the authors.

EMG In CE studies it is imperative to ensure that the unexercised muscles are relaxed during unilateral training of their contralateral homologous. This is the main reason for the frequent employment of EMG to control for any activation in the unexercised muscles. EMG is also employed to detect changes in the activation of the untrained muscles following training of the contralateral homologous ones. Although several CE studies generally report increased EMG activity in the untrained muscles (23, 25, 35, 86, 97), the pooled estimate obtained here from 11 RCTs revealed no significant change in this variable and no influence of the body region, type or intensity of the training. In agreement with these findings, not all the studies were able to demonstrate significant changes in EMG activity after resistance training, which may be due to inherent methodological limitations in surface EMG technique, as used in CE studies (1). In fact, surface EMG provides only an indirect measure of muscle activation, unlike more direct techniques such as twitch interpolation (56, 57). Other limitations of EMG data, which restrict the conclusions that can be drawn from its use, are signal amplitude cancellation, data variability as a function of subcutaneous tissue, number of motor units, and conduction velocity (44). Furthermore, skin-electrode impedance, location of electrodes over the muscle, muscle-fiber shortening, crosstalk between muscles, shift of the muscle relative to the detection system, number of recruited motor units and motor unit synchronization are to be considered among the factors that can influence surface EMG results (17), which can be influenced by any variation in any of these parameters from pre- to post-training. This may explain why Latella et al. (54) reported only modest reproducibility for EMG measurements. Taken together, these results raise the possibility that the use of surface EMG burst activity for monitoring any ongoing activity in the ‘resting’ limb is useful but it is of limited use for the quantification of neural adaptations associated with CE. However, the role of EMG remains crucial in CE studies since most of the neurophysiological techniques currently employed to probe contralateral neural adaptations are EMG-based. In the present study, other EMG-based measures such as the H-reflex and the M-wave were found unchanged after unilateral training. Based on the data available for pooling, these findings ~~seem inconclusive on~~do not allow us to make any definite conclusions about whether and how spinal circuits might contribute to CE. This contrast~~, compar~~s~~ed w~~ with more established findings of previous reports (7, 53, 55) ~~depicting~~ that depict a clearer association between the descending motor drive from supraspinal levels and CE. However, it should be noted that these EMG-based measures, particularly the M-wave, which reflects the properties of the muscle fibre action potentials, are typically used in CE studies as normalization parameters for signal changes related to other techniques such as TMS, rather than outcome measures.

MEP An increase in neural excitability at the cortical or spinal level, induced, e.g., by voluntary contraction of the target muscle, facilitates cortico-motor excitability, resulting in larger MEP amplitudes without a change in the TMS stimulus intensity (80). Although MEP amplitude is considered highly variable even with the target muscles relaxed, it is frequently employed in strength conditioning literature to detect neural adaptations associated with early improvements in strength induced by short-term training (48). However, the findings are controversial even in the directly trained muscles, with studies reporting MEP increases (28, 49, 89) and others significant reductions (8, 41) or no change at all (47, 57, 62).

When MEP amplitudes are analyzed in the context of CE studies, data are also inconsistent, with studies reporting significant increases in the untrained limb (27, 35, 46, 50, 66) or no change (12, 46, 54, 62). The pooled estimate obtained from 6 RCTs revealed a non-significant increase in MEP amplitude, with a trend to increase only after submaximal exercise. While these data suggest that the descending corticospinal volley to the untrained muscles is affected to some extent by changes in iM1 excitability, whether or not these changes may be due to improved motor unit activation which in turn may contribute to the increased strength of the untrained limb is difficult to infer.

cSP The cSP has a complex physiology and its total duration is considered to be altered only by cortical mechanisms, specifically intra-cortical inhibitory phenomena (78). In the present meta-analysis, given the high consistency in the recording of the cSP duration as well as in data reporting across the included studies, this measure was the only one for which data could be aggregated both by standardized (SMD) and absolute mean differences (MD), both resulting in significant reduction. The pooled estimate of effect showed a significant decrease of the cSP duration by 12.8-18.2 ms, depending on the body region and intensity of training. This finding suggests that a decrease in intracortical inhibition of the iM1 is associated with the observed CE effect. However, this estimate should be considered as preliminary in view of the small number of studies that could be pooled for this variable. Furthermore, as for any other variables there is no evidence that associations can be regarded as mechanistically causal.

SICI SICI is a complex inhibitory phenomenon that serves as a standard method to estimate excitability in a $GABA_A$-ergic circuit in the human cortex (79). Muscle contraction on one side tends to decrease SICI in the resting contralateral homologous muscle (40, 59, 75). The majority of the acute (40, 75) and chronic CE studies (27, 46) that examined SICI in iM1, reported significant reductions, suggesting that unilateral training can affect the synaptic efficacy of $GABA_A$ receptors of neurons forming cortico-cortical networks within iM1, releasing pyramidal neurons from inhibition (52). This position contrasts with that from chronic studies (12, 35, 62) where unilateral training produced no significant changes in SICI of the iM1. However, in some of these studies, the participants were requested to suppress intentionally any mirror activity in the resting hand (35, 62). Because volitional inhibition deepens SICI and suppresses corticospinal excitability (87), this might have contributed to the observed lack of adaptation.

Regardless of the type and intensity of training, the significant reduction in SICI was observed in both the pooled estimate of effect and the upper limb sub-group analysis, confirming previous findings outlined in previous chronic studies (27, 46). Thus, although the present estimate was calculated over a small number of studies, reduced intracortical inhibition may contribute to the CE effect. However, this conclusion should be tempered by the fact that SICI is measured at rest. Since SICI is known to change during contraction, further studies are required to test how CE might affect SICI tested in active conditions and, generally speaking, whether measures made at rest will be important during contraction. As stated previously, there is also a need to establish a direct relationship between changes in SICI in iM1 and the magnitude of CE. Until such a relationship is established, it remains tentative whether or not changes in SICI and other TMS-derived variables actually underlie the behavioural changes associated with CE.

IHI Interhemispheric inhibition refers to the neurophysiological mechanism by which one hemisphere inhibits the opposite hemisphere (72). IHI is produced by interhemispheric excitatory pathways through the corpus callosum which synapse onto local inhibitory circuits in the target M1 (22). Long-latency IHI in particular represents a complex inhibitory system projecting from various motor related cortical areas, including the dorsolateral prefrontal cortex, dorsal premotor cortex and somatosensory cortex, to the contralateral M1 (72).

There is a lack of data from chronic studies to provide evidence for a role of IHI in CE. By contrast, compelling evidence from acute experiments (35, 40, 75) clearly indicate that reduced IHI from the trained to the untrained M1 could contribute to the “irradiation” of cortical activity from the “active” to the “non-active” motor cortex giving rise to bilateral activation of both M1 (9, 87). The meta-analysis of IHI studies in chronic conditions proved inconclusive since only 2 studies (35, 62) examined IHI after chronic unilateral strength training and the excessive heterogeneity prevented us from estimating the effect size. However, unlike the other neurophysiological variables, the reduction in IHI from the trained to the untrained hemisphere correlated with the effect on strength, suggesting that changes in interhemispheric interactions accompany CE (35).

Taken all together our findings show that consistent changes occur in the ipsilateral hemisphere after contralateral training, confirming the hypothesis that CE has a neural origin. However, how these changes may contribute to CE is unknown. The lack of correlation between the significant changes in SICI and cSP in the ipsilateral hemisphere and strength increase in the untrained limb confirms the results of previous findings showing that changes in neurophysiology do not correlate with the motor behavior (3). One reason may be that most of neurophysiological measures are made at rest, so that their relevance during movement is unclear. It has been hypothesized that unilateral motor practice can upregulate, via interhemispheric pathways, the excitability of iM1, especially during muscle contraction, and improve motor behavior (35). In this light, changes in resting state excitability might become relevant when the imperative signal is given to 'move'. Patterns of neural activity at rest, which have been called "output null" patterns (43) may affect how a movement develops when it is actually triggered. Based on this population-based model, we can hypothesize that unilateral training subtly alters the pattern of resting (null-output) state activity and the consequence is a behavioral change when the movement is activated.

Neurophysiological changes in the trained side

Neurophysiological changes following unilateral chronic training were measured bilaterally in 17 (77.3%) of the 22 studies included in the present meta-analysis. After the intervention, the majority of the variables examined (70%) were found to be similarly affected (or non-affected) in the untrained and trained sides. This suggests that long term training engages homeostatic mechanisms to resolve the acute imbalance between the hemispheres and restore the physiological balance of baseline conditions (62).

The same phenomenon might account for the neurophysiological changes seen after acute training that then diminish over time towards pre-training levels. Indeed, it has been suggested that the acute effects, as probed by TMS, may be needed to initiate the CE, while persisting effects on behaviour may be consolidated in other circuits, leaving those in motor cortex available for other functions and/or learning tasks (62). The temporal pattern of neural adaptations to exercise resembles the findings obtained in animal models where the effects of learning tasks were investigated (41, 76, 77). In these studies, large changes in connectivity of the brain and changes in synaptic numbers were detected early in training, but over time they became less evident ("pruned back") eventually leaving only a few new/changed connections in the chronic state.

Study limitations

The included studies revealed a high risk of bias in important domains such as publication, allocation and detection biases, which may have led to an overestimation of effect not only for the neurophysiological changes but also for the CE effect. In fact, as already pointed out, methodological issues (heterogeneity of the training schedules and of the body region studied/type of muscle trained; unsupervised training) need to be taken into account to obtain a reliable quantification of the CE effect (65). Furthermore, it is striking that while in strength literature gender pooling is strongly discouraged, in CE studies pooling males and females' data is quite common, which may significantly affect the estimates of effect.

Due to the relatively small number of studies that entered the meta-analyses for each neurophysiological measure, the pooled estimates depicting a significant reduction in cSP duration and SICI should be considered as preliminary. Moreover, although significant, changes in cSP duration and SICI did not significantly correlate ~~to~~ with the magnitude of CE in line with a previous review reporting low or no correlation between CE and TMS-based measures changes (3). This warrants caution in the mechanistic interpretation of the results of the present meta-analysis.

Finally, the employment of the random effects model, which is employed to compare studies with methodological differences (*i.e.*, different units of measurement for the same variable; different

TMS-intensity of stimulation), may have underestimated some inconsistencies among the studies. For instance, when eliciting the cSP, studies employed a wide range of TMS intensities. Such differences may have affected the estimate derived.

Future directions

In the perspective of providing scoping lines to streamline future research, the present review indicates that in studies focused on the neural adaptations accompanying CE: *I)* cSP and SICI proved to be key parameters and thus should be included in the neurophysiological protocols. However, future studies should also attempt to address the question about the relevance of measures made at rest, such as SICI, to explain neural adaptations occurring in active states (*i.e*, during contraction); *II)* in regard to MEP amplitude, the heterogeneity across the studies still prevents us from drawing firm conclusions on its usefulness as a valid indicator of contralateral change; *III)* among the TMS-based parameters, special consideration is needed for IHI. In fact, although its investigation in chronic CE studies cannot be confidently supported, this parameter may deserve further tailored investigations, based on the promising and converging findings of acute studies (35, 40, 75) and of a previous high-quality chronic investigation proposing a putative role for IHI in CE (35). Conversely, ~~any~~ no conclusion can~~not~~ be drawn from SICF, ICF, SAI and LAI data due to the paucity of both acute and chronic studies; *IV*) the assessment of surface EMG burst activity during a MVIC attempt, at least if employed stand-alone, cannot be currently supported to probe CE due to the lack of evidence of contralateral change consistently reported ~~by~~ in a considerable number of RCTs. However, measuring the normalized EMG activity in the untrained muscles remains crucial to perform a number of EMG-based neurophysiological protocols and also to quantify *acutely* whether or not these muscles are really at rest during contralateral training.

Overall, there is a strong need for standardization of both dynamometric and neurophysiological testing protocols in order to enhance the homogeneity and relevance of the findings generated by the individual studies on neural adaptations associated to CE of strength. To maximize the quality of future research on the topic, operational steps should include the definition of homogeneous populations through adequate stratification by gender and the agreement on common experimental procedures. Appropriate statistical data analyses and presentation of the results are also critically important to allow a better understanding of the significance of group differences in neurophysiological measures and, hence, the clinical and scientific implications of that data (61, 99). Finally, the adoption of checklists of information to include when reporting data, such as the Consolidated Standards of Reporting Trials (CONSORT) would enhance the consistency and comparability among studies.

Conclusions

The present systematic review and meta-analysis is the first to provide a quantitative overview of the changes in neurophysiological variables pertinent to cross-education. Overall, the observation of significant reductions in cortical inhibitory mechanisms suggests that inhibitory phenomena occurring within iM1 may modulate corticospinal inhibition and excitability following chronic contralateral training. Specifically, interactions between GABAergic intracortical circuits mediating SICI and cSP are likely to contribute to changes in the corticospinal output to the untrained muscles.

The present results confirm that some neurophysiological measures (SICI and cSP) change consistently in the ipsilateral hemisphere. While providing some insight into the types of changes that are associated with the CE, these findings do not allow ~~to infer that~~us to conclude definitively that the circuits involved ~~the circuits involved~~ necessarily contribute to CE.

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No conflicts of interest, financial or otherwise, are declared by the authors.

Author contributions

A.M. and F.D. conceived and designed study; A.M., T.H. and F.D. analyzed data; A.M. and F.D. prepared figures; A.M., T.H. and F.D. drafted manuscript; A.M., T.H., F.D. and J.R. edited and revised manuscript; A.M., T.H., F.D. and J.R. approved final version of manuscript.

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Figure legends

Figure 1 Study flow chart. NFS, neurophysiology; CE, cross education; ST, strength training.

Figure 2 Risk of bias graph: review authors' judgments about each risk of bias item presented as percentages across all included studies.

Figure 3 Funnel plot displaying the risk for publication bias in the 22 studies included.

Figure 4 Forest plots showing the effect of unilateral resistance training on neurophysiological outcomes measured in the contralateral untrained limb. Std, standardized mean difference; IV, inverse variance; Random, random effect model; CI, confidence interval; df, degrees of freedom; I^2, inconsistency statistic. Significance set at $p<0.05$.

A, Electromyography (11 studies, 255 subjects); **B**, Maximal wave (5 studies, 112 subjects); **C**, Hoffmann reflex (2 studies, 42 subjects).

Figure 5 Forest plots showing the effect of unilateral resistance training on neurophysiological measures relative to the ipsilateral (untrained) hemisphere. Std, standardized mean difference; IV, inverse variance; Random, random effect model; CI, confidence interval; df, degrees of freedom; I^2, inconsistency statistic. Significance set at $p<0.05$.

A, Motor evoked potential (7 studies, 159 subjects; 6 studies and 145 subjects after sensitivity analyses were performed); **B**, Cortical silent period as pooled by standardized mean difference (5 studies, 114 subjects); **C**, Cortical silent period as pooled by mean difference (5 studies, 114 subjects); **D**, Short-interval intracortical inhibition (4 studies for a total of 95 subjects); **E**, Intracortical facilitation (2 studies, 54 subjects); **F,** Interhemispheric inhibition (2 studies, 54 subjects). Excessive heterogeneity (I^2=97%) prevented to obtain a definite estimate from this comparison.

Table legends

Table 1 - Excluded studies (n = 33)

Table 2. Characteristics of the studies included in the qualitative analysis (*n* = 22).

CE, Cross education; CI, 95% confidence interval; yrs, years; reps, repetitions; RM, repetition maximum; wk, week; MVIC, maximal voluntary isometric contraction; s, second; °/s, degree/seconds of angular velocity. [#]Magnitude of the cross-education effect as reported by the authors of the individual studies for the intervention group (within-subjects results). EMG, electromyography; cSP, cortical silent period; M-wave, maximum direct motor response; H-reflex, Hoffmann reflex; V-wave, volitional wave; MEP, motor evoked potential; SICF, short-interval intracortical inhibition; SICI, short-interval intracortical inhibition; ICF, intracortical facilitation; IHI, interhemispheric inhibition; SAI, Short afferent intracortical inhibition; LAI, Long latency intracortical inhibition; SIT, super-imposed twitch amplitude; SIB, super-imposed burst technique obtained by delivering a supramaximal electrical stimulus at MVIC. Changes in neurophysiological measures as reported in the full-text manuscript: ↑, increase; ↓, decrease; *significant for p <0.05; =, no change; n.r., not reported.