Determining the potential sites of neural adaptation to cross-education: Implications for the cross-education of muscle strength.

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Abstract

Cross-education describes the strength gain in the opposite, untrained limb following a unilateral strength training program. Since its discovery in 1894, several studies now confirm the existence of cross-education in contexts that involve voluntary dynamic contractions, eccentric contraction, electrical stimulation, whole-body vibration and, more recently, following mirror feedback training. Although many aspects of cross-education have been established, the mediating neural mechanisms remain unclear. Overall, the findings of this review show that the neural adaptations to cross-education of muscle strength most likely represent a continuum of change within the central nervous system that involves both structural and functional changes within cortical motor and non-motor regions. Such changes are likely to be the result of more subtle changes along the entire neuroaxis which include, increased corticospinal excitability, reduced cortical inhibition, reduced interhemispheric inhibition, changes in voluntary activation and new regions of cortical activation. However, there is a need to widen the breadth of research by employing several neurophysiological techniques (together) to better understand the potential mechanisms mediating cross-education. This fundamental step is required in order to better prescribe targeted and effective guidelines for the clinical practice of cross-education. There is a need to determine whether similar cortical responses also occur in clinical populations where, perhaps, the benefits of cross-education could be best observed.

Key words: Connectivity, Cross-education, Mirror neurons, Excitability, Inhibition, Twitch force.
List of Abbreviations

1-RM: one-repetition maximum
BOLD: blood oxygenation level dependent
FCR: flexor carpi radialis
fMRI: functional magnetic resonance imaging
FDI: First Dorsal Interosseous
GABA: γ-Aminobutyric acid
IHI: interhemispheric inhibition
LICI: long-interval intracortical inhibition
MEPs: motor-evoked potentials
MNS: mirror neuron system
MRI: magnetic resonance imaging
MVC: maximal voluntary isometric contraction
M1: primary motor cortex
rTMS: repetitive transcranial magnetic stimulation
sEMG: surface electromyography
SICI: short intracortical inhibition
tDCS: transcranial direct current stimulation
TMS: transcranial magnetic stimulation
VA_TMS: voluntary activation measured with TMS
WBV: whole-body vibration training
Introduction

There is compelling evidence to support the hypothesis that a change in habitual physical activity, such as strength training, can induce adaptations in the nervous system (Duchateau et al. 2006; Gabriel et al. 2006; Carroll et al. 2011). One common observation that underscores the complexities of neural adaptations following a period of strength training is cross-education. This phenomenon specifically refers to the increase in muscle strength in one limb following unilateral strength training of the opposite limb (Manca et al. 2017a). Since the original observation by Scripture et al. (1894), there are now many published reports that have confirmed that the cross-education of muscle strength is a real effect (Manca et al. 2017a); however, the neural mechanisms mediating cross-education are less clear (Ruddy and Carson 2013). Over the last 30 years, several studies have used surface electromyography (sEMG) recordings (Cannon and Cafarelli 1987, 1992; Mason et al. 2017a), electrical stimulation of peripheral nerves (Dragert and Zehr 2011; Fimland et al. 2009; Lagerquist et al. 2006), transcranial magnetic stimulation (TMS) (Goodwill et al. 2012; Hortobágyi et al. 2011; Manca et al. 2016a; Mason et al. 2017a), voluntary activation measured with TMS (VA_TMS) (Lee et al. 2009) and functional magnetic resonance imaging (fMRI) (Farthing et al. 2007; Palmer et al. 2013; Ruddy et al. 2017) to study the neural adaptations to cross-education. Unfortunately, the neural mechanisms associated with cross-education remain elusive. Reports in the literature are markedly different and range from no substantial change (Latella et al. 2012) to persistent bilateral increases in corticospinal excitability (Hendy et al. 2015), reductions in corticospinal inhibition (Coombs et al. 2016), and short-interval intracortical inhibition (Hortobágyi et al 2011). The complexity is further magnified because emerging evidence suggests some structural changes occur in both motor and non-motor areas (Ruddy et al. 2017). Furthermore, recent work has suggested that other brain regions and systems, such as the mirror neuron system (MNS) (Zult et al. 2016) and functional and structural connectivity (Ruddy et al. 2017)
could be important neural adaptations to cross-education. Subsequently, the aim of this
narrative review is to critically evaluate and elucidate some of the reported important neural
mechanisms and adaptations that have been implicated in the cross-education of muscle
strength, and the potential implications of this phenomenon to clinical populations. This will
be achieved by exploring the idea that the neural adaptations to cross-education are due to
structural and functional changes within cortical motor and non-motor regions and subtle
changes along the entire neuroaxis. This will be addressed by critically appraising the literature
where neuroimaging and electrophysiological techniques, such as fMRI, TMS, VATMS and
spinal cord reflex studies have been used to explore the potential sites of neural adaptations to
cross-education. Secondly, interventions which have been utilised to enhance the cross-
education effect are then considered including, the mirror neuron system, non-invasive brain
stimulation techniques such as transcranial direct current stimulation (tDCS),
electromyostimulation and vibration training. Lastly, the potential clinical implications of
cross-education are considered.

Evidence for cross-education of muscular strength

Since the first documentation of cross-education (Scripture et al. 1894), there has been a
widespread fascination among the scientific community about the phenomenon whereby
strength training of one limb results in an increase in strength of the opposite, untrained limb
(Carroll et al. 2006; Munn et al. 2004). This phenomenon has been summarised in a recent
meta-analysis by Manca et al. (2017a) that revealed a pooled cross-education effect on muscle
strength of 11.9%. Following a quantitative analysis of 31 studies which drew data from 785
subjects, the authors found a 9.4% effect for the upper limb and 16.4% for the lower limb (see
Table 1A-D). The pooled contralateral increase in strength was substantially greater than
originally reported (a pooled 7.8% increase (3.8% for the upper limb and 10.4% for the lower
limb) from 16 studies involving 449 subjects (Carroll et al. 2006)). Of particular interest,
Manca et al. (2017a) demonstrated that the cross-education effect was not restricted to a particular contraction type, but is applicable to the entire muscle action spectrum across both upper and lower limbs. Although the magnitude of cross-education does differ between contraction type (isometric 8.2%; concentric 11.3%; eccentric 17.7%; isotonic-dynamic training 15.9%), all modes significantly increase contralateral strength following unilateral strength training (Manca et al. 2017a). Based upon these findings, it is likely that cross-education could be applied in context-specific environments, such as during different phases of musculoskeletal rehabilitation, in an attempt to maximize the cross-education of muscular strength and to attenuate strength loss and muscle atrophy following injury.

Upper and lower limb effects

The net effect of unilateral strength training of the upper limb is a contralateral increase in strength of 9.4% (Manca et al. 2017a). Of the 13 studies (two studies were excluded due to high heterogeneity) included by Manca et al. (2017a), five studies examined the hand/wrist muscles (Cannon and Cafarelli 1987; Coombs et al. 2016; Farthing et al. 2005; Manca et al. 2016a; Yue and Cole 1992), two examined the wrist muscles (Kidgell et al. 2015; Lee et al. 2009b) and six examined the elbow flexor muscles (Farthing and Chilibeck 2003; Kidgell et al. 2011; Meyers 1966; Munn et al. 2005; Shaver 1970, 1975). Although the cross-education model evidently exists in the upper limb, it appears that the effect is greater for the lower-limb musculature with a 16.4% increase in contralateral strength reported (15 studies [one study excluded due to high heterogeneity]) (Manca et al. 2017a). Most of the studies that were included in this recent meta-analysis involved training the knee extensors (12 studies) (Abazović et al. 2015; Carolan and Cafarelli 1992; Coratella et al. 2015; Garfinkel and Cafarelli 1992; Goodwill et al. 2012; Hortobágyi et al. 1996, Hortobágyi et al. 1999; Kannus et al. 1992;
Latella et al. 2012; Lepley and Palmieri-Smith 2014; Weir et al. 1995, Weir et al. 1997), whilst the remaining studies examined the ankle muscles (four studies) (Fimland et al. 2009; Lagerquist et al. 2006; Manca et al. 2015; Shima et al. 2002). Interestingly, the magnitude of strength transfer was considerably more variable in the upper limb with a heterogeneity of 26% compared to the lower limb of 9% (Manca et al. 2017a). Although both heterogeneity scores are on the lower end of the continuum, it appears that the responses following strength training of the lower limb are uniform across different muscles and contraction modes. This difference may reside in the capacity to voluntarily activate the muscle of the upper or lower limb. The level of neural drive (i.e., motor output) to a muscle, which is also known as ‘voluntary activation’, can be assessed with the twitch interpolation technique (Allen et al. 1998) and via TMS cortical voluntary activation (e.g., VA\textsubscript{TMS}) (Carroll et al. 2008). Incomplete voluntary activation is associated with a reduction in the ‘voluntary’ force generating capacity of the muscle due to reduced neural drive at or above the site of stimulation of the motor nerve. It has been suggested that the capacity to increase muscle strength in upper-limb muscles may be limited when compared to lower-limb muscles due to high pre-existing levels of voluntary activation (Lee et al. 2009a) when compared to the lower-limb muscles (Ross et al. 2007; Sidhu et al. 2009). However, at present, there are not enough high-quality studies that have measured voluntary activation to conclude any differences, despite the recent finding, for at least the trained-limb voluntary activation is improved in the upper limb following strength training (Nuzzo et al. 2017). Although the magnitude of cross-education appears to be different between the upper and lower-limb muscles, other factors, such as contraction type (i.e., isometric, concentric, eccentric and isotonic) and the novelty of the training task are likely to influence the magnitude of cross-education (Farthing et al. 2005; Manca et al. 2017a).

**Contraction type**
At present, the greater part of cross-education studies has focused on the use of isometric and concentric training (Carroll et al. 2006; Manca et al. 2017a; Munn et al. 2004). However, eccentric contractions are known to provide a powerful stimulus for strength increases in the exercised and non-exercised limbs (Hortobágyi et al. 1997). This is supported by the finding of Manca et al. (2017a) who reported that eccentric and dynamic training protocols (i.e., concentric and eccentric contractions) induced significantly greater contralateral gains in strength than isometric contractions (eccentric 17.7%, dynamic 15.9% vs isometric 8.2%). However, the number of studies using eccentric and dynamic training protocols is considerably less than those using isometric and concentric training protocols (Manca et al. 2017a). Irrespective of this, the available evidence clearly indicates that the contralateral increase in muscle strength is real. This effect is strongest when the training employed has been matched to the strength outcome, showing that training specificity is very important (Kidgell et al. 2015; Manca et al. 2017a).

In the clinical context, it would be desirable to utilise a cross-education model that is likely to impart the greatest amount of strength transfer, thus training and testing specificity must be considered. Because the cross-education literature suggests that the magnitude of transfer is contraction-mode specific, the true benefits of cross-education will depend on how effective this contraction-mode training is within clinical populations. For example, it would be beneficial to explore whether eccentric training also transfers the largest amount of strength in different clinical populations. Furthermore, understanding the clinical effects of cross-education is important because the ability to activate muscles and produce force is critical for a number of activities of daily living. For example, there is a good correlation that exists between muscle strength and several clinical outcomes such as, gait speed (Suzuki et al. 2002), decreased risk of falls (Spink et al. 2011), and better balance (Moreland et al. 2004); also, people with greater strength levels tend to live longer (Legrand et al. 2014). In this regard,
understanding the neurophysiological changes that occur in the corticospinal tract and within motor and non-motor regions following a cross-education intervention is important, as it will advance our understanding of the neural adaptations that accompany the cross-education of muscle strength.

Importance of the corticospinal tract as a potential site for neural adaptation to cross-education

The spinal cord is under the control of a number of neurones that descend from the primary motor cortex (M1). The largest of these are the corticospinal neurones that have their origins in layer V of the cerebral cortex and extend to form the bulk of the corticospinal or pyramidal tract (Porter, 1985). Although corticospinal neurones are located within six cortical regions, the M1 has the largest concentration (Porter, 1985). Within the M1, these corticospinal neurones are functionally organised to project to motoneurones that control specific muscle groups (Porter, 1985; He et al. 1993). Corticospinal neurones that arise within the M1 descend through the internal capsule, brainstem, and medulla oblongata to continue to descend in the dorsolateral funiculi of the spinal cord (Alawieh et al. 2017).

As the corticospinal neurones leave the M1 and descend to the medulla, they are organised somatotopically. At the medullary spinal junction, approximately 85-90% of the corticospinal neurones cross the midline to form the motor pyramidal decussation (Alawieh et al. 2017), where they continue as the dorsolateral funiculi of the spinal cord and converge onto motoneurones within the ventral horn of the spinal cord that innervate limb muscles (Alawieh et al. 2017). Anatomical mapping studies reveal that the connectivity of the corticospinal tract suggests that the remaining uncrossed ipsilateral corticospinal tract fibres descend primarily in the dorsolateral lateral or ventral funiculi of the spinal cord (Alawieh et al. 2017).
The ipsilateral corticospinal tract projections could have important implications for the cross-education of muscle strength as there is emerging evidence that suggests a potential site for adaptation could reside within the ipsilateral M1 itself (Goodwill et al. 2012; Kidgell et al. 2011; Lee et al. 2010; Mason et al. 2017a). A small proportion of corticospinal tract fibres do not crossover at the pyramidal decussation at the medulla; rather, they project to ipsilateral spinal motoneurons, where they could alter the excitability of ipsilateral pathways (Alawieh et al. 2017; Carson, 2005). In the clinical neurophysiology literature, it has been suggested that increased utilisation of the ipsilateral pathway may provide a viable method for re-establishing motor control of upper-limb muscles following lesions to the M1 (Alawieh et al. 2017).

Techniques to investigate the functional properties of the brain

The ability to examine the human central nervous system (CNS) has developed remarkably over the last 30 years. Imaging techniques such as fMRI and positron emission topography (PET) indirectly measure the changes in blood flow associated with neural activity while participants perform a particular motor task (Jenkins et al. 1994). For example, there is a relationship between isometric force production, pre-movement activity and actual movement execution that results in increased cortical activity in the M1, supplementary motor area (SMA) and the dorsal portion of the anterior cingulate cortex (Dettmers et al. 1995; Thickbroom et al. 1999; Farthing et al. 2007). Recently, neuroimaging techniques have been used to provide insight into the potential neural adaptations to cross-education (Farthing et al. 2007, Palmer et al. 2013; Ruddy et al. 2017). Although these studies demonstrate changes in blood flow during movement preparation and execution, they do not provide any objective data concerning the excitatory and inhibitory synaptic events specific to the M1 during movement (Hallett 2007). TMS, a non-invasive neurostimulatory technique, can provide a solution to
some of these limitations as it allows the net excitatory and inhibitory synaptic activity within
the M1 and corticospinal tract to be determined with excellent time resolution (Hallett 2007).

There is now a growing body of evidence that shows that there are changes in the efficacy of
neural transmission along the corticospinal tract following cross-education (Goodwill et al.
2012; Kidgell et al. 2011; Lee et al. 2009; Mason et al. 2017a) and new regions of activation
as assessed by fMRI (Farthing et al. 2007; Palmer et al. 2013; Ruddy et al. 2017).

Structural and functional cortical activity and cross-education

The cross-education of muscle strength has historically been associated with changes in
the neural control of the untrained limb, namely, increased capacity to voluntarily activate the
untrained muscle. This observation is based upon the change in strength of an untrained limb
which occurs in the absence of muscle hypertrophy, although changes in the intrinsic properties
of the untrained homologous muscle cannot be excluded (Hendy and Lamon 2017). Despite
possible muscle effects, the consensus suggests that the cross-education effect, in part, is
mediated by changes in the activity of neural circuits, but the site of such change (i.e., cortical
or subcortical) remains unclear. It is not known whether bilateral corticospinal excitability and
inhibition generated during unilateral motor training are the genesis for concurrent neural
adaptations in both cerebral hemispheres (Hellebrandt 1951). Although studies have reported
bilateral activation of the M1 during unilateral movements (Kobayashi et al. 2003; Ruddy et al.
2016), to date, there are few studies that have reported bilateral cortical activity of the M1
following cross-education of muscle strength (Frazer et al. 2017; Hendy and Kidgell 2014;
Hendy et al. 2015; Mason et al. 2017a). An emerging approach that may add new knowledge
regarding the neural adaptations underpinning the cross-education is the use of neuroimaging
techniques. There are now several neuroimaging studies that show, that during unilateral
movements, several regions of activation within pre-motor areas outside of the M1 are involved. These recent findings suggest that there is a greater need to understand the structural and functional bases of cross-education (Farthing et al. 2007; Koeneke et al. 2004) and their causal effect in increasing muscle strength of the untrained limb.

Neuro-imaging and cross-education

Evidence suggests that the cerebral responses to cross-education are controlled centrally by communication between the cerebral hemispheres, via interhemispheric communication and bilateral cortical activity (Hortobágyi et al. 2011; Perez and Cohen 2009). There is a growing need to obtain detailed knowledge of the structural and functional connectivity patterns within the brain following cross-education training. In vivo neuroimaging, such as spatiotemporally resolved magnetic resonance imaging (MRI), alongside its functional variant, fMRI, is one of the pre-eminent tools that is capable of capturing the interaction between neural substrates and action in humans. fMRI is a non-invasive technique that detects changes in regional blood flow within cortical structures in response to a motor task and is quantified as a change in the blood oxygenation level dependant (BOLD) contrast or ratio of deoxygenated haemoglobin (Kelly and Garavan 2005). Changes in the BOLD response are representative of a change in the activation of neural circuits (Xiong et al. 2009), but the BOLD response is not a direct measure of synaptic activity or the presence action potential activity. Thus, any interpretation from BOLD changes should be made with caution as a number of biological process may be involved. For example, changes in activation could be accompanied by changes in synaptic activity within brain structures that are involved in movement (Palmer et al. 2013). Nevertheless, fMRI has been used to detect temporal correlations in spontaneous BOLD responses while subjects remain at rest. These BOLD responses reveal that multiple cortical brain regions are
functionally connected forming resting-state networks. Interestingly, the level of functional connectivity within resting-state networks suggests the existence of direct structural (i.e., neuroanatomical) connections between functionally-linked brain regions (Cordes et al. 2001). Such structural and functional connectivity facilitates the ongoing inter-regional neuronal communication. Importantly, the structural integrity of white-matter tracts, which are structural neural pathways within the brain, facilitates the transmission of neural communication from one brain region to another (Greicius et al. 2009). Understanding changes in structural and functional connectivity following cross-education will add new knowledge regarding the potential sites and mechanism of strength development for the untrained limb.

**Regions of cortical activation and their implications for cross-education**

There is experimental evidence that suggests regions outside, but functionally connected to the M1, may mediate the neural adaptations of cross-education (Farthing et al. 2007; Ruddy et al. 2017; see Table 1). For example, during unilateral movements, there are larger increases in the BOLD response within the premotor cortex (Koenke et al. 2004), supplementary motor area (Grafton et al. 2002) and cingulate motor area (Kermadi 2000). Structural connectivity reveals that the dorsal premotor cortex, supplementary motor area and cingulate motor area have dense structural white-matter connections within the homologous zone in the opposite cerebral hemisphere (Ruddy et al. 2017). At a minimum, this suggests that a structural basis exists for cross-education and may be a specific neural strategy used by the nervous system for increasing the strength of an untrained limb. This line of inquiry is supported when assessing the functional importance of these structural connections. The supplementary motor area is a critical region for motor learning as it plays an important role in modulating the timing of force output (Haller et al. 2009) and is associated with cortical plasticity following motor learning (Grafton et al. 1995, 2002; Perez et al. 2007b). In addition, the supplementary motor area controls both the contralateral and ipsilateral limbs during...
sequence motor learning (Brinkman and Kuypers 1973). Perez et al. (2007b) showed that when repetitive TMS (rTMS) was applied to the supplementary motor area, it abolished the cross-transfer of a sequenced motor-learning task to an untrained limb, but it had no effect on the performance gains of the trained limb. Recent evidence now confirms that the structural connectivity of bilateral supplementary motor areas is substantially greater than any other bilateral regions of the motor cortical network (e.g., M1; Ruddy et al. 2017). In support of this, Ruddy et al. (2017) showed that, following an acute bout of 300 ballistic wrist-flexion movements, motor performance of the untrained limb improved and was associated with an increase in functional connectivity between the right and left supplementary motor area. These observations show that unilateral motor-training engages interhemispheric pathways and that the level of structural connectivity (i.e., the connecting white-matter pathways) influences the magnitude of cross-education (Ruddy et al. 2017). Although this provides new evidence and a good rationale for other cortical structures to be involved in the cross-education of muscle strength, to date, there have been no long-term cross-education studies to show that there are persistent changes in connectivity between left and right supplementary motor areas.

The dorsal premotor region has strong bilateral connections to the prefrontal cortex, parietal cortex and striatum, and this may also serve as a pathway mediating cross-education of strength. In primates, stimulation of the premotor cortex leads to an observable twitch response, showing its contribution to motor output during movement (Dum and Strick 2005; He et al. 1995). Further, because the dorsal premotor cortex has direct projections to the spinal motoneurone pool and shared connections with the ipsilateral M1, it is a candidate structure that could modulate the output from the M1 following cross-education (He et al. 1993). Neuroimaging data support strong interhemispheric bilateral connections between the dorsal premotor cortices (Fling et al. 2011), and structural connectivity reveals that the anterior dorsal premotor area has connections to the prefrontal cortex, whilst the posterior regions have direct
projections to the M1 and spinal cord (Schubotz and von Cramon 2002). These structural and functional connections provide cortical loci that could mediate cross-education. However, a caveat to this interpretation is that there have been no cross-education studies that have examined the pattern of activation within the dorsal premotor cortex. Rather, most of the current understanding for this region is based upon the motor-learning literature (Hardwick et al. 2013). A significant drawback with imaging is that changes in the fMRI BOLD signal do not reveal any changes in the synaptic behaviour regarding these new regions of cortical connectivity.

A cortical network, not thoroughly explored, that could contribute to cross-education is the cingulate motor area. The cingulate motor area is active during unilateral limb movements but, importantly, the level of activation increases as a function of increased force output (Winterer et al. 2002). It is likely that chronic high-force unilateral training may result in persistent changes in connectivity of the cingulate motor area. This hypothesis is possible because of the strong connections between the cingulate motor area and the homologous region in the opposite cerebral hemisphere (Ruddy et al. 2017).

**Neuroimaging studies and cross-education**

The origins of structural and functional connectivity provide the opportunity to explore brain networks following cross-education; however, to date, there has only been one acute cross-education study (i.e., a single training bout) (Ruddy et al. 2017) and two long-term cross-education studies (six and four week training interventions) (Farthing et al. 2007; Palmer et al. 2013) that have used neuroimaging to determine potential sites and patterns of cortical activation.
Using a ballistic training task for the left-wrist flexors, Ruddy et al. (2017) demonstrated that the increase in performance of the untrained limb was 83% of that observed for the trained limb, and that there was a significant increase in functional connectivity in the resting motor network between the right and left supplementary motor areas. Interestingly, the increase in functional connectivity was not associated with the individual level of cross-education. Intriguingly, fibre density in the neural tracts (white matter) connecting the bilateral supplementary motor areas was negatively correlated with cross-education. Although this was only an observation following an acute bout of unilateral motor training, the finding suggests that interhemispheric pathways and the structural integrity of the connecting white-matter pathways of the supplementary motor area play a crucial role in cross-education. Although this is an interesting finding, it remains unclear if repeated high-force voluntary contractions performed over 2-4 weeks lead to a similar effect in structural and functional connectivity.

Farthing et al. (2007) were the first to examine the training-related effects of cross-education on patterns of brain activation following a six-week unilateral strength-training intervention. Strength of the left untrained limb improved by 47% and there was a persistent increase in activation of the sensorimotor cortex and bilateral M1, which is consistent with the motor-learning literature (Grafton et al. 2002). The cross-education of strength was also accompanied by an increase in the pattern of activation within the temporal lobe and the right and left cerebellum (Farthing et al. 2007). Because there was an increase in activation of the M1, supplementary motor area and temporal region following cross-education, this finding supports the recent evidence from Ruddy et al. (2017) that there are structural and functional changes within cortical networks following unilateral motor practice.

Palmer et al. (2013) examined the effects of four-weeks of strength training of the lower limb on structural connectivity. Training consisted of six sets of six maximal voluntary contractions (MVCs) of the dominant-leg plantar flexors. MVC was measured pre and post for
the trained and untrained leg. Strength training increased plantar flexion strength by 30% in
the untrained limb; however, this change was not accompanied by any structural brain changes
which is in contrast to the findings of Farthing et al. (2007). Physiological interpretation of
changes in regions of brain activation obtained from fMRI should be made with caution as
there could be a range of other physiological processes that supplement changes in brain
activation, such as neuronal activation from between and within brain regions that occur during
movement. Therefore, there is a need to examine the muscle-specific pattern of cortical activity
following upper- and lower-limb cross-education using multiple techniques.

In light of the above, given the recent emergence of neuroimaging data, it seems
reasonable to suggest that the cross-education of strength is associated with patterns of brain
activation because of repeated training. The finding of increased activation of the temporal
region following cross-education has important implications for understanding the mechanisms
of strength transfer (Farthing et al. 2007). For some time, cross-education has been considered
a form of motor learning and there are several lines of evidence that the temporal region is
important for memory recall (Martin et al. 1995). In theory, memory retrieval could be
important for cross-education in regards to providing an internal representation of a movement
previously acquired by the opposite limb (Obayashi 2004). This hypothesis supports earlier
findings within the motor-learning literature where the temporal region has been implicated
(Farthing et al. 2007). Increased activation within the right and left cerebellum also suggests
that cross-education training may improve the timing and activation of agonists, antagonists
and synergist muscle groups which improve the coordination of a movement task. There is
good evidence that cross-education training improves patterns of muscle activation (Cannon
and Cafarelli 1987; Carolan and Cafarelli 1992; Farthing et al. 2005; Fimland et al. 2009;
Garfinkel and Cafarelli 1992; Mason et al. 2017a; Perez et al. 2007a) and such changes are
likely a result of changes in activation of the cerebellum (Obayashi 2004).
Although new regions of activation have emerged as potential sites of adaptation following cross-education, fMRI is not able to determine the intracortical mechanisms underlying increased activation. For example, it remains unclear whether excitatory or inhibitory mechanisms underlie the changes in the BOLD responses (Logothetis 2003). Therefore, there is a need to combine neuroimaging techniques with electrophysiological techniques, such as single- and paired-pulse TMS and quantitative electroencephalography, to determine the spatial and temporal effects of cross-education.

**Neurophysiological mechanisms mediating cross-education**

A consistently reported neural adaptation to cross-education is bilateral cortical activity (Frazer et al. 2017; Hendy and Kidgell 2014; Lee et al. 2010) whereby, during unilateral strength training, there is concurrent activation in both cerebral hemispheres that are involved in motor output. The M1 ipsilateral to the training limb has been shown to play an important role in mediating the cross-education effect (Frazer et al. 2017; Hendy et al. 2015; Hortobágyi et al. 2011; Lee et al. 2010). Specifically, cross-education studies have reported increased corticospinal excitability (Kidgell et al. 2015), decreased corticospinal inhibition (Coombs et al. 2016; Hendy et al. 2015), reduced interhemispheric inhibition (IHI) (Hortobágyi et al. 2011; Zult et al. 2016) and increased VA_{TMS} (Lee et al. 2009b) in the M1 ipsilateral to the training limb.

**The use of TMS to examine the cortical responses to cross-education**

TMS is one of the most robust tools available to study the function of the M1 following a motor-training intervention. Briefly, TMS requires placing a wire coil over the M1 ‘hot spot’ of the target representation of the trained muscle (Hallett 2007). When the magnetic coil is
discharged over the M1, it generates a brief ($\leq 100$ µs) high-current pulse that passes painlessly through the skull (Hallett 2007; Rothwell et al. 2009). When the intensity of the TMS pulse is sufficiently large, it primarily activates cortico-cortical axons which provide excitatory inputs onto corticospinal output neurones, depolarising them trans-synaptically as I-waves, with some in close proximity to the axon hillock as D-waves (Di Lazzaro et al. 1999). The net result is the production of a series of descending volleys (action potentials) that travel along the corticospinal tract, synapsing at the appropriate lower-motoneurone pool within the spinal cord, eliciting a brief, relatively synchronous muscle response in the contralateral side of the body. The response is recorded using sEMG and is referred to as a motor-evoked potential (MEP) (Hallett 2007).

MEPs represent the balance between the net excitatory and inhibitory influences on the corticospinal tract as a whole, including those from cortical circuitry, the motoneurone pool, and spinal interneuronal relays (Hallett 2007; Kidgell et al. 2017). Therefore, it is difficult to ascertain the exact site(s) of adaptation with a single-pulse TMS protocol following a motor-training intervention; however, MEPs still provide several important physiological variables that represent the overall efficacy of neural transmission along the corticospinal tract (Rossini and Rossi 2007). The amplitude of the MEP reflects the integrity of the corticospinal tract, the excitability of the M1, and the efficiency of neural conduction along the peripheral motor pathway (Hallett 2007). Conversely, the corticospinal silent period, a period of electrical silence immediately following a MEP evoked in an active muscle, represents inhibition along the corticospinal tract (Wilson et al. 1993). The early portion (50-75 ms) is primarily caused by segmental mechanisms, such as Renshaw cell recurrent inhibition and activation of Iα interneurones by descending corticospinal volleys. The latter portion (>75 ms) is caused by cortical gamma-aminobutyric acid (GABA), specifically $\text{GABA}_B$ contributions (Fuhr et al. 1991). There are now several cross-education studies that have examined the MEP amplitude
and corticospinal silent period duration of the M1 ipsilateral to the trained limb (Coombs et al. 2016; Goodwill et al. 2012; Hortobágyi et al. 2011; Kidgell et al. 2011, 2015; Latella et al. 2012; Manca et al. 2016a; Mason et al. 2017a). However, the findings are mixed. For example, some studies have reported increased MEP amplitudes (Mason et al. 2017a; Hendy et al. 2015), whilst others have shown no change (Coombs et al. 2016; Latella et al. 2012); those that examined the corticospinal silent period have shown reductions (Coombs et al. 2016, Kidgell et al. 2015).

Although single-pulse TMS can provide useful information about the excitability of the corticospinal tract, paired-pulse TMS allows an objective assessment of the physiology of the intrinsic cortico-cortical connections within the M1 (Kujirai et al. 1993). Paired-pulse TMS that uses a sub-threshold conditioning stimulus (70-80% motor threshold), delivered 2-4 ms prior to a supra-threshold test-stimulus, results in a suppressed paired-pulse MEP compared to a baseline single-pulse MEP (Rothwell et al. 2009). This protocol allows the estimation of the excitability of GABAergic circuits within the M1 by calculating the ratio between the conditioned and unconditioned MEPs, which is known as short-interval intracortical inhibition (SICI). SICI is synaptic in origin and mediated by the activation of low-threshold inhibitory circuits that have a presence of GABA receptors from the sub-threshold conditioning stimulus (Kujirai et al. 1993). Paired-pulse TMS enables the measurement of synaptic efficacy of inhibitory neural networks detectable at the level of the M1 following cross-education (Goodwill et al. 2012). In fact, several cross-education studies have now shown that SICI is reduced within the M1 ipsilateral to the training limb (Goodwill et al. 2012; Kidgell et al. 2015).

In a similar manner, when a suprathreshold TMS pulse at an inter-stimulus interval (ISIs) of 50-200 ms is applied, MEPs are significantly reduced and are referred to as long-interval intracortical inhibition (LICI) and this is representative of a slow-phase inhibitory circuit (Valls-Solé et al. 1992). Similar to the corticospinal silent period, LICI is thought to
reflect GABA_B-mediated cortical inhibition. To date, there has only been one study that has examined the effect of cross-education on LICI and it reported no changes in LICI in the ipsilateral M1 (Manca et al. 2016a).

Although TMS is an emerging technique that can be used to provide insight into the neural adaptations to cross-education, there are several limitations that should be considered when interpreting the literature. It must be recognised that the amplitude of MEPs are influenced by numerous factors along the brain-to-muscle pathway. For example, the excitability of the corticospinal and intracortical neurones that are activated by TMS and the efficacy of the synapses between these neurones can influence MEP amplitude (Mazzocchio et al. 1994; Ugawa et al. 1995). Further, the excitability of interneurones located between corticospinal neurones and α-motoneurones, the efficacy of the corticospinal-motoneuronal synapses (Bunday and Perez, 2012; Taylor and Martin, 2009), and the excitability of the motoneurones themselves (Nielsen and Petersen, 1995; Di Lazzaro et al. 1998), all affect the amplitude of MEPs.

Ipsilateral MEP responses to cross-education

There are now several studies that have reported that cross-education of muscle strength is accompanied by an increase in ipsilateral corticospinal excitability (i.e., MEPs) following dynamic, eccentric and concentric training, (Goodwill et al. 2012; Kidgell et al. 2011; Mason et al. 2017a), eccentric (Kidgell et al. 2015) and isometric strength-training (Hortobágyi et al. 2011). However, similar to the TMS strength-training literature, this finding is not always consistent (Kidgell et al. 2017). For example, just as many studies have shown no change in ipsilateral corticospinal excitability (Coombs et al. 2016; Latella et al. 2012; Manca et al. 2016a). These inconsistencies appear to be driven by the different TMS methods employed,
muscles trained, intensity of the training stimulus, and the mode of muscle contraction used
during training.

Historically, the cross-education effect was thought to be confined to the contralateral
homologous muscle; however, recent experimental data suggested that the effects exist beyond
the contralateral agonist muscle (Mason et al. 2017a). Recently, we (Mason et al. 2017a)
examined the spatial effects of cross-education on ipsilateral corticospinal excitability. Briefly,
participants completed 3-weeks of high intensity (80% one repetition maximum [1-RM])
elbow-flexion training and we recorded MEPs from agonist and synergist muscles by
stimulating ipsilateral M1 prior to, and following, the training period. The MEPs of the
untrained elbow-flexors increased but there were no changes in MEPs of the untrained
synergistic wrist-flexors (Mason et al. 2017a), yet we showed increased strength in both the
contralateral agonist and synergist. We also reported a similar finding for corticospinal silent
period duration. These findings raise questions as to why there was not any increase in the MEP
and any decrease in corticospinal silent period duration of the synergist muscle despite
reporting an increase in strength. As alluded to earlier, it is likely the spatial effects of strength
transfer could be mediated by other cortical structures, but functionally linked to the ipsilateral
M1 that TMS is unable to detect. Overall, the ipsilateral MEP responses to cross-education are
inconsistent across studies and confined to the contralateral homologous motor-cortical
network. To this end, there is no clear evidence to support a role for increased corticospinal
excitability of the M1 ipsilateral to the training limb as a mechanism that mediates the cross-
education of strength (see Table 2).

Ipsilateral inhibitory responses to cross-education

Unlike the MEP responses to cross-education, there is stronger evidence that shows an
important neural adaptation to cross-education is a reduction in intracortical inhibition of the

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ipsilateral M1 (Coombs et al. 2016; Goodwill et al. 2012; Hortobágyi et al. 2011; Kidgell et al. 2015; Latella et al. 2012; Mason et al. 2017a). For example, work from our laboratory showed that there is a 12-18 ms reduction in the corticospinal silent period following both upper-(Coombs et al. 2016; Kidgell et al. 2015; Latella et al. 2012; Mason et al. 2017a) and lower-limb cross-education (Goodwill et al. 2012; Latella et al. 2012). There is also good evidence that shows SICI is reduced (Goodwill et al. 2012; Hendy et al. 2015; Kidgell et al. 2015), suggesting cross-education selectively reduces the synaptic efficacy of inhibitory networks within the ipsilateral M1 and corticospinal tract. Because the corticospinal silent period is modified by GABAB-mediated inhibition (Werhahn et al. 1995), it seems that cross-education specifically affects intracortical inhibitory neurones that collectively results in an improved ability to activate the spinal motorneurone pool, which could partly explain the increases in muscle strength.

The reduced corticospinal silent period and SICI show that the GABA-ergic inhibitory neural networks are important for maximal force generation and are associated with the cross-education of muscle strength (Christie and Kamen 2013; Coombs et al. 2016; Kidgell and Pearce 2010; Weier et al. 2012). This line of enquiry is supported by early theories of cross-education whereby reductions in IHI could play a pivotal role in the behavioural effects observed (Hellebrandt 1951; Hortobágyi et al. 2011). The reduction in corticospinal inhibition seems to be an important mechanism that mediates cross-education because several studies, that have used models of immobilization, have reported increased silent-period durations and reduced muscle-strength (Clark et al. 2008; Pearce et al. 2012); however, motor training seems to attenuate the prolongation of the corticospinal silent period (Clark et al. 2014).

The reduction in SICI following cross-education suggests that the excitability of short-latency intracortical inhibitory circuits are reduced, which serves to focus the excitatory drive onto corticospinal neurones within the ipsilateral M1 that produce the intended movement of
the agonist muscle (Reynolds and Ashby 1999; Ridding et al. 1995). Certainly, evidence is now available that, during increased muscle activity, the reduction in inhibition is selective and specific to the agonist muscle. This supports the findings of Mason et al. (2017a) whereby ipsilateral changes in inhibition were confined to the agonist muscle following cross-education.

To date, there has only been one cross-education study that has examined LICI (Manca et al. 2016a). Participants trained the first dorsal interosseous (FDI) muscle via a maximal pinch-force task (five sets of ten repetitions) three days a week for four-weeks. Following training, there were no changes in LICI, but the corticospinal silent period was not measured. Based upon the current systematic evidence available, in order to increase our understanding of the corticospinal responses to cross-education, there is a need to design cross-education studies that use techniques to probe both the excitatory and inhibitory cortico-cortical connections following both acute and chronic interventions. These will provide important information about the mechanisms associated with cross-education.

**Interhemispheric inhibition and cross-education**

TMS can be used to assess the mediating inhibitory pathways between cerebral hemispheres via IHI. IHI is a neurological mechanism whereby one hemisphere inhibits the opposite hemisphere and its presence is due to excitatory pathways via the corpus callosum which synapse onto local inhibitory circuits within the target M1 (Perez and Cohen 2009). Eight-weeks of maximal isometric training of the right FDI reduced IHI from the trained to the untrained M1 by 31% and, importantly, this increase was associated with the magnitude of cross-education (Hortobágyi et al. 2011). This finding supports the evidence from the examination of IHI following strong unilateral contractions and acute motor-skill training (Hortobágyi et al. 2003; Perez et al. 2007c). However, Manca et al. (2016a) recently reported that four weeks of cross-education had no effect on IHI. Currently, it is inconclusive whether
reductions in IHI mediate cross-education, but the physiological role of IHI seems to be an important modulator for cross-education. For example, one hypothesis suggests that reduced IHI from the trained to the untrained M1 would support the theory of cross-activation from the active to the non-active M1 (Lee et al. 2010), a mechanism consistently reported during maximal contraction of one limb (Hortobágyi et al. 2003) and implicated in cross-education (Frazer et al. 2017; Hendy and Kidgell 2014; Hendy et al. 2015; Mason et al. 2017a; Ruddy et al. 2017).

Collectively, the use of TMS to examine the ipsilateral corticospinal responses to cross-education has begun to provide some putative neural mechanisms that mediate a change in strength of an untrained limb. However, there is a need to establish whether there is a direct relationship between the changes in ipsilateral corticospinal responses and the magnitude of cross-education. Thus, at present, it remains tentative whether the changes in ipsilateral TMS responses actually underpin the observed changes in strength of the untrained limb. Furthermore, because of the limitations associated with TMS and the anatomy of the corticospinal tract, changes in the excitability of the spinal cord and the ability to activate the motoneurone pool (i.e., voluntary activation) may also add further insights into the ipsilateral corticospinal responses associated with cross-education.

Potential spinal mechanisms and cross-education

There is evidence to suggest that adaptations in spinal circuitry occur following strength training (Duclay et al. 2008); however, current methodology is unable to determine specific spinal pathways that may be involved in the cross-education of strength (Dragert et al. 2011; Fimland et al. 2009; Lagerquist et al. 2006). Despite this, the Hoffman reflex (H-reflex), an electrically evoked reflex used to quantify the efficacy of the 1a afferent motoneuronal synapse
(Palmeri et al. 2004), has been measured in only three cross-education studies (Dragert et al. 2011; Fimland et al. 2009; Lagerquist et al. 2006). All three studies showed no change in H-reflex amplitude following cross-education. Thus, these findings do not enable any definitive conclusions to be drawn as to whether the intrinsic circuitry of the spinal cord contributes to the cross-education of strength.

**Twitch force studies and cross-education**

The interpolated twitch technique has been used extensively to measure voluntary activation as a reflection of “neural drive” (Merton 1954). In addition to measuring sEMG changes with unilateral and contralateral musculature following training, twitch interpolation (or interpolated twitch technique) is a single-pulse protocol that allows investigations to address the question of training protocols increasing central nervous system excitability. In particular, twitch interpolation allows for the investigation of whether the motoneuron pool has been excited sufficiently by volition to evoke all the force the relevant muscle can produce (Herbert and Gandevia 1999; Gandevia, 2001; Todd et al. 2016). The amplitude of the interpolated twitch declines with increasing contraction intensity (voluntary activation) allowing for the quantification of the level of excitation of motoneurones, or “neural drive” (Herbert and Gandevia 1999).

Historically, understanding the neural mechanisms contributing to strength changes following strength training has been conducted in the primary agonist muscle (see review by Shield and Shi 2004). However, electrical twitch interpolation has also been used to assess neural adaptation in contralateral neural pathways following unilateral strength training (cross-education; see Table 3). For example, Shima et al. (2002) showed that, in healthy untrained males (n=9), voluntary activation increased in both trained and contralateral untrained leg
(plantar flexor muscles) following a six-week training period of progressive strength training of calf raises and foot-presses. Moreover, following a six-week period of detraining, the authors found that voluntary activation did not significantly change (trained: 0.3 ± 2.6%; contralateral: -0.7 ± 1.7%), suggesting that cross education of muscular strength might be explained by central neural mechanisms during training and, at least in part, during the period of detraining (Shima et al. 2002).

More recently, twitch interpolation has been used to assess neural adaptations with strength training in older populations (Tøien et al. 2017). Whilst it is known that age can attenuate efferent neural drive, even in those with a long history of strength training experience, these authors investigated whether the deterioration of neural drive impedes contralateral neural drive with increasing age (Molenaar et al. 2013). They used an older population of males (n = 23; mean age 73 ± 4 years) who undertook a three-week strength training program (nine sessions; three sessions per week) of dynamic plantar flexion involving both concentric and eccentric contractions (90% of one-repetition maximum [1RM]). Voluntary activation, measured in the contralateral soleus muscle, increased by a mean of 5.0 ± 5.5%. Tøien et al. (2017) suggested that older individuals exhibit cross-limb neural adaptations, providing a potential clinical value in unilateral high-intensity strength-training that is not limited by age.

Whilst the majority of studies using interpolated twitch technique are undertaken electrically, magnetic stimulation may also be used (Lampropoulou et al. 2012). Since 2000, there has been increased interest in using peripheral magnetic stimulation as an alternative to electrical stimulation in assessing voluntary activation (Goodall et al. 2014) in upper-limb (Harris et al. 2000; Lampropoulou et al. 2012) and lower-limb musculature (Goodall et al. 2009; Hamnegård et al. 2004; Kremenic et al. 2004; Vivodtzev et al. 2005). Comparisons between electrical and magnetic peripheral twitch interpolation during maximal voluntary contractions show similarities in the onset latencies and supramaximal twitch response in hand muscles.
(Harris et al. 2000; Olney et al. 1990) and also in biceps brachii (Lampropoulou et al. 2012).

However, peripheral twitch interpolation is limited because the exact level (or site) of adaptation within the central nervous system cannot be distinguished as the change may occur at any point, or combination of points, proximal to the position of stimulation (Goodall et al. 2014). Further, it has been argued that other limitations of peripheral stimulation, including the strength of the stimulus, can pose problems in terms of the distribution of the magnetic field, particularly if it is too high (supramaximal stimulus) and the spread of the magnetic field can conduct to tissues other than the target peripheral nerve (Hamnegård et al. 2004; Matsumoto et al. 2010; Millet et al. 2011). Conversely, the stimulus might not be of sufficient intensity if the coil position is not correctly orientated (Lampropoulou et al. 2012) to maximally stimulate all motor units, a desired outcome that might also be limited by subcutaneous adipose tissue (Lin et al. 2008; Tomazin et al. 2010, 2011). As a result, peripheral magnetic stimulation does not have extensive recognition either as a ‘gold standard’ technique or as a practical alternative to electrical twitch interpolation (Lampropoulou et al. 2012).

Magnetic stimulation has also been shown to elicit a twitch response when stimulation is applied over the M1. There are many studies that utilise TMS to quantify adaptations of the corticospinal pathway following short-term strength-training, not only in the trained (Kidgell and Pearce 2010; Kidgell et al. 2010; Leung et al. 2017; Mason et al. 2017b; Weier et al. 2012) but also in the untrained contralateral arm (Coombs et al. 2016; Kidgell et al. 2011, 2015) and leg (Goodwill and Kidgell 2012; Goodwill et al. 2012; Latella et al. 2012). However, these studies generally used sub-maximal contractions to measure MEPs and corticospinal silent period as determinants of neural adaptations in the contralateral untrained limb. While maximal voluntary activation of human elbow-flexors can be assessed with TMS (\(VA_{\text{TMS}}\)) (Todd et al. 2004), few studies have employed the technique of \(VA_{\text{TMS}}\) to assess the neural adaptations to cross-education (Lee et al. 2009b). This is surprising because an advantage of \(VA_{\text{TMS}}\) is that it
allows the assessment of supraspinal contributions for the volitional drive to the muscle during a maximal contraction (Lee et al. 2008). However, the technique itself is technically difficult and has its own limitations. For example, it is imperative that the TMS stimulus produces a maximal response in the target muscle (Carroll et al. 2008). In addition, changes in VA_{TMS} are likely to be more specific to the testing condition (e.g., isometric), thus training interventions that do not match the conditions of testing prove problematic when interpreting the data.

Despite the theoretical contribution of VA_{TMS}, there is a paucity of research using this technique, particularly for contralateral neural adaptations following cross-education strength training. To date, only one study has utilized TMS twitch interpolation to assess neural adaptations in the contralateral arm (Lee et al. 2009b). Following four weeks of unilateral wrist extension strength training, a significant increase in contralateral voluntary activation in the untrained wrist was observed (2.9 ± 3.5%). These authors concluded that unilateral maximal strength training improved motor output from the M1 to the homologous untrained muscles. Given the potential of using VA_{TMS} to assess the ability of the M1 to drive the motoneurone pool, future studies should consider incorporating this technique.

**Interventions to enhance the cross-education effect**

Recently, there have been some innovative techniques used to enhance the cross-education effect. There is now some preliminary evidence to show that the cross-education of strength may be enhanced by mirror feedback. This intervention activates the mirror neuron system (MNS) which is known to have projections to motor regions (Zult et al. 2014). In addition, there are emerging studies that have used tDCS, a simple and cost effective technique whereby electrodes are placed over the M1 of a target muscle and low levels of electricity are passed through to the underlying cortical neurones (Frazer et al. 2017). Both acute and chronic
studies now show that the cross-education of strength is enhanced when tDCS is applied to the ipsilateral M1 before or during the training intervention (Frazer et al. 2017; Hendy and Kidgell 2014; Hendy et al. 2015). In a similar manner, applying electrical stimulation over the training muscles (Hortobágyi et al. 1999) and whole-body vibration training (WBV) have also been used to enhance the cross-education effect (Goodwill and Kidgell 2012; Lapole et al. 2013).

**Mirror neurones and mirror box training to augment cross-education**

The aforementioned work reporting cross-education of strength is compelling. When coupled with evidence showing that this phenomenon can attenuate strength loss and reduce the magnitude of atrophy during short-term immobilisation in healthy individuals with (Magnus et al. 2013) and without (Farthing et al. 2009, 2011; Pearce et al. 2012) a fracture, this opens up exciting possibilities for cross-education as a therapeutic intervention in clinical populations with unilateral orthopaedic and neurological dysfunction. These preliminary data are promising, but many clinical populations have unilateral dysfunction for periods of time more extended than those studied in the literature. Consequently, cross-education might not be as efficacious in some scenarios. Given the potential clinical importance of cross-education, it would be of interest to explore methods that have the potential to further augment the magnitude of the cross-education of muscle strength. Because sensory feedback during motor practice can increase motor output, one possibility is to activate neurones involved in the transfer that might also be activated by other means, thereby resulting in a synergistic effect on motor output and, hence, increase the transfer of strength (Howatson et al. 2013).

Previous research (Carson and Ruddy 2012; Farthing et al. 2007, 2011; Hortobágyi et al. 2011) has shown that, following strength training and the subsequent cross-education, there is overlap in the activation of brain areas that contain mirror neurones. The MNS is a network of neurones that are active during perceptual and actual execution of motor activities (Iacoboni 1999, 2005; Rizzolatti et al. 1996). In a recent hypothesis (Howatson et al. 2013) and systematic
review (Zult et al. 2014), the possibility of activating the MNS concurrently with performing a cross-education strength task was proposed to increase the magnitude of cross-education.

The MNS is distributed across numerous areas of the cerebral cortex and provides a neuroanatomical basis for ‘action observation’ whereby observation and/or imitation of an act can develop motor learning and skill acquisition (Rizzolatti et al. 1999; Sakadjian et al. 2014). In brief, it is thought that the MNS is activated when performing a similar task that has been observed. Specifically, the MNS is activated during the observation of a self-performed act, observing a third person, imitation of an observed task, perceptual input and the performance of the practised movement (di Pellegrino et al. 1992; Heyes 2010; Ray and Heyes 2011). These activities are very common in the arts and athletic performance where extensive observed practice is used to master skills that stimulate areas common to motor cortical areas and the MNS which are detailed elsewhere (Howatson et al. 2013; Zult et al. 2014). Previous experience of the motor activity seems important to modulate and engage the MNS (Beudel et al. 2011); naïve participants, when compared to skilled dancers and musicians, show greater MNS activation when observing dancers and musicians, respectively (Heyes 2010). Therefore, practice of a task will likely engage these neural networks and improve motor performance (Howatson et al. 2013; Zult et al. 2014).

Conceptually, the observation of a relatively simple, self-performed, unilateral motor-task (like resistance exercise) might enhance the cross-education effect by viewing the performed task in a mirror. In this concept, a reflected image (with the use of a mirror) is superimposed over the non-exercising limb to provide the illusion that the non-active limb is moving. The reality is that the participant is actually viewing a reflection of the exercising limb and, hence, receives the visual sensation of the non-active limb actually moving (Nojima et al. 2012; Small et al. 2012). Previous work using mirror training showed increased ipsilateral brain activity (Garry et al. 2005; Matthys et al. 2009), skill acquisition of the non-practised hand in
healthy participants (Hamzei et al. 2012; Läppchen et al. 2012; Nojima et al. 2012), reduced phantom-limb pain (Ramachandran and Rogers-Ramachandran 1996), and enhanced stroke recovery (Sütbeyaz et al. 2007; Yavuzer et al. 2008). The brain structures thought to be implicated in cross-education have neuroanatomical commonality with those of the MNS. This makes the expectation tenable that observing the moving limb in a mirror could increase the brain activity controlling the resting limb and, thereby, increase the magnitude of cross-education (Howatson et al. 2013; Zult et al. 2014). Until recently, this idea had not been explored experimentally.

In two subsequent studies arising from the aforementioned concept, we explored the cortical excitability and intra and interhemispheric connectivity: 1) when observing forceful contractions in a mirror; and 2) to assess the magnitude of cross-education when resistance training was performed using a mirror. The first of these studies (Zult et al. 2015) was cross-sectional in nature to examine the cortical networks hypothesised to share commonality between the MNS and networks thought to be involved in cross-education. In this work, 27 right-handed male volunteers had corticospinal and motor-cortical responses to TMS recorded in the left flexor carpi radialis (FCR). This was done with and without viewing a mirror, at rest and during a forceful shortening contraction of the right-wrist flexors. Corticospinal excitability in the resting FCR increased during the contraction (similar to that observed previously) but was not different between mirror and no-mirror conditions. However, SICI was significantly lower (~9% release of SICI) illustrating that GABA-ergic inhibitory networks were implicated in the cross-education phenomenon (Goodwill et al. 2012; Perez and Cohen 2008). This first step in support of the hypothesis (Howatson et al. 2013) provided evidence that neural networks implicated in cross-education can also be modulated by viewing a reflection of the exercising limb superimposed on the resting limb. In a follow-up training study that further tested this idea, Zult and colleagues (2016) took 24 right-handed volunteers to
undertake either mirror training or no-mirror training. Specifically, all participants completed 15 resistance-training bouts (six sets of eight repetitions isokinetic, concentric actions [20°/s] at 80% MVC) of the right-wrist flexors over a three-week period. Critically, the training resulted in improved performance in both groups, but the mirror group had a 13% greater increase ($P = 0.03$) than the no-mirror group in strength of the untrained limb. These strength improvements in the untrained limb were accompanied with a mean reduction in the contralateral silent period of 30 ms, which was thought to be indicative of greater inhibition of GABA$_B$-mediated networks. In addition, there were reductions in interhemispheric inhibition, a GABA-ergic network between cortical hemispheres which is also implicated in the MNS from a neuroanatomical perspective (Zult et al. 2014).

Although the potential for using a mirror to augment the cross-education effect is just emerging, it provides a potentially exciting field of research. Volunteers in these studies (Zult et al. 2015, 2016) verbally reported sensations of the left, non-active limb moving when observing strong monotonic contractions of the right limb in a mirror that were superimposed on the non-active limb. So, conceptually, there could be a placebo effect that has limited the neurophysiological basis (at least in the measure collected). Notwithstanding, these data are particularly important because of the implications for clinical populations, whereby an increase in the cross-education phenomenon is amplified. This effect should be investigated further to explore its application to unilateral orthopaedic or neurological impairment. Importantly, the use of neuroimaging techniques to establish the role of the MNS and other regions of the cerebral cortex seems to be an important progression in understanding the mediating mechanisms of cross-education (Rizzolatti and Craighero 2004).
tDCS has emerged as a promising, non-invasive technique to improve motor performance in both young and older adults (Goodwill et al. 2015; Kidgell et al. 2013). The application of tDCS over the M1 induces transient, polarity-specific changes in the neuronal resting membrane potential (Nitsche et al. 2008), with increases in excitability and performance improvements lasting up to 90 min following the cessation of stimulation (Lang et al. 2005). However, similar to the TMS strength-training studies (Kidgell et al. 2017), the reproducibility of neuroplasticity inducing protocols, like tDCS, remains a challenge (Heroux et al., 2015; Heroux et al., 2017). However, recently, tDCS has been used experimentally to enhance the cross-education of muscle strength. For example, Hendy and Kidgell (2014) reported an increase in maximal strength and cross-activation of the contralateral untrained limb (left hand) following a single session of anodal tDCS applied to the ipsilateral right M1 during strength training of the right hand (Hendy and Kidgell 2014). In a follow-up study, Hendy et al. (2015) applied anodal tDCS to the ipsilateral right M1 during a two-week strength-training intervention and showed that the effects of cross-education were prolonged, and that tDCS retained strength of the untrained limb compared to sham tDCS and strength-training (Hendy et al. 2015).

Although this data is interesting and has potential applications in the clinical environment, there is a greater need to identify the optimal timing of tDCS to the ipsilateral M1 (i.e., before, during or after training). In an attempt to address this, we recently demonstrated a substantial increase in maximum strength of the untrained left biceps brachii when anodal tDCS was applied to the ipsilateral M1 (right hemisphere), prior to a single bout of strength training of the right arm only, exploiting the principles of homeostatic meta-plasticity (Frazer et al. 2017). Although preliminary evidence indicates that tDCS is a promising tool, the timing of application needs to be rigorously investigated following both single-session and longer-term training periods (>2 weeks). Undoubtedly, combining robust
investigation techniques, such as TMS and fMRI, would aid in quantifying the potential opportunity to augment the cross-education of muscle strength.

**Electromyostimulation during cross-education**

Similar to voluntary contractions evoked during unilateral strength training, there is good evidence to show that the application of electrostimulation during strength training increases MVC force production of an untrained homologous muscle following unilateral strength training (Bezerra et al. 2009; Hortobágyi et al. 1999). There are now several studies that have revealed electrical stimulation of a muscle, compared with voluntary contraction, evokes specific effects at the level of the cerebral cortex and increases force in an untrained limb (Bezerra et al. 2009; Hortobágyi et al. 1999). Hortobágyi et al. (1999) reported that electrical muscle stimulation induced a contralateral increase in strength of 21% following four-weeks of isometric strength training, which was comparable to that induced by voluntary isometric strength training alone. In addition, six weeks of eccentric strength training with electrical muscle stimulation induced an increase in strength of 104% compared to 23% for voluntary eccentric training alone (Hortobágyi et al. 1999; Oakman et al. 1999). Interestingly, electrical stimulation training is also more effective than voluntary strength training when imparting a cross-education effect (Bezerra et al. 2009). Because the cross-education effect following electrical stimulation training is not associated with any changes in the cross-sectional area of the contralateral untrained muscle (Bezerra et al. 2009), the physiological mechanisms underpinning the changes in strength seem to reside within the cerebral cortex. For example, when electrical muscle stimulation is used to induce left-wrist flexion, both TMS induced MEPs and the H-reflex increase in the right resting-wrist flexors (Hortobágyi et al. 2003). In a similar manner, when electrical muscle stimulation is applied during voluntary contraction of the left-wrist flexors, TMS-induced MEPs are increased, but the H-reflex in the right resting-wrist flexors is reduced (Hortobágyi et al. 2003). These observations suggest that
electrical muscle stimulation and voluntary contractions are affected differently at a supraspinal level in contralateral homologous muscles. This difference is likely the result of increased sensory and nociceptive inputs that act at a cortical level following electrical stimulation. Perhaps such inputs modify motor output and interhemispheric paths, which lead to an increase in strength of the trained and untrained limb. Certainly, this hypothesis is supported by changes in IHI following unilateral training (Hortobágyi et al. 2011; Howatson et al. 2011; Lee et al. 2010).

**Whole-body vibration training and cross-education**

The recent emergence of WBV as a training technique has been of interest to researchers due to its potential to improve neuromuscular function. Many studies have reported increases in strength following an acute bout of WBV. Similarly, increases in strength have also been demonstrated following a period of strength training with the addition of WBV (Issurin 2005; Nordlund and Thorstensson 2007; Rittweger 2010), suggesting that WBV training may be an effective and alternative training technique for strength development (Rittweger et al. 2003) and for enhancing cross-education (Goodwill and Kidgell 2012). Given that the magnitude of strength gain in the trained limb is an important proxy for strength transfer to the untrained limb, we recently examined the effect of unilateral strength training with superimposed WBV on the magnitude of cross-education (Goodwill and Kidgell 2012). Healthy participants completed unilateral strength training with or without the application of WBV (35 Hz; 2.5 mm amplitude), three times per week for three weeks. Strength increased by 41% in the trained limb following strength training without WBV and by 55% with WBV. Interestingly, the cross-transfer of strength was greater for the untrained limb (52%) following WBV, with only a 35% transfer following training without WBV. Further, after WBV training, there was an increase in corticospinal excitability and a reduction in SICI of the ipsilateral M1 suggesting that WBV training had a cortical effect (Goodwill and Kidgell 2012). In a similar
manner, 14 days of Achilles-tendon vibration also increased the strength of a vibrated gastrocnemius and the non-vibrated gastrocnemius muscle. The increase in strength of the non-vibrated gastrocnemius was associated with a 41% increase in the volitional wave (a measure of neural drive), but the H-reflex remained unchanged (Lapole et al. 2013). These observations suggest that there could be additional cross-education benefits following the application of vibration to the training limb.

Clinical application of cross-education

In clinical practice, unilateral injuries, such as knee osteoarthritis, tendinopathy, fracture, stroke, and cerebral palsy are extremely common. In the Western world, 6% of women will have sustained a distal radius fracture by the age of 80, and 9% by the age of 90. Regardless of whether these fractures are treated surgically or by casting, patients are immobilised for two to six weeks (or more). Physical and occupational therapy, as a key element in rehabilitation, typically only starts following the period of immobilisation. During the period of immobilisation, patients often keep their injured limb in rigid postures, and the involved ligaments of the joints become shortened (Freeland and Luber 2005). Different methods of treatment, but especially the long immobilisation periods, lead to a high incidence of complications which are typically associated with poor functional outcomes (McKay et al. 2001). These complications include complex and regional pain syndrome, stiffness, nerve injury, tendon and ligament injuries, and a large reduction in range of motion and muscle strength (Diaz-Garcia et al. 2011). In addition, recovery of the strength loss and muscle atrophy experienced following a unilateral injury and period of immobilisation is often hampered by patients’ and therapists’ inability to effectively exercise the involved body part. As a result, final function of the injured limb is often suboptimal, highlighting the critical need to
implement strategies such as cross-education to attenuate the loss of function that occurs with immobilisation.

Although there is a strong consensus that unilateral motor practice is a viable candidate to reduce unilateral loss of muscle strength and motor function during a period of immobilisation, there is limited evidence to support this notion. To date, just five studies have investigated the effects of cross-education in healthy participants undergoing a period of immobilisation (Andrushko et al. 2017; Farthing et al. 2009, 2011; Papandreou et al. 2013; Pearce et al. 2012). All five investigations found that a cross-education intervention attenuated the strength loss in the immobilised limb, with four of the investigations also displaying a sparing effect for muscle size (Andrushko et al. 2017; Farthing 2009; Papandreou et al. 2013; Pearce et al. 2012). Of particular interest, this cross-education model was successfully translated into a clinical population of women older than 50 years who suffered a unilateral distal radius fracture (Magnus et al. 2013). Magnus et al. (2013) demonstrated an increase in strength and range of motion of injured wrists 12-weeks post-fracture, providing preliminary evidence of the clinical efficacy of cross-education for immobilised patients. Nevertheless, there is still insufficient empirical evidence demonstrating that unilateral training of the non-injured limb during the early rehabilitation period can attenuate atrophy and strength loss associated with immobilisation. The successful implementation of such an intervention, and subsequent exploitation of the cross-education effect, could accelerate recovery by enabling patients to maintain a higher level of function in the injured limb prior to remobilisation. This would have the dual benefit of improving functional outcomes in the immediate period post-injury and facilitating the execution of rehabilitation exercises designed to mobilise, reduce atrophy and strengthen the injured limb.

Another highly-plausible application of cross-education is the restoration of bilateral limb symmetry following stroke (Dragert and Zehr 2013). A recent meta-analysis has shown
the positive effect of cross-education on muscle strength in patients who have suffered a stroke (Ehrensberger et al. 2016). Although only two studies were analysed, post-stroke hemiplegic patients demonstrated a 31.4% and 45.5% strength increase in the untrained, more-affected dorsiflexor muscle following unilateral training (Dragert and Zehr 2013; Kim et al. 2015).

Indeed, these results are promising; however, attention needs to shift beyond the focus of demonstrating a transfer in strength to gains in functional tasks and recovery of motor function (Ehrensberger et al. 2016). Furthermore, there has been recent preliminary evidence suggesting that cross-education might aid in the management of limbs severely weakened because of multiple sclerosis (Dragert and Zehr 2013; Manca et al. 2016b, 2017b). Of note, none of the trials investigating the efficacy of cross-education in neurological patients employed a training design using eccentric or dynamic contraction modes. Given the recent finding that eccentric and dynamic contractions induce significantly greater contralateral gains in strength in healthy subjects (Manca et al. 2017a), it would be interesting to examine whether the transfer of strength in neurological subjects may be further enhanced by changing the type of training contraction employed. This highlights the urgency to not only investigate the role of cross-education in the rehabilitation and management of neurological patients, but also to further enhance our understanding of best prescription variables for this population. Overall, there is a great need to examine the efficacy of cross-education in clinical populations with an emphasis on using interventions which are known to maximise the cross-education effect.

**General summary and conclusions**

Cross-education is a phenomenon that has been shown to occur following different unilateral strength-training interventions. Although some variability exists regarding the magnitude of the cross-education effect between the upper and lower limbs, critically, there is
a lack of correlation between the reported cross-education effect and the changes in the nervous system. Overall, it seems that the neural adaptations to cross-education of muscular strength most likely represent a continuum of change within the central nervous system that involves both structural and functional changes within cortical motor and non-motor regions. Such changes are likely to be the result of more subtle changes along the entire neuroaxis which include increased corticospinal excitability, reduced cortical inhibition, reduced IHI, changes in VA\textsubscript{TMS} and new regions of cortical activation (see Fig. 1).

Insert Figure 1.

Notwithstanding, there is a need to widen the breadth of research that collectively employs several neurophysiological techniques to better understand the potential mechanisms mediating cross-education. This fundamental step is required in order to better prescribe targeted and effective guidelines for the clinical practice of cross-education. There is a need to determine whether similar cortical responses also occur in clinical populations where, perhaps, the benefits of cross-education could be of most benefit.
References:


<table>
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<th>Citation</th>
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Figure 1. Potential sites of neural adaptation to cross education include (1) changes in (A) supplementary motor area, (B) primary motor cortex, (C) middle temporal gyrus, (D) inferior temporal gyrus, (E) occipital lobe, (F) cerebellum, (2) changes in interhemispheric inhibition, (3) changes in TMS measures confined to the ipsilateral “untrained” primary motor cortex (SICI), (4) changes along the corticospinal tract ipsilateral to the trained limb (excitability and inhibition), (5) changes in motoneurone excitability, and (6) changes in $V_{A_{TMS}}$, raw EMG response (MEP) produced by cortical stimulation during maximal contraction, as well as the superimposed twitch produced by cortical stimulation during maximal contraction, (right).
Table 1: Summary of studies examining the effect of unilateral motor-training on structural connectivity and patterns of brain activation.

<table>
<thead>
<tr>
<th>Study</th>
<th>Subject Details</th>
<th>Muscle Group</th>
<th>Methods</th>
<th>Training Details</th>
<th>Training Intensity</th>
<th>Strength Gain</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ruddy et al. 2017</td>
<td>44 subjects (20 control, 24 experimental)</td>
<td>Wrist flexors</td>
<td>rs-fMRI and DWI</td>
<td>One session</td>
<td>300 ‘fast as possible’ wrist flexion</td>
<td>↑ 83% peak acceleration</td>
<td>↑ rs-fMRI of right/left SMA; DWI was associated with increased transfer</td>
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<tr>
<td>Farthing et al. 2007</td>
<td>23 subjects (12 physical training, 11 imagery)</td>
<td>Flexor Carpi Ulnaris</td>
<td>fMRI patterns of activation, maximal isometric muscle activation (EMG)</td>
<td>6 wks of unilateral isometric training</td>
<td>Isometric MVC 6 × 8 reps</td>
<td>↑ 47.1% MVC</td>
<td>↑ muscle activation ↑ activation M1 and SMA, left ventral M1 and SMA, left anterior and posterior middle temporal gyrus, left inferior temporal gyrus, medial occipital cortex and posterior medial and lateral cerebellum</td>
</tr>
</tbody>
</table>

DWI: diffusion weighted imaging; EMG: electromyography; M1: motor cortex; MVC: maximum voluntary contraction; rs-fMRI: resting-state functional magnetic resonance imaging; SMA: supplementary motor area; wks: weeks
Table 2: Summary of studies examining the effect of cross-education training on cortical excitability and inhibition.

<table>
<thead>
<tr>
<th>Study</th>
<th>Participant Details</th>
<th>Muscle Group</th>
<th>Training Details</th>
<th>TMS Method</th>
<th>TMS Outcome for untrained limb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hortobágyi et al. 2011</td>
<td>20 participants (12 trained, 8 control)</td>
<td>First dorsal interosseous</td>
<td>5 × 10 MVCs performed at 80%, 3/wk × 8 wks</td>
<td>MEPs, SICI, ICF &amp; IHI during 20 and 80% MVC</td>
<td>↑ MEPs at 20 and 80% MVC, ↓ IHI</td>
</tr>
<tr>
<td>Kidgell et al. 2011</td>
<td>26 participants (13 trained, 13 control)</td>
<td>Biceps brachii</td>
<td>4 × 6-8 bicep curls at 80% 1-RM, 3/wk × 4 wks</td>
<td>MEPs and CSP during 10%rmsEMG</td>
<td>↑ MEPs during, ↔ CSP</td>
</tr>
<tr>
<td>Latella et al. 2012</td>
<td>18 participants (9 trained, 9 control)</td>
<td>Rectus Femoris</td>
<td>3 × 8 leg presses at 78%-88.5% 1-RM, 3/wk × 8 wks</td>
<td>MEPs and CSP during 10%rmsEMG</td>
<td>↔ MEPs during, 10%rmsEMG, ↓ CSP</td>
</tr>
<tr>
<td>Goodwill et al. 2012</td>
<td>14 participants (7 trained, 7 control)</td>
<td>Rectus Femoris</td>
<td>4 × 8 single-leg squats at 80% 1-RM, 3/wk × 4 wks</td>
<td>MEPS and SICI during 10% MVC</td>
<td>↑ MEPmax, ↓ SICI</td>
</tr>
<tr>
<td>Kidgell et al. 2015 (concentric)</td>
<td>18 participants (9 trained, 9 control)</td>
<td>Wrist flexors</td>
<td>4 × 8 maximal concentric wrist flexion performed at 20°/s</td>
<td>MEPS, SICI and CSP during 5, 20 &amp; 40% MVC</td>
<td>↔ MEPs at 5, 20 &amp; 40% MVC, ↔SICI at 5, 20 &amp; 40% MVC, ↔CSP at 5, 20 &amp; 40% MVC</td>
</tr>
<tr>
<td>Kidgell et al. 2015 (eccentric)</td>
<td>18 participants (9 trained, 9 control)</td>
<td>Wrist flexors</td>
<td>4 × 8 maximal eccentric wrist flexion performed at 20°/s</td>
<td>MEPS, SICI and CSP during 5, 20 &amp; 40% MVC</td>
<td>↑MEPs at 40% MVC, ↔SICI at 40% MVC, ↓CSP at 5 &amp; 20% MVC</td>
</tr>
<tr>
<td>Coombs et al. 2016 (right-hand)</td>
<td>15 participants (8 trained, 7 control)</td>
<td>Wrist extensors</td>
<td>4 × 6-8 wrist extensions at 70% 1-RM for the right arm only</td>
<td>MEPS, SICI and CSP during 5%rmsEMG</td>
<td>↔MEPs, ↔SICI, ↓CSP at MEPmax</td>
</tr>
<tr>
<td>Coombs et al. 2016 (left arm)</td>
<td>15 participants (8 trained, 7 control)</td>
<td>Wrist extensors</td>
<td>4 × 6-8 wrist extensions at 70% 1-RM for the left arm only</td>
<td>MEPS, SICI and CSP during 5%rmsEMG</td>
<td>↔MEPs, ↔SICI, ↔CSP</td>
</tr>
<tr>
<td>Manca et al. 2016a</td>
<td>24 subjects (17 trained, 17 control)</td>
<td>First dorsal interosseous</td>
<td>5 × 10 isometric pinch contractions, 3/wk × 4 wks</td>
<td>MEPS at rest and during 10% MVC, SICI, ICF, SICF, SIHI and LIHI</td>
<td>↔MEPs, ↔SICF, ↔SICI, ↔ICF, ↔LIHI and ↔LIH</td>
</tr>
<tr>
<td>Mason et al. 2017b</td>
<td>20 participants (10 trained, 10 control)</td>
<td>Biceps brachii</td>
<td>4 × 6-8 bicep curls at 80% 1-RM, 3/wk × 3 wks</td>
<td>MEPs and CSP during 5%rmsEMG</td>
<td>↑MEPs and ↓CSP</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Study</th>
<th>Participant Details</th>
<th>Muscle Group</th>
<th>Training Details</th>
<th>Twitch Method</th>
<th>Twitch Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lee et al. 2009b</td>
<td>20 participants (10 trained, 10 control)</td>
<td>Wrist extensors</td>
<td>4 × 10 brief (1-2 s) isometric wrist extension, 3/wk × 4 wks</td>
<td>TMS cortical voluntary activation</td>
<td>↓ superimposed twitch evoked during extension MVC</td>
</tr>
<tr>
<td>Shima et al. 2002</td>
<td>15 participants (9 trained, 6 control)</td>
<td>Gastrocnemius</td>
<td>3 × 10-12 reps at 70-75% 1-RM, 4/wk × 6 wks</td>
<td>Interpolated twitch technique</td>
<td>↔ voluntary activation</td>
</tr>
<tr>
<td>Tøien et al. 2017</td>
<td>23 participants (11 trained, 12 control)</td>
<td>Gastrocnemius</td>
<td>4 × 4 reps at 90% 1-RM, 3/wk × 3 wks</td>
<td>Interpolated twitch technique</td>
<td>↑ voluntary activation</td>
</tr>
<tr>
<td>Fimland et al. 2009</td>
<td>26 participants (15 trained, 11 control)</td>
<td>Gastrocnemius</td>
<td>7 × 1 min isometric contractions at 30% MVC, increasing to 15 sets of 1 min, 7/wk × 8 wks</td>
<td>Maximal twitch tension</td>
<td>↔ twitch force</td>
</tr>
</tbody>
</table>

1-RM: one-repetition maximum; MVC: maximum voluntary contraction; TMS: transcranial magnetic stimulation; wk: week; wks: weeks