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

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18 **Running head:** Motor learning and cross-limb transfer

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29 **Abstract**

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

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

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New & Noteworthy

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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 indirectly 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.

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Introduction

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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.

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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).

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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)

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

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

107

108

“Figure 1 about here”

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

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120

Methods

121 *Participants*

122 Thirty-six healthy, right-handed young adults (Oldfield, 1971) were randomly
123 assigned to either a cM1 (n = 12, 5 males, average age = 26.2 years, SD = 5.6), iM1 (n = 12, 6
124 males, average age = 24.4 years, SD = 5.9), or a sham group (n = 12, 5 males, average age =
125 24.4 years, SD = 5.0) where cM1, iM1 and sham refer to the nature of the applied stimulus
126 following unilateral practice (see Task and procedure). All participants gave written informed
127 consent, and completed a medical history questionnaire which confirmed the absence of any
128 known neurological and neuromuscular dysfunction and any contraindications to TMS. All
129 procedures were approved by the Tasmanian Human Research Ethics Committee Network.

130

131 *Task and procedure*

132 The experiment was designed to use non-invasive brain stimulation to interact with
133 training-induced plasticity in the trained and untrained hemisphere following unilateral
134 motor training. We aimed to determine whether training-induced performance gains in the
135 trained (direct learning) and untrained hands (cross-limb transfer) are driven by similar, LTP-
136 like, neural adaptations in trained and untrained motor cortices, respectively. Figure 2
137 outlines the experimental procedure. Following Hinder et al. (2011, 2013) and Lee et al.
138 (2010), participants practiced a ballistic abduction of the right index finger (audio-paced at
139 0.5 Hz) where the performance goal was to maximise peak horizontal (abduction)
140 acceleration of each movement. This type of motor learning paradigm represents an ideal
141 model to study the mechanisms of adaptation and transfer for many reasons. Substantial
142 performance gains are exhibited within a single session, which simplifies the use of brain
143 stimulation methods such as transcranial magnetic stimulation (TMS) to assess the neural
144 underpinnings of adaptation (Carroll et al. 2008). Moreover, the neural responses to ballistic
145 motor training are similar to those observed after strength training (Selvanayagam et al.
146 2011). Accordingly the model provides a window into the mechanisms underlying an
147 important physical attribute that often limits function in old age and in patients with
148 neurological disorders. Triaxial accelerometers (Dytran Instruments, Chatsworth, CA;
149 Endevco, San Juan Capistrano, CA) were mounted to plastic splints and taped to the top of
150 the left and right index fingers such that one of the orthogonal axes of each accelerometer
151 was aligned to measure horizontal acceleration. A custom written Signal (CED) script (see
152 Hinder et al. 2011, 2013) allowed us to detect the first peak of the acceleration trace and
153 provide this information to participants as visual performance feedback according to the
154 feedback design (see below).

155

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

180 dorsal interosseus (FDI) muscle (cM1 group), the untrained FDI muscle (iM1 group), or over
181 the vertex (handle backwards) with the coil tilted by 90° (coil surface orthogonal to the scalp
182 surface) with one side of the coil remaining contact with the head (sham group; Mistry et al.
183 2012). The active motor threshold was defined as the minimum stimulator intensity required
184 to evoke MEPs of $\geq 200 \mu\text{V}$ (in three out of five trials) (Huang et al. 2005) during a light
185 isometric contraction of the corresponding FDI muscle at about 10 % of maximum force.

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

196

197 ***Recording of muscle activity***

198 In order to quantify muscle activity (during the execution of the motor task and in
199 response to suprathreshold pulses of TMS) we recorded EMG activity with Ag/AgCl
200 electrodes (Meditrace 130, Tyco Healthcare, Mansfield, MA) from the FDI in both hands in a
201 belly-tendon montage (as per Hinder et al. 2011, 2013). EMG signals were fed into a CED
202 1401 amplifier (Cambridge, UK), where a notch filter (50 Hz) was applied before
203 amplification (gain 300–1,000), and stored for off-line analysis. Participants' EMG activity

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

206

207 ***Transcranial magnetic stimulation***

208 Transcranial magnetic stimulation (TMS) was delivered using two Magstim 200² units
209 (Magstim Company, Dyfed, UK) connected via a Bistim² unit and a single figure-of-eight coil
210 (70 mm external diameter). Motor 'hotspots' for the left and the right FDI (with posterior- to
211 anterior-induced current in the cortex) were determined and resting motor thresholds
212 (RMT) were established as the minimum intensities required to elicit MEPs > 50 μ V in the
213 right and left FDI muscles in three out of five consecutive trials when stimulating at the
214 hotspots (Carroll et al. 2001; Hinder et al. 2010). Participants were instructed to relax their
215 limbs during RMT determination and visual feedback of muscle activity helped to keep
216 muscle activity to a minimum.

217 During TMS test blocks, 30 stimulations (with an interstimulus interval of 4-6 s) were
218 administered to the right (untrained) or left (trained) motor hotspots, respectively. Half of
219 the stimulations involved a single 'test' pulse (130 % RMT) to assess the net excitability of
220 the corticospinal projections to the trained/untrained hand, while half of the trials involved
221 paired-pulse stimulation (Kujirai et al. 1993) in which a subthreshold conditioning pulse (70
222 % RMT) preceded the same test pulse. The ratio of the average MEP evoked following
223 paired-pulse trials (within one TMS test block) to the average MEP amplitude evoked in the
224 single-pulse trials (within the same TMS test block) is referred to as the short-interval
225 intracortical inhibition ratio, SICI (Kujirai et al. 1993), and reflects activity of intracortical
226 inhibitory circuits. The order of single- and paired-pulse stimulations was randomised within
227 each TMS block.

228

229 ***Control Experiment***

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

243

244 ***Data analysis***

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

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

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

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

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

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

301

302 ***Statistical analysis***

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

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

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

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

348

349

Results

350 ***Motor performance***

351 *Training-induced effects on motor performance*

352 Average peak accelerations at pre-test were $20.5 \pm 9.4 \text{ ms}^{-2}$, $20.9 \pm 4.6 \text{ ms}^{-2}$ and 14.8
353 $\pm 2.4 \text{ ms}^{-2}$ for the right hand and were $18.9 \pm 5.9 \text{ ms}^{-2}$, $20.1 \pm 3.7 \text{ ms}^{-2}$ and $14.4 \pm 2.7 \text{ ms}^{-2}$ for
354 the left hand for the cM1, iM1 and sham groups, respectively. Upon completion of the first
355 training block (i.e., at pre-iTBS), peak acceleration of the index finger had increased to $36.5 \pm$
356 10.0 ms^{-2} , $40.1 \pm 6.7 \text{ ms}^{-2}$ and $28.5 \pm 8.4 \text{ ms}^{-2}$ in the trained hand, and to $27.8 \pm 7.6 \text{ ms}^{-2}$, 31.3
357 $\pm 7.1 \text{ ms}^{-2}$ and $21.7 \pm 4.3 \text{ ms}^{-2}$ in the untrained hand in the cM1, iM1 and sham groups,
358 respectively. ANOVA revealed a significant time x hand interaction, $F(1,33) = 25.62$, $p < .001$,
359 $\eta_p^2 = .44$, with posthoc pairwise comparisons revealing a significant increase in performance
360 from pre-test (trained: $18.8 \pm 3.7 \text{ ms}^{-2}$, untrained: $17.8 \pm 2.6 \text{ ms}^{-2}$) to pre-iTBS (trained: $35.0 \pm$
361 5.2 ms^{-2} , untrained: $26.9 \pm 4.0 \text{ ms}^{-2}$) for both, trained and untrained, hands (both $p < .001$, d
362 $> .89$) averaged across all three groups. Peak acceleration did not differ significantly between
363 hands at pre-test ($p = .39$, $d = .09$; pairwise Sidak adjusted post-hoc tests confirmed that
364 baseline performance was not significantly different between any of the groups, all $p > 0.27$),
365 but was greater for the trained as compared to the untrained hand after motor training at
366 pre-iTBS ($p < .001$, $d = .58$). ANOVA revealed no significant main or interaction effects
367 including the factor group.

368

369 *iTBS-induced effects on motor performance*

370 To assess the impact of the different iTBS protocols on trained and untrained hand
371 performance, we compared normalized peak acceleration values (relative to pre-test) of
372 cM1, iM1 and sham group participants before and after the application of iTBS. As seen in
373 Figure 3, iTBS resulted in a *reduction* of normalized performance (relative to pre-test) of the
374 trained hand from 2.14 (± 0.47) to 1.77 (± 0.47) for the cM1 group, whereas normalized
375 performance of the trained hand in the iM1 group (pre-iTBS: 2.01 \pm 0.25; post-iTBS: 1.88 \pm
376 0.40) and sham group (pre-iTBS: 2.02 \pm 0.53; post-iTBS: 2.05 \pm 0.62) was much less affected
377 by iTBS. In contrast, iTBS *increased* normalized performance in the untrained hand from 1.62
378 (± 0.26) at pre-iTBS to 2.03 (± 0.54) at post-iTBS in the iM1 group, whereas normalized
379 performance of the untrained hand appeared to be unaffected by iTBS in the cM1 (pre-iTBS:
380 1.57 \pm 0.21; post-iTBS: 1.49 \pm 0.25) and sham groups (pre-iTBS: 1.51 \pm 0.22; post-iTBS: 1.66 \pm
381 0.37). ANOVA conducted on normalized performance revealed a significant time x hand x
382 group interaction, $F(2,33) = 3.75$, $p = .03$, $\eta_p^2 = .19$, confirming the changes described above;
383 i.e., performance changes in response to iTBS were hand- and iTBS location (group)-specific.
384 Indeed, posthoc pairwise comparisons revealed that the performance *decrease* of the
385 trained hand in the cM1 group ($p = .004$, $d = .43$) and the performance *increase* of the
386 untrained hand in the iM1 group ($p = .002$, $d = .52$) following iTBS were statistically
387 significant; all other pairwise comparisons were not statistically significant (all $p > .23$, $d <$
388 .26).

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

394 training block, the greater the subsequent iTBS-induced improvements in that hand. That is,
395 we observed a positive relationship between the extent of iTBS-induced change in
396 performance in the untrained hand and the extent of the previous performance gains in the
397 untrained hand (i.e., as a result of cross-limb transfer) following the first training block ($r =$
398 $0.72, p = .004$). In contrast, for the trained hand of the cM1 group, there was a negative
399 correlation between iTBS-induced performance changes and the previous use-dependent
400 performance gains as a result of the first training block ($r = -0.55, p = .03$). This illustrates
401 that the greater the use-dependent performance increase in the trained hand following the
402 first training block, the more performance of that hand is reduced following the application
403 of iTBS.

404

405 *“Figure 3 about here”*

406

407 *iTBS-induced effects on subsequent motor training*

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

418 induced change in performance (trained hand: $r = -0.57$, $p < .001$; untrained hand: $r = -0.49$,
419 $p = .001$). That is, the greater the iTBS-induced performance decrement in the trained hand,
420 the greater the subsequent learning in that hand in the second block. For the untrained
421 hand, the greater the iTBS-induced gain, the lower the subsequent performance gain in that
422 hand resulting from the second training block.

423

424 ***Neurophysiological measures***

425 Corticospinal excitability

426 *Training-induced effects on corticospinal excitability*

427 RMTs (as a % of maximum stimulator output, \pm 95% CI) for the right hand were $42.5 \pm$
428 4.2% , $40.7\% \pm 3.5\%$ and $43.1 \pm 3.5\%$; and were $42.1 \pm 4.1\%$, $40.8 \pm 4.0\%$ and $44.1 \pm 2.4\%$ for
429 the left hand for cM1, iM1 and sham group participants, respectively. There were no
430 significant differences between groups, $F(2,33) = 0.60$, $p = .56$, $\eta_p^2 = .04$, or hands, $F(1,33) =$
431 0.11 , $p = .75$, $\eta_p^2 = .003$, and no interaction between hand and group, $F(1,33) = 0.30$, $p = .75$,
432 $\eta_p^2 = .02$. AMTs were $48.0 \pm 3.4\%$, $47.2 \pm 2.5\%$ and $49.4 \pm 3.6\%$ of maximum stimulator
433 output for cM1, iM1 and sham group participants, respectively, and did not differ between
434 groups, $F(2,33) = 0.45$, $p = .65$, $\eta_p^2 = .03$. (NB: AMT appears higher than RMT because it was
435 determined on the less powerful Magstim Super Rapid² stimulator which was used to
436 subsequently administer iTBS, whereas RMT and single/paired pulse TMS was administered
437 using two Magtim 200² units connected with a BiStim module). Average MEP amplitudes at
438 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
439 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
440 group participants respectively. Upon completion of the first training block (i.e., at pre-iTBS),
441 cM1, iM1 and sham group participants' excitability increased to 2.18 ± 0.70 mV, 1.94 ± 0.74

442 mV and 1.25 ± 0.35 mV in the trained hand, respectively. However, excitability of the
443 untrained hand was relatively unaffected by training in all three groups (cM1: 1.41 ± 0.80
444 mV, iM1: 1.42 ± 0.61 mV, sham: 1.36 ± 0.33 mV). ANOVA revealed a significant main effect
445 for time, $F(1,33) = 13.11$, $p = .001$, $\eta_p^2 = .28$; averaged across both hands excitability
446 increased from pre-test (1.33 ± 0.28 mV) to pre-iTBS (1.59 ± 0.37 mV); however the
447 significant time x hand interaction, $F(1,33) = 18.05$, $p < .001$, $\eta_p^2 = .35$ indicates that this
448 effect was driven by changes in excitability in the trained hand. Indeed, posthoc pairwise
449 comparisons revealed that (averaged across all groups) a significant increase in corticospinal
450 excitability occurred from pre-test to pre-iTBS for the trained hand ($p < .001$, $d = .54$), but
451 not for the untrained hand ($p = .87$, $d = .02$). There were no significant differences between
452 the excitability of trained and untrained hands at pre-test ($p = .25$, $d = .19$); however, the
453 trained hand exhibited greater excitability at pre-iTBS than the untrained hand ($p = .03$, $d =$
454 $.35$). All other main or interaction effects were not significant (all $F < 2.28$, $p > .12$, $\eta_p^2 < .12$).

455

456 *iTBS-induced effects on corticospinal excitability*

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

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

475 Correlation analyses revealed that trained and untrained hand excitability gains were
476 not significantly related to each other following the first training block at pre-iTBS ($r = .12$, p
477 $= .50$) or following iTBS at post-iTBS ($r = .29$, $p = .09$). For the iM1 group, there was a
478 marginal positive correlation between the extent of iTBS-induced change in performance in
479 the untrained hand, and the extent of iTBS-induced change in excitability (i.e., at post-iTBS)
480 in the untrained motor cortex ($r = .52$, $p = .08$), but not for the trained motor cortex and
481 hand ($r = -.29$, $p = .36$). Also there were no such associations between performance and
482 excitability changes following iTBS for the cM1 group's trained ($r = .09$, $p = .78$) or untrained
483 hand ($r = .33$, $p = .29$) at post-iTBS.

484

485 *"Figure 4 about here"*

486

487 *iTBS-induced effects on subsequent motor training*

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

490 the trained hand increased to $1.51 (\pm 0.48)$ in the cM1 group, to $1.10 (\pm 0.19)$ in the iM1
491 group and to $1.17 (\pm 0.22)$ in the sham group. Normalized excitability of the untrained hand
492 (relative to post-iTBS) at post-test was $0.97 (\pm 0.18)$ in the cM1 group, $1.01 (\pm 0.17)$ in the
493 iM1 group and $1.23 (\pm 0.28)$ in the sham group. Despite the apparent differences between
494 groups and hands described qualitatively above, ANOVA conducted to assess the effect of
495 the second training bout (i.e., post-test excitability normalized to post-iTBS excitability)
496 revealed no significant differences between groups ($p = .50$). The main effect of hand,
497 $F(1,33) = 3.45, p = .07, \eta_p^2 = .10$, and the interaction of hand and group were marginal,
498 $F(2,33) = 3.05, p = .06, \eta_p^2 = .16$. Post-hoc tests showed that the marginal interaction was
499 driven by the greater excitability gain in cM1 group's trained hand at post-test as compared
500 to their untrained hand ($p = .005$).

501 Correlation analyses revealed a significant relationship between the extent of
502 excitability increases in cM1 group's trained hand induced as a result of the second training
503 period and the extent of the previous iTBS-induced change (reduction) in excitability ($r = -$
504 $0.74, p = .003$). That is, the greater the reduction in excitability induced by iTBS, the greater
505 the subsequent increase in excitability as a result of motor learning.

506

507 *Intracortical inhibition*

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

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

517

518 ***Multiple Regression analysis to elucidate predictors of cross-limb transfer***

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

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

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

545

546 *“Figure 5 about here”*

547

548 **Control Experiment**

549 The results of the main experiment showed that both performance and MEPS
550 increased in the untrained hand after iTBS to the untrained M1, but it is unclear whether this
551 performance gain reflected a general improvement in motor function due to enhanced
552 excitability, or an interaction with the recently transferred motor skill. We therefore
553 analyzed the iTBS-induced change in excitability and motor performance (normalized values
554 relative to pre-test) at post-iTBS (i.e. following iTBS over right M1) in a control group that
555 performed no prior motor training. Normalized excitability of circuits projecting to the right
556 and left hands at post-iTBS was $0.95 (\pm 0.12)$ and $1.13 (\pm 0.18)$ respectively (see Figure 6A).
557 Normalized motor performance following iTBS was $0.93 (\pm 0.11)$ for the right hand and was
558 $0.86 (\pm 0.07)$ for the left hand (see Figure 6B). One-sample t-Tests (against pre-test level, i.e.
559 1) revealed the decrease in left hand performance to be significant, $t(11) = -3.71$, $p = .003$,
560 but not the increase in left hand (right M1) excitability, $t(11) = 1.407$, $p = .18$. Thus, the
561 expected LTP-like effect of iTBS was not statistically significant for the entire group due to

562 inter-subject variability (as has been reported previously, Hamada et al., 2013; Hinder et al.,
563 2014). We therefore looked at the subset of six control participants who showed the largest
564 MEP changes for the left hand, to be sure that an iTBS-induced increase in MEP amplitude
565 does not change motor performance. Average normalized excitability of the sub-sample was
566 $0.98 (\pm 0.13)$ and $1.37 (\pm 0.23)$ for circuits projecting to right and left hands respectively (see
567 Figure 6C). Average normalized performance following iTBS for the subset of best
568 ‘responders’ was $0.98 (\pm 0.16)$ for the right hand and was $0.88 (\pm 0.08)$ for the left hand (see
569 Figure 6D). One-sample t-Tests revealed both the increase in left hand (right M1) excitability,
570 $t(5) = 2.84$, $p = .04$ and the decrease in left hand performance to be significant, $t(5) = -2.85$,
571 $p = .04$. Moreover, changes in MEP amplitude and motor performance following iTBS were
572 not associated, neither across the entire group of 12 subjects (left hand: $r = .05$, $p = .89$; right
573 hand: $r = -.38$, $p = .22$), nor in the subset of participants that exhibited the largest MEP
574 changes in the left hand following iTBS (left hand: $r = .03$, $p = .96$; right hand: $r = -.11$, $p =$
575 $.84$). Taken together, the data imply that there was no tendency towards increased motor
576 performance simply as a result of increased excitability produced by iTBS in the absence of
577 training.

578

579

Discussion

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585

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

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

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

621

622 *Effects of iTBS on subsequent performance and learning*

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

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

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

651

652 *What type of ipsilateral adaptations mediate untrained hand performance?*

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

658 cortex is consistent with evidence from sequencing tasks (Camus et al. 2009; Pascual-Leone
659 et al. 1995; Perez et al. 2007). Moreover, previous work has also shown that transfer of
660 ballistic motor skill can even be accompanied by decreases in excitability of the untrained
661 hemisphere (Duque et al. 2008). A likely mechanism that contributes to enhanced
662 performance in the untrained limb is reduced inter-hemispheric inhibition, which is reduced
663 from the trained to the untrained M1 after various types of sequence learning (Camus et al.
664 2009; Perez et al. 2007), and after strength training (Hortobagyi et al. 2011; see Ruddy and
665 Carson 2013 for a review). Thus, while ballistic motor training reliably potentiates
666 corticospinal excitability in the trained M1 (cf. Liepert et al. 1998; Muellbacher et al. 2001),
667 untrained, left hand performance gains following unilateral practice (i.e., as a result of cross-
668 limb transfer) are not necessarily accompanied by overt changes in excitability in the
669 untrained, right M1. However, the fact that iTBS applied to the untrained hemisphere
670 amplified untrained hand performance gains in the current study suggests that some form of
671 adaptation occurred within the untrained M1 which mediated performance improvements
672 in the untrained hand. In support of this view there is evidence from neuroimaging data on
673 the encoding of (sequential) single finger movements (Diedrichsen et al. 2013; Wiestler et al.
674 2014) demonstrating similar (mirrored) representation patterns in both motor cortices (and
675 sensory motor cortices) that include the same fine-grained details of the movement, but
676 with suppressed BOLD signals (relative to resting baseline) in the motor cortex ipsilateral to
677 the active hand. This raises the possibility that multiple processes may influence ipsilateral
678 cortical function, including generalised suppression of activation (which might underlie the
679 lack of corticospinal excitability we observed) and patterned activation specifically associated
680 with task performance (which might underlie transfer of performance to the untrained limb).

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

704 With respect to potential non-primary contributions to transfer, it has indeed been
705 demonstrated that a broad neural network is involved in cross-limb transfer (Gerloff and
706 Andres 2002; Rizzolatti et al. 1998; Ruddy and Carson 2013), with evidence for bilateral
707 changes in different secondary motor areas (Hardwick et al. 2013; Wiestler and Diedrichsen
708 2013). As such, neural adaptations in other brain regions beyond M1 ipsilateral to the
709 trained limb could account for cross-limb transfer effects observed in the current study. In
710 this case, the stimulation protocol used in the present study would only have affected a part
711 of the acquired skill representation for the untrained limb, and thereby limiting the
712 conclusions drawn here to features of the task represented within M1. For example, Romei
713 and colleagues (Romei et al. 2009) provided evidence that M1 contributes to intrinsic (i.e.,
714 knowledge represented in body-centred coordinates; muscle and joint based) but not
715 extrinsic components (i.e., world-centred coordinates; movement features in external space)
716 of motor skill learning. Therefore, future studies should examine the relative contribution of
717 a more extensive brain network in the untrained cortex to cross-limb adaptations following
718 unilateral practice of different motor tasks (e.g., ballistic, sequential, or reaction time tasks).
719

720 *Conclusions*

721 In sum, the present study suggests that, while occurring simultaneously, motor
722 learning and cross-limb transfer represent distinct neural adaptation processes which
723 interact differently with iTBS. The typical effect of an LTP-like inducing brain stimulation
724 protocol were reversed in the hemisphere projecting to the trained hand, consistent with
725 the suggestion that LTP contributes to ballistic motor learning. That is, motor learning resulted
726 in subsequent iTBS having a LTD-like effect in the trained M1. In contrast, LTP-like effects
727 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

866

867

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

879

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

885

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

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

893

894 **Figure 4. Average normalized MEPs relative to pre-test for cM1, iM1 and sham group**

895 **participants.** MEP amplitudes were tested on the trained (right) hand (circles) and on the
896 untrained (left) hand (triangles) before a first training block (T1), before and after iTBS and
897 after a second training block (T2). MEP amplitudes were normalized to pre-test values. Error
898 bars indicate 95% CI and the X-axis represents pre-test values.

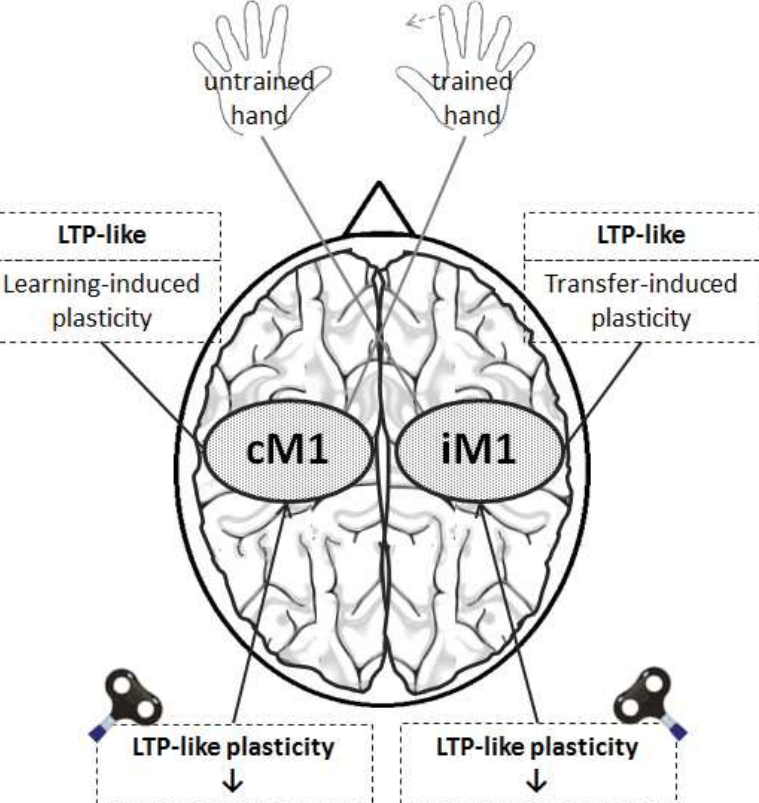
899

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

909

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

A) Learning and Transfer represent similar neural mechanisms



B) Learning and Transfer represent distinct neural mechanisms

