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Motor learning and cross-limb transfer rely upon distinct neural adaptation processes

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Running head: Motor learning and cross-limb transfer

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Abstract

Performance benefits conferred in the untrained limb after unilateral motor practice are termed cross-limb transfer. Although the effect is robust, the neural mechanisms remain incompletely understood. Here we use non-invasive brain stimulation to reveal that the neural adaptations that mediate motor learning in the trained limb are distinct from those that underlie cross-limb transfer to the opposite limb. Thirty-six participants practiced a ballistic motor task with their right index finger (150 trials), followed by intermittent-theta burst stimulation (iTBS) applied to the trained (contralateral) primary motor cortex (cM1 group), the untrained (ipsilateral) M1 (iM1 group), or the vertex (sham group). Following stimulation, another 150 training trials were undertaken. Motor performance and corticospinal excitability were assessed before motor training, pre- and post-iTBS, and following the second training bout. For all groups, training significantly increased performance and excitability of the trained hand, and performance, but not excitability, of the untrained hand, indicating transfer at the level of task performance. The typical faciltatory effect of iTBS on MEPs was reversed for cM1, suggesting homeostatic metaplasticity, and prior performance gains in the trained hand were *degraded*, suggesting that iTBS interfered with learning. In stark contrast, iM1 iTBS *facilitated* both performance and excitability for the untrained hand. Importantly, the effects of cM1 and iM1 iTBS on behaviour were exclusive to the hand contralateral to stimulation, suggesting that adaptations within the untrained M1 contribute to cross-limb transfer. However, the neural processes that mediate learning in the trained hemisphere versus transfer in the untrained hemisphere appear distinct.

Keywords: ballistic motor learning, inter-limb transfer, non-invasive brain stimulation, corticospinal excitability, motor performance

New & Noteworthy

In the present study we observed that non-invasive brain stimulation interacted differently with motor practice when applied to the motor cortex projecting to the trained versus the untrained limb. This suggests that distinct neural processes underlie learning obtained via direct motor practice, and learning conferred indirecty from practice with the opposite limb (i.e. cross-limb transfer). The results provide a step forward in using non-invasive brain stimulation methods to promote cross-limb transfer in motor rehabilitation.

Introduction

Generalization of learned actions is critical for flexible and adaptive human behavior; it is clearly advantageous to be able to apply motor skill obtained in one context to alternative spatial locations, movement directions and effectors. Cross-limb transfer describes the behavioral benefit conferred in the untrained limb (i.e., inter-limb generalization) following unilateral motor practice. Although this effect has been studied for over a century (see Carroll et al. 2006; Farthing et al. 2009; Ruddy and Carson 2013 for overviews) the neural mechanisms mediating performance gains in the untrained limb remain incompletely understood.

While adaptations at the spinal level cannot be excluded, the available evidence suggests that adaptations within cortical networks that project to the untrained limb are likely to be primarily responsible for the phenomenon of cross-limb transfer (see Ruddy and Carson 2013 for an overview). The data are consistent with Parlow and Kinsbourne's (1989) cross-activation hypothesis, which suggests that during motor learning, task-relevant information is simultaneously stored in both the trained and untrained hemispheres (also Cramer et al. 1999; Dettmers et al. 1995). Transcranial magnetic stimulation (TMS) studies also show that activation of one limb results in contraction intensity dependent excitability changes of the pathways projecting to the opposite limb (e.g., Hess et al. 1986; Liepert et al. 2001); the stronger the contraction of one limb, the greater the change in excitability observed in the projections to the opposite limb (Perez and Cohen 2008).

Motor learning paradigms utilizing simple ballistic movements, in which participants aim to maximize the rate of force development or acceleration of the upper limb or hand (e.g. Classen et al. 1998), represent an ideal model to study the mechanisms of adaptation and transfer. Using a "virtual lesion" TMS approach in this paradigm, Lee et al. (2010)

showed that adaptations within each hemisphere specifically mediate performance improvements of the contralateral limb, irrespective of whether the performance gains are due to direct practice or transfer. However, it remains unknown whether the synaptic mechanisms of adaptation are similar in the two hemispheres.

Here we used a non-invasive brain stimulation (NBS) protocol that induces effects that resemble long-term potentiation (LTP) in the resting brain (intermittent theta-burst stimulation, iTBS; Huang et al. 2005), to study the synaptic mechanisms that underlie performance improvements in the trained and untrained limbs. Specifically, following unilateral ballistic motor learning, we administered iTBS to the trained (contralateral) or untrained (ipsilateral) primary motor cortex. When applied following motor training, the 'expected' effects of NBS protocols that induce LTP-like effects at baseline can be occluded or reversed (Rosenkranz et al. 2007; Stefan et al. 2006; Ziemann et al. 2004) according to principles of homeostatic plasticity (i.e., Müller-Dahlhaus and Ziemann 2015 for a review), which provides evidence that learning is driven by LTP-like plastic changes. Here, we tested whether training-induced performance gains in the trained (direct learning) and untrained hands (cross-limb transfer) are driven by similar, LTP-like, neural adaptations in the trained and untrained motor cortices, respectively. If the synaptic mechanisms of learning and transfer are similar in each hemisphere, then the LTP-like effects of iTBS should be reduced or reversed in both the trained and untrained motor cortices (see Figure 1A). If however, transfer represents a distinct neural process to learning, then iTBS applied to the untrained hemisphere following training would be predicted to induce similar effects as when applied in isolation (Figure 1B).

"Figure 1 about here"

Because it is of practical interest, for potential therapeutic applications, to understand the impact of plasticity-inducing NBS on the capacity for subsequent performance improvements via transfer, we also assessed performance changes due a second block of unimanual training performed after iTBS. Prior induction of LTP-like plasticity can enhance subsequent *learning* for the contralateral limb via non-homeostatic processes (Teo et al. 2011), but the effects of synaptic plasticity induction upon subsequent *transfer* have not been reported. If similar mechanisms apply to cross-limb transfer, we should see the effects of iTBS to the untrained M1 reflected in subsequent performance gains according to non-homeostatic processes (i.e. LTP-like effects should result in enhanced subsequent performance gains, whereas LTD-like effects should impair subsequent performance gains).

Methods

Participants

Thirty-six healthy, right-handed young adults (Oldfield, 1971) were randomly assigned to either a cM1 (n = 12, 5 males, average age = 26.2 years, SD = 5.6), iM1 (n = 12, 6 males, average age = 24.4 years, SD = 5.9), or a sham group (n = 12, 5 males, average age = 24.4 years, SD = 5.0) where cM1, iM1 and sham refer to the nature of the applied stimulus following unilateral practice (see Task and procedure). All participants gave written informed consent, and completed a medical history questionnaire which confirmed the absence of any known neurological and neuromuscular dysfunction and any contraindications to TMS. All procedures were approved by the Tasmanian Human Research Ethics Committee Network.

Task and procedure

The experiment was designed to use non-invasive brain stimulation to interact with training-induced plasticity in the trained and untrained hemisphere following unilateral motor training. We aimed to determine whether training-induced performance gains in the trained (direct learning) and untrained hands (cross-limb transfer) are driven by similar, LTP-like, neural adaptations in trained and untrained motor cortices, respectively. Figure 2 outlines the experimental procedure. Following Hinder et al. (2011, 2013) and Lee et al. (2010), participants practiced a ballistic abduction of the right index finger (audio-paced at 0.5 Hz) where the performance goal was to maximise peak horizontal (abduction) acceleration of each movement. This type of motor learning paradigm represents an ideal model to study the mechanisms of adaptation and transfer for many reasons. Substantial performance gains are exhibited within a single session, which simplifies the use of brain stimulation methods such as transcranial magnetic stimulation (TMS) to assess the neural underpinnings of adaptation (Carroll et al. 2008). Moreover, the neural responses to ballistic motor training are similar to those observed after strength training (Selvanayagam et al. 2011). Accordingly the model provides a window into the mechanisms underlying an important physical attribute that often limits function in old age and in patients with neurological disorders. Triaxial accelerometers (Dytran Instruments, Chatsworth, CA; Endevco, San Juan Capistrano, CA) were mounted to plastic splints and taped to the top of the left and right index fingers such that one of the orthogonal axes of each accelerometer was aligned to measure horizontal acceleration. A custom written Signal (CED) script (see Hinder et al. 2011, 2013) allowed us to detect the first peak of the acceleration trace and provide this information to participants as visual performance feedback according to the feedback design (see below).

156 *"Figure 2 about here"*

157

158 Participants undertook a total of 300 practice trials within two training blocks, each
159 consisting of 150 movements (cf. Hinder et al. 2011, 2013). 30s rest breaks were provided
160 every 15 movements (i.e., ten 15-movement sub-blocks per block) to avoid fatigue. Visual
161 feedback of the movement outcome was provided on 50% of the movements (i.e., odd-
162 numbered sub-blocks) to assist in promoting performance gain (Winstein and Schmidt,
163 1990).

164 In order to specifically interact with the neural adaptations mediating performance
165 gains in the trained hand (i.e., direct motor learning gains) and the untrained hand (i.e.,
166 cross-limb transfer), we applied intermittent theta burst stimulation (iTBS) to the trained or
167 untrained M1, or to the vertex as a 'sham' condition, after the first training block. iTBS has
168 been shown to increase motor evoked potential (MEP) amplitude in a manner consistent
169 with LTP-like plasticity (Huang et al. 2005). Consistent with principles of homeostatic
170 plasticity (Müller-Dahlhaus and Ziemann 2015, as well as Karabanov et al. 2015 for an
171 overview), we postulated that the LTP-like effects of iTBS on MEPs would be reduced or
172 reversed in both the trained or untrained motor cortices, if both learning and transfer are
173 driven by LTP-like plastic changes. If untrained hand performance gains following unilateral
174 ballistic practice (cross-limb transfer) are not driven by LTP-like plastic changes in the
175 untrained motor cortex, iTBS should be able to act in the 'expected' direction (Huang et al.
176 2005) and facilitate MEPs within the cortical network that projects to the untrained limb.
177 iTBS (600 pulses, 190s stimulation; cf. Huang et al. 2005) was administered (Magstim Super
178 Rapid² stimulator and 70mm figure-of-eight-coil) at an intensity of 80% of active motor
179 threshold (AMT) over the motor hotspot (coil handle 45° to the midline) of the trained first

dorsal interosseus (FDI) muscle (cM1 group), the untrained FDI muscle (iM1 group), or over the vertex (handle backwards) with the coil tilted by 90° (coil surface orthogonal to the scalp surface) with one side of the coil remaining contact with the head (sham group; Mistry et al. 2012). The active motor threshold was defined as the minimum stimulator intensity required to evoke MEPs of ≥ 200 μV (in three out of five trials) (Huang et al. 2005) during a light isometric contraction of the corresponding FDI muscle at about 10 % of maximum force.

Motor performance (i.e., peak acceleration in 10 test movements per hand) and neurophysiological measures (i.e., cortical excitability and intracortical inhibition as assessed with TMS) were obtained for both hands/ motor cortices before motor training commenced (pre-test), after the first motor training block but before iTBS administration (pre-iTBS), immediately following iTBS (post-iTBS), and following completion of the second training block (post-test). TMS testing always preceded motor performance testing at each of the time points such that changes in neurophysiological measures could be attributed to the unilateral training block rather than the test phases conducted with both hands; the hand-order during motor performance and TMS testing was counterbalanced across participants within each group.

Recording of muscle activity

In order to quantify muscle activity (during the execution of the motor task and in response to suprathreshold pulses of TMS) we recorded EMG activity with Ag/AgCl electrodes (Meditrace 130, Tyco Healthcare, Mansfield, MA) from the FDI in both hands in a belly-tendon montage (as per Hinder et al. 2011, 2013). EMG signals were fed into a CED 1401 amplifier (Cambridge, UK), where a notch filter (50 Hz) was applied before amplification (gain 300–1,000), and stored for off-line analysis. Participants' EMG activity

was constantly monitored by the experimenter to guarantee strong movement-related FDI bursts in the activated hand and a relaxation of the muscle between trials.

Transcranial magnetic stimulation

Transcranial magnetic stimulation (TMS) was delivered using two Magstim 200^2 units (Magstim Company, Dyfed, UK) connected via a Bistim^2 unit and a single figure-of-eight coil (70 mm external diameter). Motor 'hotspots' for the left and the right FDI (with posterior- to anterior-induced current in the cortex) were determined and resting motor thresholds (RMT) were established as the minimum intensities required to elicit MEPs > 50 μV in the right and left FDI muscles in three out of five consecutive trials when stimulating at the hotspots (Carroll et al. 2001; Hinder et al. 2010). Participants were instructed to relax their limbs during RMT determination and visual feedback of muscle activity helped to keep muscle activity to a minimum.

During TMS test blocks, 30 stimulations (with an interstimulus interval of 4-6 s) were administered to the right (untrained) or left (trained) motor hotspots, respectively. Half of the stimulations involved a single 'test' pulse (130 % RMT) to assess the net excitability of the corticospinal projections to the trained/untrained hand, while half of the trials involved paired-pulse stimulation (Kujirai et al. 1993) in which a subthreshold conditioning pulse (70 % RMT) preceded the same test pulse. The ratio of the average MEP evoked following paired-pulse trials (within one TMS test block) to the average MEP amplitude evoked in the single-pulse trials (within the same TMS test block) is referred to as the short-interval intracortical inhibition ratio, SICI (Kujirai et al. 1993), and reflects activity of intracortical inhibitory circuits. The order of single- and paired-pulse stimulations was randomised within each TMS block.

Control Experiment

Because the results of the main experiment showed that both performance and MEPs increased in the untrained hand after iTBS to the untrained M1, it was important to determine whether the performance gain reflected a general improvement in motor function due to enhanced excitability, or was due to an interaction with the recently transferred (improved) motor skill. We therefore conducted a control experiment, for which another cohort of healthy, right-handed young adults (n = 12, 3 males, average age = 25.9 years, SD = 7.3) was recruited. Here we examined the effects of iTBS delivered to the right M1 (N.B. to correspond with the untrained hemisphere in the main experiment) without prior motor training. MEP amplitudes and motor performance were assessed for right and left hands before and after iTBS, in the absence of a preceding training block. Thus, the results of the control experiment allowed us to isolate the effects of iTBS, applied over the right M1, on MEP amplitudes and motor performance without being influenced by prior motor training.

Data analysis

Acceleration data were low-pass filtered at 20 Hz prior to analysis. As per Hinder et al. (2011, 2013) peak acceleration of the ballistic abduction was determined as the first peak in the horizontal acceleration for each movement trial (referred to as ACC). Performance of right and left hand movements at each test phase was calculated as the average peak acceleration across the 10 trials in each test for the respective hand. Performance at pre-iTBS and post-iTBS was subsequently normalized to pre-test values (referred to as nACC [nACC > 1 indicating increased performance and nACC < 1 decreased performance relative to

performance at pre-test]) to explore the effect of iTBS on prior motor training-induced changes in the trained and untrained hands. Performance data at post-test were normalized to values obtained at post-iTBS to examine the influence of iTBS on changes in performance in both hands following a second block of motor training. Performance of right hand movements during training was expressed as the average peak acceleration across the 15 trials in each sub-block. Average performance of the trained, right hand in the penultimate, ninth sub-block was then normalized to the average performance obtained during the first sub-block of training for the right hand as a measure of training-related changes in trained hand performance (referred to as $nACC_{training}$). The penultimate block was chosen such that we compared sub-blocks in which visual feedback was consistent (i.e., visual feedback of performance was provided in both the first and ninth sub-block, but not the tenth sub-block).

Responses to TMS were sampled at 10 kHz from 3 s before to 2 s after the test pulse. Trials in which background root mean square EMG exceeded 25 μV in a 40 ms time window immediately prior to TMS stimulation were excluded from further analysis. The peak-to-peak amplitudes of the motor evoked response (MEP) were measured in a window 15–50 ms after stimulation in the limb contralateral to the stimulated cortex. For both neurophysiological measures (MEP, SICI), data at pre-iTBS and post-iTBS were normalized to those values obtained at pre-test (referred to as nMEP [nMEP > 1 indicates a facilitatory change while nMEP < 1 indicates suppression of evoked responses, relative to pre-test responses] and nSICI [nSICI > 1 indicates a release of inhibition and nSICI < 1 indicates increased inhibition relative to pre-test]) to explore the effect of iTBS on prior motor training-induced changes in excitability and inhibition in both motor cortices. Post-test

values were normalized to those values obtained at post-iTBS to examine the influence of iTBS on changes in excitability/inhibition following a second block of motor training.

As potential predictors of cross-limb transfer, we quantified the training-induced change in FDI activity of the trained (right) and untrained (left) hands, as well as a measure of relative mirror muscle activity of the left hand during right hand movements. As per Hinder et al. (2011, 2013) individual EMG signals of trained and untrained FDIs assessed during task execution were processed to only represent movement-related muscle activity during the ballistic action. That is, movement-related EMG data during training trials was rectified and low-pass filtered (20 Hz) before determining the peak EMG amplitude in the active FDI (i.e., of the hand performing the ballistic abduction). Movement onset was defined as the time when FDI activity in the active hand first exceeded 4 times background EMG determined 50–100 ms before the 'go' tone. Movement offset was defined as the time when FDI activity in the hand performing the task first dropped below 0.2 times the peak amplitude (Carroll et al. 2008; Hinder et al. 2011). In this time window, the average burst-related EMG of the FDI in the hand performing the task was calculated minus the average value of background EMG. During the same time window the average FDI EMG in the contralateral hand (i.e., mirror activity) was determined. EMG values were then averaged over the 15 trials of each sub-block of the training. The average values for both the trained and untrained FDI of the penultimate, ninth sub-block were normalized to the average EMG values obtained during the first sub-block of training for the respective hand as a measure of training-related changes in FDI activity in the trained and untrained hands (referred to as nEMG [nEMG > 1 indicating increased FDI activity and nEMG < 1 decreased FDI activity relative to the first sub-block). Additionally, FDI activity of the untrained hand averaged across the 150 training trials was normalized to FDI activity of the trained hand averaged

across these 150 trials as a measure of relative mirror muscle activity during training (EMG_{mirror}).

Statistical analysis

To ascertain that 1) pre-test values (relating to both behaviour and cortical excitability/inhibition) and training-induced changes from pre-test to pre-iTBS in these parameters were similar across groups and 2) to ensure significant learning and transfer effects following the first motor training block were apparent, we separately submitted raw (non-normalized) peak acceleration, MEP and SICI values to time (pre-test, pre-iTBS) x hand (trained, untrained) x group (cM1, iM1, sham) ANOVAs. Subsequently, normalized (relative to pre-test) performance (nACC) and TMS measures (nMEP, nSICI) were subjected to time (pre-iTBS, post-iTBS) x hand (trained, untrained) x group (cM1, iM1, sham) ANOVAs (for each dependent variable separately) to examine the effect of iTBS on prior motor learning gains of the trained and untrained hands, and associated changes in excitability/inhibition of the corresponding motor cortices. Additionally, separate hand (trained, untrained) x group (cM1, iM1, sham) ANOVAs were performed on the (normalized) post-test values for performance and TMS measures to examine the impact of iTBS on subsequent motor training gains (i.e., gains in block 2 normalized to post-iTBS values). Significant main or interaction effects were further explored using post hoc pairwise comparisons (using the Sidak adjustment). Main inferential analyses (ANOVA) were complemented by correlation statistics (with Benjamini-Hochberg procedure applied to correct for multiple comparisons) where appropriate (e.g., to explore the nature of the interaction between use-dependent and iTBS-induced changes in performance and corticospinal excitability).

To benefit from cross-limb transfer effects (e.g., in rehabilitation settings) it is critical to know which factors (e.g., motor learning itself, mirror muscle activity, corticospinal excitability) predict and mediate performance gains in an untrained hand. It is also important to know whether performance gains in an untrained limb after unilateral practice are driven by adaptations in the untrained hand/ motor cortex during training (i.e., via cross activation) or in the trained hand/ motor cortex upon retrieval (i.e., via callosal access). To this end, a multiple regression analysis was employed to identify the main predictors of cross-limb transfer (i.e., normalized performance gains of the untrained hand relative to pre-test performance of that hand) following an initial unilateral practice period (i.e., at pre-iTBS), and to study their relative predictive strength (when controlling for other predictor variables). Two regression models were tested. The first one of these models included three variables derived from the trained (active hand). These were: the normalized performance change of the trained hand from the first to the penultimate, ninth training sub-block ($nACC_{training}$), the change in burst-related FDI activity of the active (trained) hand from the first to the ninth training block ($nEMG_{trained}$), and the training-induced change in corticospinal excitability of the trained M1 from pre-test to pre-iTBS ($nMEP_{trained}$). The second model was complemented by the inclusion of three additional variables related to the untrained hand. Specifically, we considered the change in FDI activity of the untrained hand (as defined above, $nEMG_{untrained}$), relative mirror muscle activity during training (EMG_{mirror}) and the training-induced change in untrained M1 excitablity (see above, $nMEP_{untrained}$).

All data are reported as normalized values: to assess the effects of the first training block and subsequent iTBS, behavioural and neurophysiological parameters are expressed relative to the correspsonding pre-test value; to assess the affect of the second training block, post-training values are expressed relative to post-iTBS values. Corresponding 95 %

confidence intervals (CI) provide a measure of variability, while partial eta-squared (η_p^2) and Cohen's *d* are reported as measures of effect size.

Results

Motor performance

Training-induced effects on motor performance

Average peak accelerations at pre-test were 20.5 ± 9.4 ms^{-2}, 20.9 ± 4.6 ms^{-2} and 14.8 ± 2.4 ms^{-2} for the right hand and were 18.9 ± 5.9 ms^{-2}, 20.1 ± 3.7 ms^{-2} and 14.4 ± 2.7 ms^{-2} for the left hand for the cM1, iM1 and sham groups, respectively. Upon completion of the first training block (i.e., at pre-iTBS), peak acceleration of the index finger had increased to 36.5 ± 10.0 ms^{-2}, 40.1 ± 6.7 ms^{-2} and 28.5 ± 8.4 ms^{-2} in the trained hand, and to 27.8 ± 7.6 ms^{-2}, 31.3 ± 7.1 ms^{-2} and 21.7 ± 4.3 ms^{-2} in the untrained hand in the cM1, iM1 and sham groups, respectively. ANOVA revealed a significant time x hand interaction, $F(1,33) = 25.62$, $p < .001$, $\eta_p^2 = .44$, with posthoc pairwise comparisons revealing a significant increase in performance from pre-test (trained: 18.8 ± 3.7 ms^{-2}, untrained: 17.8 ± 2.6 ms^{-2}) to pre-iTBS (trained: 35.0 ± 5.2 ms^{-2}, untrained: 26.9 ± 4.0 ms^{-2}) for both, trained and untrained, hands (both $p < .001$, $d > .89$) averaged across all three groups. Peak acceleration did not differ significantly between hands at pre-test ($p = .39$, $d = .09$; pairwise Sidak adjusted post-hoc tests confirmed that baseline performance was not significantly different between any of the groups, all $p > 0.27$), but was greater for the trained as compared to the untrained hand after motor training at pre-iTBS ($p < .001$, $d = .58$). ANOVA revealed no significant main or interaction effects including the factor group.

iTBS-induced effects on motor performance

To assess the impact of the different iTBS protocols on trained and untrained hand performance, we compared normalized peak acceleration values (relative to pre-test) of cM1, iM1 and sham group participants before and after the application of iTBS. As seen in Figure 3, iTBS resulted in a *reduction* of normalized performance (relative to pre-test) of the trained hand from 2.14 (± 0.47) to 1.77 (± 0.47) for the cM1 group, whereas normalized performance of the trained hand in the iM1 group (pre-iTBS: 2.01 ± 0.25; post-iTBS: 1.88 ± 0.40) and sham group (pre-iTBS: 2.02 ± 0.53; post-iTBS: 2.05 ± 0.62) was much less affected by iTBS. In contrast, iTBS *increased* normalized performance in the untrained hand from 1.62 (± 0.26) at pre-iTBS to 2.03 (± 0.54) at post-iTBS in the iM1 group, whereas normalized performance of the untrained hand appeared to be unaffected by iTBS in the cM1 (pre-iTBS: 1.57 ± 0.21; post-iTBS: 1.49 ± 0.25) and sham groups (pre-iTBS: 1.51 ± 0.22; post-iTBS: 1.66 ± 0.37). ANOVA conducted on normalized performance revealed a significant time x hand x group interaction, $F(2,33) = 3.75$, $p = .03$, $\eta_p^2 = .19$, confirming the changes described above; i.e., performance changes in response to iTBS were hand- and iTBS location (group)-specific. Indeed, posthoc pairwise comparisons revealed that the performance *decrease* of the trained hand in the cM1 group ($p = .004$, $d = .43$) and the performance *increase* of the untrained hand in the iM1 group ($p = .002$, $d = .52$) following iTBS were statistically significant; all other pairwise comparisons were not statistically significant (all $p > .23$, $d < .26$).

Correlation analyses revealed significant (positive) relationships between trained and untrained hand performance gains following motor training at pre-iTBS ($r = .68$, $p < .001$) across all participants. That is, the greater trained hand performance improvements, the greater improvements in the untrained hand. Moreover, for the iM1 group, analyses revealed that the greater the increase in untrained hand performance following the first

training block, the greater the subsequent iTBS-induced improvements in that hand. That is, we observed a positive relationship between the extent of iTBS-induced change in performance in the untrained hand and the extent of the previous performance gains in the untrained hand (i.e., as a result of cross-limb transfer) following the first training block (r = 0.72, p = .004). In contrast, for the trained hand of the cM1 group, there was a negative correlation between iTBS-induced performance changes and the previous use-dependent performance gains as a result of the first training block (r = -0.55, p = .03). This illustrates that the greater the use-dependent performance increase in the trained hand following the first training block, the more performance of that hand is reduced following the application of iTBS.

"Figure 3 about here"

iTBS-induced effects on subsequent motor training

To test for the influence of iTBS on subsequent learning and transfer, post-test data following the second training block were analysed relative to post-iTBS values. Upon completion of the second training block, the normalized performance of participants in the cM1, iM1 and sham groups increased (relative to post-iTBS) to 1.64 (± 0.26), 1.34 (± 0.24) and 1.18 (± 0.13) in the trained hand, and to 1.29 (± 0.21), 1.15 (± 0.25) and 1.11 (± 0.06) in the untrained hand, respectively. ANOVA revealed the greater performance gains of the cM1 group as compared to iM1 and sham groups to be significant averaged across hands (main effect for group: $F(2,33) = 4.22$, p = .023, η_p^2 = .20). Interestingly, across all participants, and for both the trained and untrained hands, there were negative correlations between the performance gains as a result of the second training block, and the extent of previous iTBS-

induced change in performance (trained hand: r = -0.57, p < .001; untrained hand: r = -0.49, p = .001). That is, the greater the iTBS-induced performance decrement in the trained hand, the greater the subsequent learning in that hand in the second block. For the untrained hand, the greater the iTBS-induced gain, the lower the subsequent performance gain in that hand resulting from the second training block.

Neurophysiological measures

Corticospinal excitability

Training-induced effects on corticospinal excitability

RMTs (as a % of maximum stimulator output, ± 95% CI) for the right hand were 42.5 ± 4.2%, 40.7% ± 3.5% and 43.1 ± 3.5%; and were 42.1 ± 4.1%, 40.8 ± 4.0% and 44.1 ± 2.4% for the left hand for cM1, iM1 and sham group participants, respectively. There were no significant differences between groups, $F(2,33) = 0.60$, $p = .56$, $\eta_p^2 = .04$, or hands, $F(1,33) = 0.11$, $p = .75$, $\eta_p^2 = .003$, and no interaction between hand and group, $F(1,33) = 0.30$, $p = .75$, $\eta_p^2 = .02$. AMTs were 48.0 ± 3.4%, 47.2 ± 2.5% and 49.4 ± 3.6% of maximum stimulator output for cM1, iM1 and sham group participants, respectively, and did not differ between groups, $F(2,33) = 0.45$, $p = .65$, $\eta_p^2 = .03$. (NB: AMT appears higher than RMT because it was determined on the less powerful Magstim Super Rapid² stimulator which was used to subsequently administer iTBS, whereas RMT and single/paired pulse TMS was administered using two Magtim 200² units connected with a BiStim module). Average MEP amplitudes at pre-test were 1.49 ± 0.51 mV, 1.34 ± 0.56 mV and 0.90 ± 0.17 mV for the right FDI and were 1.37 ± 0.63 mV, 1.47 ± 0.52 mV and 1.40 ± 0.32 mV for the left FDI for cM1, iM1 and sham group participants respectively. Upon completion of the first training block (i.e., at pre-iTBS), cM1, iM1 and sham group participants' excitability increased to 2.18 ± 0.70 mV, 1.94 ± 0.74

mV and 1.25 ± 0.35 mV in the trained hand, respectively. However, excitability of the untrained hand was relatively unaffected by training in all three groups (cM1: 1.41 ± 0.80 mV, iM1: 1.42 ± 0.61 mV, sham: 1.36 ± 0.33 mV). ANOVA revealed a significant main effect for time, $F(1,33) = 13.11$, $p = .001$, $\eta_p^2 = .28$; averaged across both hands excitability increased from pre-test (1.33 ± 0.28 mV) to pre-iTBS (1.59 ± 0.37 mV); however the significant time x hand interaction, $F(1,33) = 18.05$, $p < .001$, $\eta_p^2 = .35$ indicates that this effect was driven by changes in excitability in the trained hand. Indeed, posthoc pairwise comparisons revealed that (averaged across all groups) a significant increase in corticospinal excitability occurred from pre-test to pre-iTBS for the trained hand ($p < .001$, $d = .54$), but not for the untrained hand ($p = .87$, $d = .02$). There were no significant differences between the excitability of trained and untrained hands at pre-test ($p = .25$, $d = .19$); however, the trained hand exhibited greater excitability at pre-iTBS than the untrained hand ($p = .03$, $d = .35$). All other main or interaction effects were not significant (all $F < 2.28$, $p > .12$, $\eta_p^2 < .12$).

iTBS-induced effects on corticospinal excitability

To assess the impact of the different iTBS protocols on trained and untrained hand excitability, we compared the normalized excitability (relative to pre-test) of cM1, iM1 and sham groups before and after the application of iTBS. As shown in figure 4, iTBS *reduced* normalized excitability of circuits projecting to the trained hand from 1.50 (± 0.26) at pre-iTBS to 1.23 (± 0.24) at post-iTBS when delivered to the motor cortex contralateral to the trained hand (cM1 group), but had little effect when delivered to the ipsilateral motor cortex (iM1 group; pre-iTBS: 1.51 ± 0.30; post-iTBS: 1.41 ± 0.21) or the vertex (sham group; pre-iTBS: 1.40 ± 0.30; post-iTBS: 1.58 ± 0.43). In contrast, iTBS *increased* normalized excitability of the untrained hand from 0.98 (± 0.19) at pre-iTBS to 1.38 (± 0.39) at post-iTBS in the iM1

group, whereas normalized excitability of the untrained hand was less affected by iTBS delivered to the cM1 (pre-iTBS: 1.04 ± 0.24; post-iTBS: 1.14 ± 0.28) or to the vertex (sham group; pre-iTBS: 1.03 ± 0.18; post-iTBS: 1.13 ± 0.14). ANOVA conducted on nMEP values revealed a significant time x hand x group interaction, $F(2,33) = 4.31$, $p = .02$, $\eta_p^2 = .21$, indicating that the hand- and group-specific effects described above were statistically significant. Posthoc pairwise comparisons revealed the *decrease* in excitability in the cM1 groups' trained hand ($p = .04$, $d = .57$) and the *increase* in excitability in the iM1 groups' untrained hand ($p < .001$, $d = .69$) following iTBS to be significant, while no other pairwise comparisons reached significance (all $p > .17$, $d < .26$).

Correlation analyses revealed that trained and untrained hand excitability gains were not significantly related to each other following the first training block at pre-iTBS ($r = .12$, $p = .50$) or following iTBS at post-iTBS ($r = .29$, $p = .09$). For the iM1 group, there was a marginal positive correlation between the extent of iTBS-induced change in performance in the untrained hand, and the extent of iTBS-induced change in excitability (i.e., at post-iTBS) in the untrained motor cortex ($r = .52$, $p = .08$), but not for the trained motor cortex and hand ($r = -.29$, $p = .36$). Also there were no such associations between performance and excitability changes following iTBS for the cM1 group's trained ($r = .09$, $p = .78$) or untrained hand ($r = .33$, $p = .29$) at post-iTBS.

"Figure 4 about here"

iTBS-induced effects on subsequent motor training

The effect of the second training block was to increase trained hand excitability (relative to values observed at post-iTBS) in all groups. Specifically, normalized excitability of

the trained hand increased to 1.51 (± 0.48) in the cM1 group, to 1.10 (± 0.19) in the iM1 group and to 1.17 (± 0.22) in the sham group. Normalized excitability of the untrained hand (relative to post-iTBS) at post-test was 0.97 (± 0.18) in the cM1 group, 1.01 (± 0.17) in the iM1 group and 1.23 (± 0.28) in the sham group. Despite the apparent differences between groups and hands described qualitatively above, ANOVA conducted to assess the effect of the second training bout (i.e., post-test excitability normalized to post-iTBS excitability) revealed no significant differences between groups (p = .50). The main effect of hand, $F(1,33) = 3.45$, p = .07, η_p^2 = .10, and the interaction of hand and group were marginal, $F(2,33) = 3.05$, p = .06, η_p^2 = .16. Post-hoc tests showed that the marginal interaction was driven by the greater excitability gain in cM1 group's trained hand at post-test as compared to their untrained hand (p = .005).

Correlation analyses revealed a significant relationship between the extent of excitability increases in cM1 group's trained hand induced as a result of the second training period and the extent of the previous iTBS-induced change (reduction) in excitability (r = -0.74, p = .003). That is, the greater the reduction in excitability induced by iTBS, the greater the subsequent increase in excitability as a result of motor learning.

Intracortical inhibition

Average SICI ratios at pre-test were 0.56 ± 0.13, 0.71 ± 0.18 and 0.66 ± 0.17 for the right FDI and were 0.64 ± 0.19, 0.72 ± 0.17 and 0.72 ± 0.25 for the left FDI for cM1, iM1 and sham group participants respectively. There were no differences in SICI at pre-test between the groups (p > .45). ANOVA conducted on non-normalized SICI ratios of trained and untrained hands (before and after the first training block) revealed a marginal main effect of time, $F(1,33) = 3.17$, p = .08, η_p^2 = .09, indicating a small (and non-significant) increase in the

level of inhibition (averaged over all groups and both hands) as a result of the first training block (pre-test: 0.67 ± 0.11; pre-iTBS: 0.61 ± 0.09). All other main effects and interactions were not statistically significant (all $p > .17$).

Multiple Regression analysis to elucidate predictors of cross-limb transfer

Averaged across the three groups (n=36), performance of the untrained, left hand increased by 56.6% (± 13.3%) as a result of unilateral, right hand motor training from pre-test to pre-iTBS; this is equivalent to 61.2 ± 28.6% of the gains observed in the *trained* hand (i.e., untrained hand normalized performance gains relative to trained hand gains following the first block of motor training). To identify the main predictors of (normalized) untrained hand performance gains at pre-iTBS and to assess their relative predictive strength, we employed a multiple regression analysis. Initially, we entered predictor variables that were directly related to the excitability change and dynamics of the muscle bursts in *untrained* hand (i.e., $nMEP_{untrained}$, $nEMG_{untrained}$ and EMG_{mirror}, respectively). A second model also included predictor variables that were related to the *trained* hand performance, muscle activity and excitability changes (i.e., $nACC_{training}$, $nMEP_{trained,}$ $nEMG_{trained,}$) to additionally account for the impact of adaptations in the trained hand on adaptations in the untrained limb.

Untrained hand performance gains at pre-iTBS (i.e., cross-limb transfer) were significantly predicted by model 2 ($\Delta R^2 = 0.49$, $\Delta F(3,29) = 10.06$, $\Delta p < 0.001$), but not by model 1 (adjusted $R^2 = -0.06$, $F(3,32) = .38$, $p = 0.77$). Normalized performance gains of the trained hand during training ($nACC_{training}$, $\beta = 0.66$, $t(35) = 4.71$, $p < .001$) and training-induced excitability changes of the trained hand ($nMEP_{trained}$, $\beta = 0.37$, $t(35) = 2.86$, $p = .008$) explained 43.6 % and 13.7 % of the variance in untrained hand performance gains following

unilateral practice, respectively, when controlled for the other variables in the equation. The analysis also revealed a marginal (unique) contribution of the training-induced excitability changes in the untrained hand ($nMEP_{untrained}$, β = 0.25, $t(35) = 1.89$, $p = .07$) explaining at least 6.4 % of the variance in normalized untrained hand performance following motor practice at pre-iTBS. Partial regression plots for the variables that have been shown to explain significant (marginal) portions of variance in untrained hand performance gains at pre-iTBS are displayed in Figure 5.

"Figure 5 about here"

Control Experiment

The results of the main experiment showed that both performance and MEPs increased in the untrained hand after iTBS to the untrained M1, but it is unclear whether this performance gain reflected a general improvement in motor function due to enhanced excitability, or an interaction with the recently transferred motor skill. We therefore analyzed the iTBS-induced change in excitability and motor performance (normalized values relative to pre-test) at post-iTBS (i.e. following iTBS over right M1) in a control group that performed no prior motor training. Normalized excitability of circuits projecting to the right and left hands at post-iTBS was 0.95 (± 0.12) and 1.13 (± 0.18) respectively (see Figure 6A). Normalized motor performance following iTBS was 0.93 (± 0.11) for the right hand and was 0.86 (± 0.07) for the left hand (see Figure 6B). One-sample t-Tests (against pre-test level, i.e. 1) revealed the decrease in left hand performance to be significant, t(11) = -3.71, p = .003, but not the increase in left hand (right M1) excitability, t(11) = 1.407, p = .18. Thus, the expected LTP-like effect of iTBS was not statistically significant for the entire group due to

inter-subject variability (as has been reported previously, Hamada et al., 2013; Hinder et al., 2014). We therefore looked at the subset of six control participants who showed the largest MEP changes for the left hand, to be sure that an iTBS-induced increase in MEP amplitude does not change motor performance. Average normalized excitability of the sub-sample was 0.98 (± 0.13) and 1.37 (± 0.23) for circuits projecting to right and left hands respectively (see Figure 6C). Average normalized performance following iTBS for the subset of best 'responders' was 0.98 (± 0.16) for the right hand and was 0.88 (± 0.08) for the left hand (see Figure 6D). One-sample t-Tests revealed both the increase in left hand (right M1) excitability, $t(5) = 2.84$, $p = .04$ and the decrease in left hand performance to be significant, $t(5) = -2.85$, $p = .04$. Moreover, changes in MEP amplitude and motor performance following iTBS were not associated, neither across the entire group of 12 subjects (left hand: $r = .05$, $p = .89$; right hand: $r = -.38$, $p = .22$), nor in the subset of participants that exhibited the largest MEP changes in the left hand following iTBS (left hand: $r = .03$, $p = .96$; right hand: $r = -.11$, $p = .84$). Taken together, the data imply that there was no tendency towards increased motor performance simply as a result of increased excitability produced by iTBS in the absence of training.

Discussion

The present study used non-invasive brain stimulation to probe the neural mechanisms underpinning motor learning and cross-limb transfer. The major novel finding was that when applied following an initial period of motor learning, brain stimulation that induces LTP-like plasticity in the resting-state motor cortex (iTBS) had unilateral effects on motor performance and corticospinal excitability, the nature of which differed depending on which cortex was stimulated. Specifically, iTBS applied to the trained cortex (cM1 group)

586 resulted in statistically significant *reductions* of both prior training-induced performance
587 gains (Figure 3A) and corticospinal excitability increases (Figure 4A) in the trained hand and
588 motor cortex, without affecting performance in, or corticospinal projections to, the
589 untrained hand. The reversal of the typical facilitatory effect of iTBS on corticospinal
590 excitability (Huang et al. 2005) is consistent with homeostatic plasticity (see Müller-Dahlhaus
591 and Ziemann 2015 for a review), whereas the reduction in training-induced performance
592 gains suggests that non-invasive brain stimulation interfered with circuits involved in storage
593 or retrieval of the new motor memory (Muellbacher et al. 2002). In contrast, iTBS applied to
594 the untrained hemisphere (iM1 group), resulted in *improved* motor performance (Figure 3B)
595 and *increased* corticospinal excitability (Figure 4B) in the untrained hand and motor cortex
596 without affecting the performance or projections to the trained hand (see Figure 1B for that
597 prediction). Moreover, these changes in performance and excitability seem functionally
598 related; the extent of performance transfer to the untrained hand predicted the magnitude
599 of excitability increases. The *distinct* effects of iTBS on performance in the trained
600 (performance decrements) and untrained (performance gains) cortices is highly suggestive
601 that different mechanisms mediate motor learning and cross-limb transfer. Importantly, the
602 observed differences in the manner in which iTBS affected performance in the trained and
603 untrained hands appeared despite the fact that both hands had exhibited increases in
604 performance following the initial unilateral motor learning.
605
606 *Homeostatic versus non-homeostatic processes in the trained and untrained M1s*
607 The interaction between the mechanisms underpinning motor learning in the trained
608 hand and iTBS is consistent with the notion of homeostatic metaplasticity. In this instance,
609 rather than LTP-like plasticity from motor learning and iTBS accumulating, the prior motor

learning reversed, or occluded, the 'expected' effects of a subsequent LTP-inducing protocol (in this case, iTBS; see Di Lazzarro et al. 2008; Huang et al. 2005) applied to the trained hemisphere (see Müller-Dahlhaus and Ziemann 2015 for a review; Rosenkranz et al. 2007; Stefan et al. 2006; Stöckel et al. 2015; Ziemann et al. 2004).

In contrast, the increases in corticospinal excitability observed in the untrained hemisphere following iTBS applied to the untrained M1 (iM1 group), reflect an apparent LTP-like effect. This is consistent with iTBS effects observed when applied in isolation (Huang et al. 2005). Conceivably, because the behavioral gains in the untrained hand (following motor training) were not accompanied by increases in excitability of the untrained hemisphere (Figure 4), the iTBS protocol was still able to act in the 'expected' direction and induce facilitation of MEPs.

Effects of iTBS on subsequent performance and learning

Because NBS is a potential candidate to augment neuro-rehabilitation (Müller-Dahlhaus and Ziemann 2015; Ridding and Rothwell 2007), it is important to consider its effects on subsequent motor performance and learning. Previous work shows that learning can be enhanced in the trained limb when iTBS is applied to the contralateral M1 (cM1; trained hemisphere) prior to practice (Teo et al. 2010; c.f. Agostino et al. 2008 for a contradictory report, note that their experiments involved either a short training protocol that caused limited learning, or a small sample of n = 5). In the current study, performance was reduced when assessed without feedback immediately after iTBS to the contralateral M1, but rapidly increased during the second learning bout such that final performance was no different from a group that received sham stimulation. However, disentangling the influence of iTBS on subsequent learning was complicated in the current study by the fact

that motor practice was also performed *prior* to iTBS delivery. The rapid recovery of performance during the first few trials of T2 could be viewed as an increase in learning rate following iTBS (as per Teo et al. 2010), or the dissipation of a homeostatic interaction between iTBS and prior training (Stöckel et al. 2015).

More importantly, we were interested in the influence of iTBS to the ipsilateral M1 (iM1; untrained hemisphere) on subsequent performance and *transfer* from the trained limb, as to our knowledge, this effect has not been previously investigated. The increase in ipsilateral excitability that we observed after iTBS to the ipsilateral M1 appeared to drive further performance gains in the untrained hand. Because neither excitability nor performance were significantly increased following iTBS in the non-training control group, we propose that the effects of prior training with the opposite limb interact with iTBS delivered to the untrained M1. In particular, it appears in this case that the NBS-induced facilitatory effect summated with the transfer-induced performance gains. Similar to the results for the trained hand, however, final performance measured in the untrained hand after T2 was not different between groups. This indicates that the immediate performance benefit conferred by iTBS to the ipsilateral M1 failed to improve subsequent performance gains due to transfer from the opposite limb.

What type of ipsilateral adaptations mediate untrained hand performance?

Unlike previous research demonstrating bilateral increases in corticospinal excitability following unilateral, ballistic motor practice (Carroll et al. 2008; Lee et al. 2010; Hinder et al. 2011), substantial performance improvements in the untrained hand were not accompanied by increased excitability of corticospinal projections to the untrained hand in the current study. Transfer of performance without changes in excitability of the untrained

cortex is consistent with evidence from sequencing tasks (Camus et al. 2009; Pascual-Leone et al. 1995; Perez et al. 2007). Moreover, previous work has also shown that transfer of ballistic motor skill can even be accompanied by decreases in excitability of the untrained hemisphere (Duque et al. 2008). A likely mechanism that contributes to enhanced performance in the untrained limb is reduced inter-hemispheric inhibition, which is reduced from the trained to the untrained M1 after various types of sequence learning (Camus et al. 2009; Perez et al. 2007), and after strength training (Hortobagyi et al. 2011; see Ruddy and Carson 2013 for a review). Thus, while ballistic motor training reliably potentiates corticospinal excitability in the trained M1 (cf. Liepert et al. 1998; Muellbacher et al. 2001), untrained, left hand performance gains following unilateral practice (i.e., as a result of cross-limb transfer) are not necessarily accompanied by overt changes in excitability in the untrained, right M1. However, the fact that iTBS applied to the untrained hemisphere amplified untrained hand performance gains in the current study suggests that some form of adaptation occurred within the untrained M1 which mediated performance improvements in the untrained hand. In support of this view there is evidence from neuroimaging data on the encoding of (sequential) single finger movements (Diedrichsen et al. 2013; Wiestler et al. 2014) demonstrating similar (mirrored) representation patterns in both motor cortices (and sensory motor cortices) that include the same fine-grained details of the movement, but with suppressed BOLD signals (relative to resting baseline) in the motor cortex ipsilateral to the active hand. This raises the possibility that multiple processes may influence ipsilateral cortical function, including generalised suppression of activation (which might underlie the lack of corticospinal excitabitly we observed) and patterned activation specifically associated with task performance (which might underlie transfer of performance to the untrained limb).

The finding that untrained hand performance gains following motor training are only affected (i.e. up-regulated) by iTBS applied to the untrained, but not the trained, M1 is strongly suggestive of a contribution of the ipsilateral M1 to cross-limb transfer. In line with the cross-activation hypothesis (Cramer et al. 1999; Dettmers et al. 1995; Parlow and Kinsbourne 1989) and previous experimental work using the same motor learning task (Lee et al. 2010), our results suggest that task-related information stored in the *untrained* hemisphere during motor learning is subsequently retrieved to drive cross-limb adaptations when the task is undertaken with the untrained limb. The fact that training related improvements in the untrained hand are positively correlated with subsequent iTBS-induced improvements in this same hand is further evidence to suggest that cross-limb improvements (if governed by LTP-like processes) and iTBS interact in a non-homeostatic manner. Alternatively, because the untrained limb improvements were not associated with excitability increases, it may be that transfer itself is not driven by LTP-like effects, and hence the subsequent iTBS LTP-inducing protocol was able to act without being affected by a prior history of LTP. Although our evidence strongly suggests that the ipsilateral, untrained M1 is involved in transfer, the lack of change in MEP, compared with MEP increases in the contralateral, trained M1, implies either that 1) the majority of neurons that contribute to the peripheral responses to TMS (i.e. MEPs) are not involved in transfer, or 2) other non-primary areas also contribute substantially to transfer. Finally, it should be noted that the current cohort consisted of right handed adults who trained with their dominant hand. Thus, although we feel it unlikely, the possibility exists, that influences of hand dominance and hemisphere-specific effects of iTBS in the right and left M1 (irrespective of which hand/hemisphere had trained) may have had a small effect on the results.

With respect to potential non-primary contributions to transfer, it has indeed been demonstrated that a broad neural network is involved in cross-limb transfer (Gerloff and Andres 2002; Rizzolatti et al. 1998; Ruddy and Carson 2013), with evidence for bilateral changes in different secondary motor areas (Hardwick et al. 2013; Wiestler and Diedrichsen 2013). As such, neural adaptations in other brain regions beyond M1 ipsilateral to the trained limb could account for cross-limb transfer effects observed in the current study. In this case, the stimulation protocol used in the present study would only have affected a part of the acquired skill representation for the untrained limb, and thereby limiting the conclusions drawn here to features of the task represented within M1. For example, Romei and colleagues (Romei et al. 2009) provided evidence that M1 contributes to intrinsic (i.e., knowledge represented in body-centred coordinates; muscle and joint based) but not extrinsic components (i.e., world-centred coordinates; movement features in external space) of motor skill learning. Therefore, future studies should examine the relative contribution of a more extensive brain network in the untrained cortex to cross-limb adaptations following unilateral practice of different motor tasks (e.g., ballistic, sequential, or reaction time tasks).

Conclusions

In sum, the present study suggests that, while occurring simultaneously, motor learning and cross-limb transfer represent distinct neural adaptation processes which interact differently with iTBS. The typical effect of an LTP-like inducing brain stimulation protocol were reversed in the hemisphere projecting to the trained hand, consistent with the suggestion that LTP contributes to ballistic motor learning. That is, motor learning resulted in subsequent iTBS having a LTD-like effect in the trained M1. In contrast, LTP-like effects following iTBS were observed in the hemisphere projecting to the transfer hand, suggesting

728 either that LTP within the untrained M1 does not underlie cross limb transfer, or that the
729 majority of neurons that contribute to the peripheral responses to TMS (applied to the
730 untrained M1) are not involved in transfer. Importantly, iTBS had a unilateral effect on both
731 the training and transfer process, offering further support that transfer is governed by the
732 cross-activation hypothesis (Cramer et al. 1999; Dettmers et al. 1995; Lee et al. 2010).
733

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865

Figure captions

Figure 1. Predictions of the study. Panel **A**) predicts that, if learning and transfer are both mediated by LTP-like processes, then the effect of a subsequent intervention that induces LTP-like effects in the absence of training (here iTBS) should be reduced or reversed due to homeostatic plasticity in both the trained or untrained hemispheres. Panel **B**) predicts that, if learning relies on LTP-like processes, then it should cause a reduction or reversal in the expected LTP-like effects induced by iTBS to the contralateral M1 (cM1; trained hemisphere). However, if the processes that mediate transfer are not LTP-like, then the typical LTP-like effect of iTBS to the ipsilateral M1 (iM1; untrained hemisphere) should be observed. (↓ indicates reduction of iTBS-induced LTP-like effect due to homeostatic interaction of learning/transfer with iTBS; ↔ indicates maintenance of the expected iTBS-induced LTP-like effects).

Figure 2. Experimental procedure. Transcranial magnetic stimulation (TMS) and motor performance (MP) measures were assessed before a first training block, before and after applying intermittent theta-burst stimulation (iTBS) and after a second training block for right and left hands and motor cortices. Depending on group affiliation, iTBS was induced over the trained or untrained motor cortex or over the vertex.

Figure 3. Normalized performance relative to pre-test. Following a first training block of ballistic right hand practice (150 trials), iTBS was applied to the contralateral, trained (cM1) [panel A], the ipsilateral, untrained motor cortex (iM1) [panel B], or over the vertex (sham group) [panel C]. Performance was tested in participants trained (right) hand (circles) and untrained (left) hand (triangles) before the first training block, before and after iTBS and after

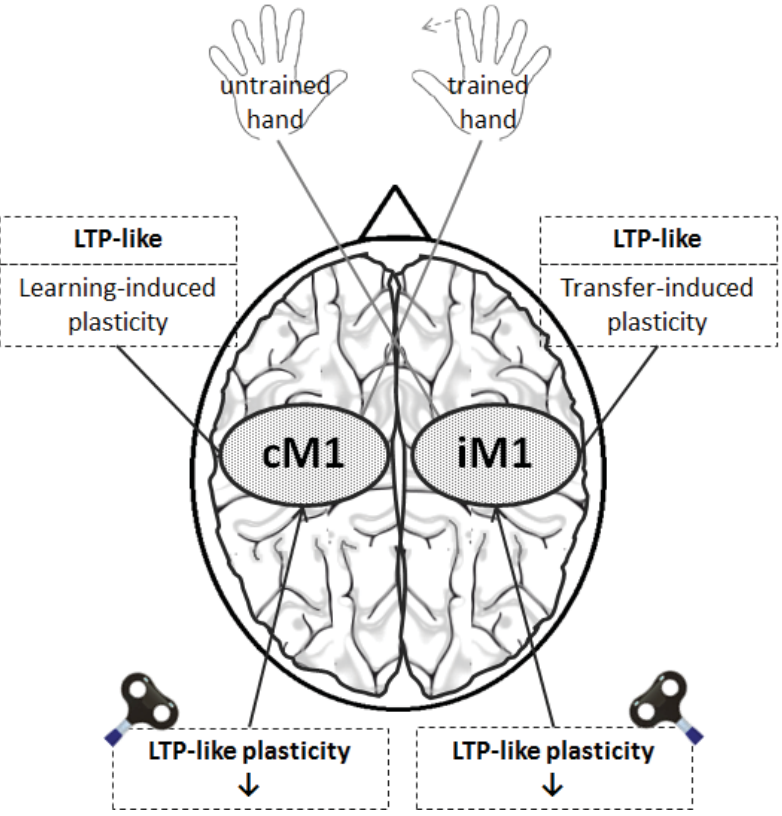
a second training block. Performance values were normalized to the corresponding pre-test performance for that hand (as indicated by the X-axis). Error bars indicate 95% CI.

Figure 4. Average normalized MEPs relative to pre-test for cM1, iM1 and sham group participants. MEP amplitudes were tested on the trained (right) hand (circles) and on the untrained (left) hand (triangles) before a first training block (T1), before and after iTBS and after a second training block (T2). MEP amplitudes were normalized to pre-test values. Error bars indicate 95% CI and the X-axis represents pre-test values.

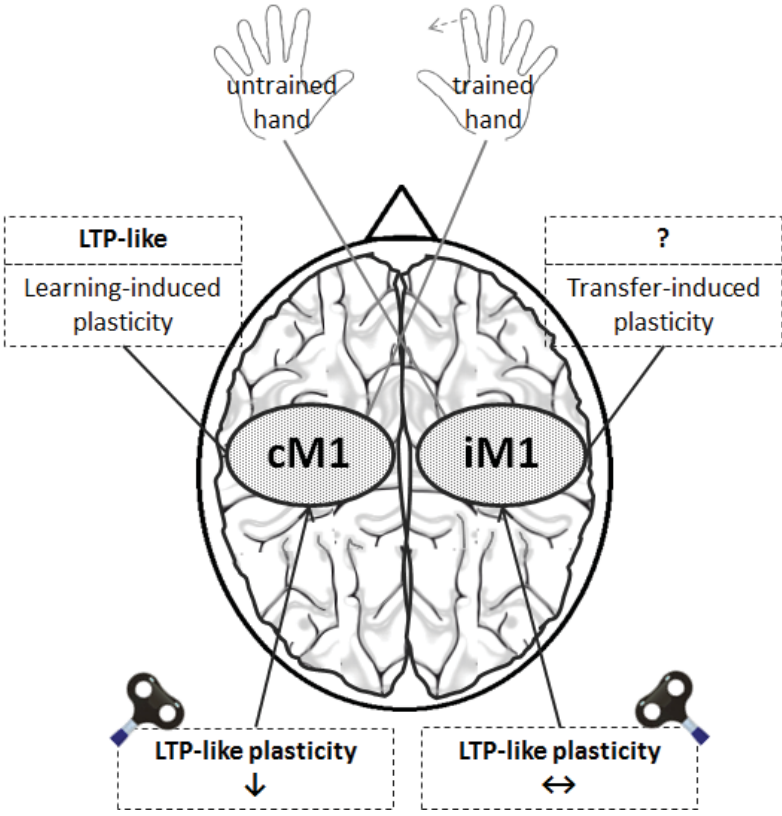
Figure 5. Partial regressions. Multiple regression analysis was performed to identify main predictors of cross-limb transfer (i.e., normalized untrained hand performance) following unilateral practice (i.e., at pre-iTBS) and to study their relative strength. Displayed are partial regressions of the (**A**) normalized performance of the trained hand during the first training block, (**B**) normalized MEPs of the trained hand following the first training block, and (**C**) normalized MEPs of the untrained hand at pre-iTBS; all of which contributed unique variance to the regression model, i.e. were identified as potential predictors of cross-limb transfer. Relations between the measures are displayed by linear trend lines. X- and Y-axes represent respective pre-test values.

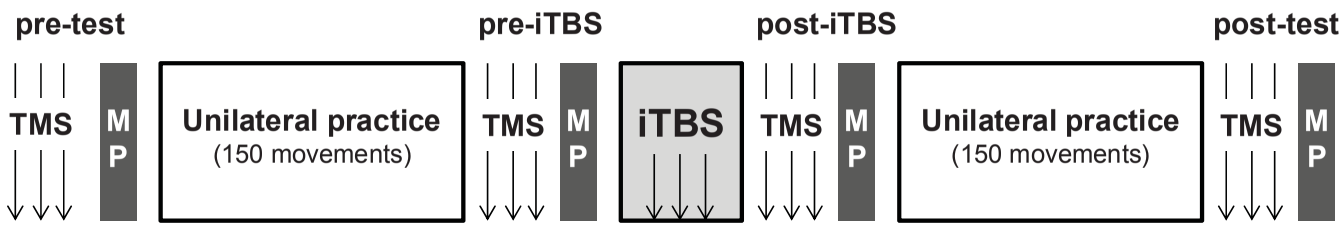
Figure 6. Control experiment data summary. (**A**) MEP and (**B**) performance changes in right and left hands following right M1 iTBS averaged across all participants of the no-training control group (n = 12)**.** (**C**) MEP and (**D**) performance changes in the right and left hands averaged across the 6 best responders to iTBS (out of the 12 no-training control group participants). MEP amplitudes and performance values were normalized to pre-test values (before iTBS). Error bars indicate 95% CI.

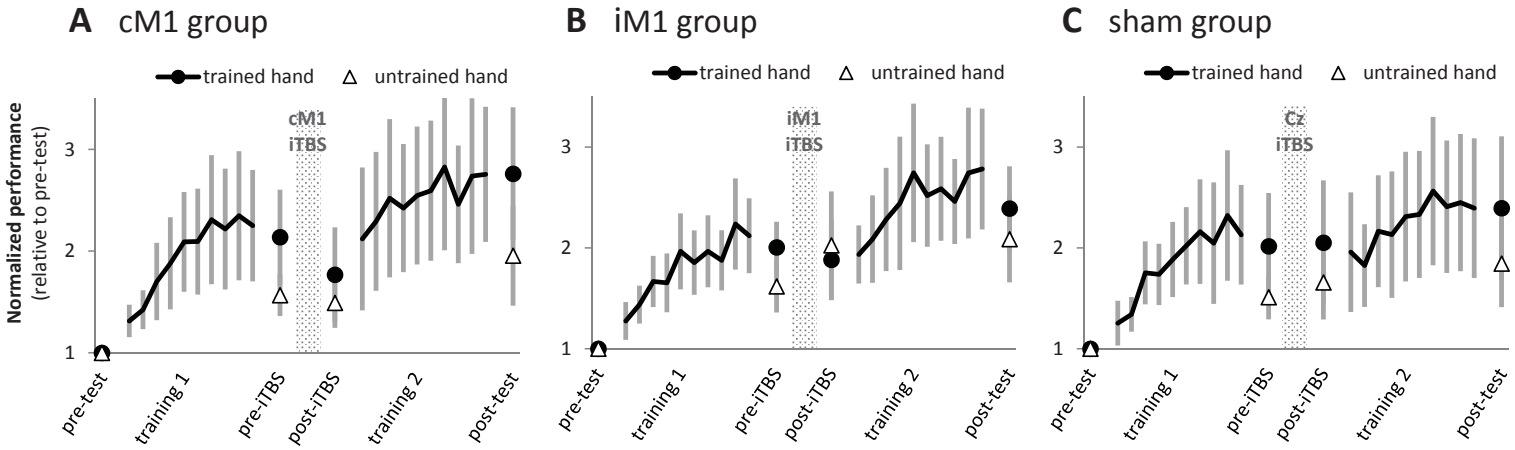
A) Learning and Transfer represent *similar* neural mechanisms

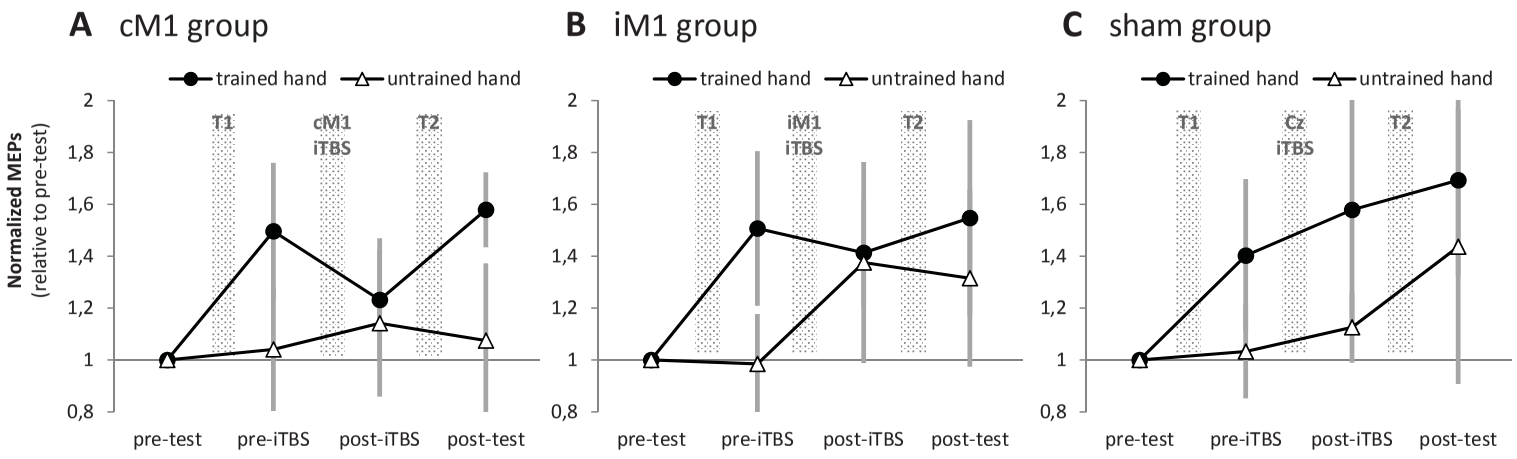


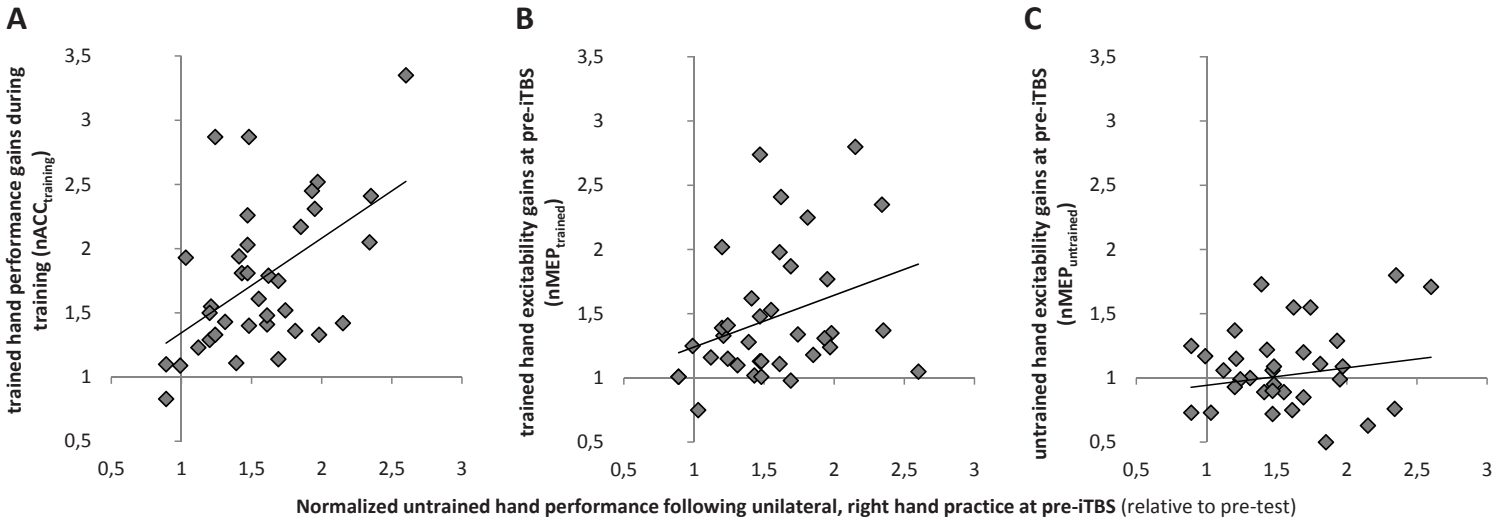
B) Learning and Transfer represent *distinct* neural mechanisms

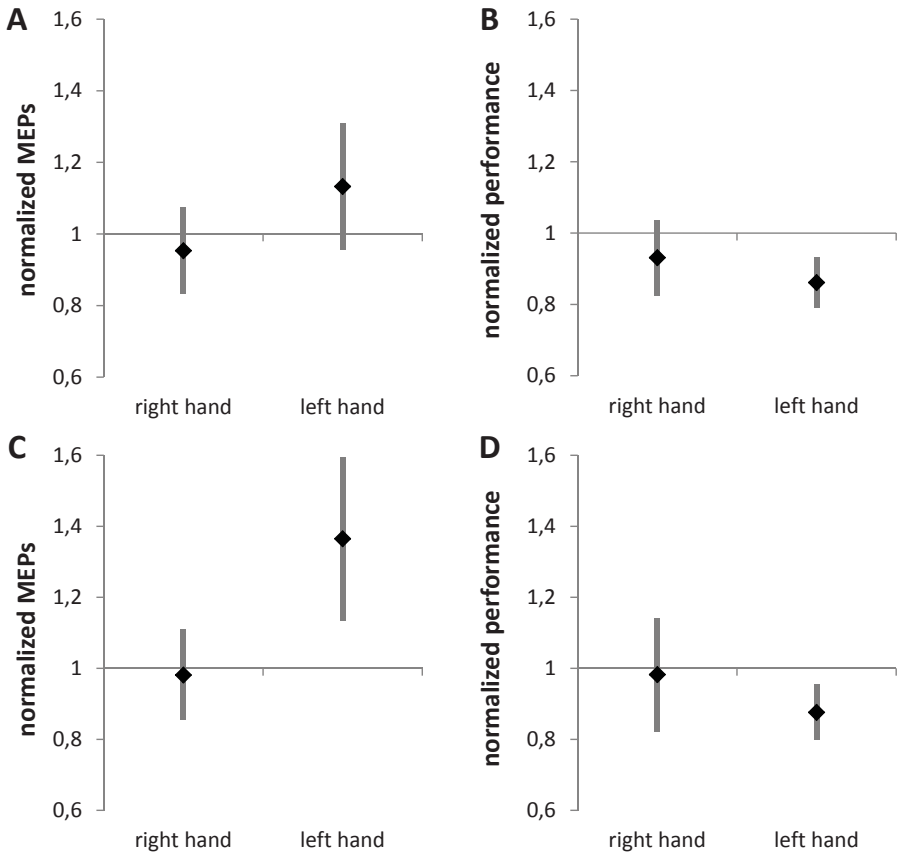



pre-test
pre-iTBS
post-iTBS
post-test
TMS
MP
Unilateral practice
(150 movements)
TMS
MP
iTBS
TMS
MP
Unilateral practice
(150 movements)
TMS
MP


A cM1 group
B iM1 group
C sham group
trained hand
untrained hand
Normalized performance
(relative to pre-test)
cM1 iTBS
iM1 iTBS
Cz iTBS
1
2
3
pre-test
training 1
pre-iTBS
post-iTBS
training 2
post-test


A cM1 group
B iM1 group
C sham group
trained hand
untrained hand
Normalized MEPs
(relative to pre-test)
2
1,8
1,6
1,4
1,2
1
0,8
T1
cM1
iTBS
T2
iM1
Cz
pre-test
pre-iTBS
post-iTBS
post-test


A
B
C
trained hand performance gains during training (nACC$_{training}$)
trained hand excitability gains at pre-iTBS (nMEP$_{trained}$)
untrained hand excitability gains at pre-iTBS (nMEP$_{untrained}$)
3,5
3
2,5
2
1,5
1
0,5
0,5
1
1,5
2
2,5
3
Normalized untrained hand performance following unilateral, right hand practice at pre-iTBS (relative to pre-test)


A
normalized MEPs
1,6
1,4
1,2
1
0,8
0,6
right hand
left hand
B
normalized performance
1,6
1,4
1,2
1
0,8
0,6
right hand
left hand
C
normalized MEPs
1,6
1,4
1,2
1
0,8
0,6
right hand
left hand
D
normalized performance
1,6
1,4
1,2
1
0,8
0,6
right hand
left hand