Mirror illusion reduces motor cortical inhibition in the ipsilateral primary motor cortex during forceful unilateral muscle contractions

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Running title: Mirror-viewing reduces ipsilateral M1 inhibition

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Abstract

Forceful, unilateral contractions modulate corticomotor paths targeting the resting, contralateral hand. However, it is unknown if mirror-viewing of a slowly moving but forcefully contracting hand would additionally affect these paths. Here we examined corticospinal excitability and short-interval intracortical inhibition (SICI) of the right-ipsilateral primary motor cortex (M1) in healthy young adults under a no-mirror and mirror condition at rest and during right wrist flexion at 60% maximal voluntary contraction (MVC). During the no-mirror conditions, neither hand was visible, whereas in the mirror conditions, participants looked at the right hand's reflection in the mirror. Corticospinal excitability increased during contractions in the left flexor carpi radialis (FCR) (contraction: 0.41 mV vs. rest: 0.21 mV) and extensor carpi radialis (ECR) (contraction: 0.56 mV vs. rest: 0.39 mV) but there was no mirror effect (FCR: P=0.743; η_P^2=0.005, ECR: P=0.712; η_P^2=0.005). However, mirror-viewing of the contracting and moving wrist attenuated SICI relative to test pulse in the left FCR by ~9% compared with the other conditions (P<0.05; d≥0.62). Electromyographic activity in the resting left hand prior to stimulation was not affected by the mirror (FCR: P=0.255; η_P^2=0.049; ECR: P=0.343; η_P^2=0.035), but increased two-fold during contractions. Thus, viewing the moving hand in the mirror and not just the mirror image of the non-moving hand seems to affect motor cortical inhibitory networks in the M1 associated with the mirror image. Future studies should determine if the use of a mirror could increase inter-limb transfer produced by cross-education, especially in patients groups with unilateral orthopaedic and neurological conditions.

Keywords: cross-education, strength training, mirror training, mirror-neuron system, primary motor cortex, transcranial magnetic stimulation

1. Introduction

Action observation generates an internal replica of that action in the observer's motor system without causing overt motor actions (4, 5). Observation of a motor act performed by oneself, observation of a motor act performed by someone else, viewing a motor act in a mirror (which is often the case in dance and sport practice) all activate the same neural structures as the actual movement execution, producing subliminal facilitation of neurons forming the motor neural network (7, 12, 44). The subliminal engagement of neurons might have an adaptive role in motor learning (34) and therefore action observation seems to be a potential tool to facilitate motor learning.

A specific form of motor practice that makes use of action observation is mirror training. In mirror training, the practicing limb's image is superimposed over the resting limb (40, 49), creating the illusion in the mirror that the resting limb is moving. Mirror training is known to reduce phantom limb pain (54, 55), enhance recovery of motor function of the paretic lower (65) and upper extremity (42, 71) following a stroke, and can also facilitate skill acquisition of the non-trained hand in healthy participants (24, 37, 49). The benefits of mirror training are widely accepted but the mechanisms responsible for these beneficial effects are unclear. Although viewing a movement in the form of action observation can activate, for example, the primary motor cortex (M1); but whether or not and how such activation serves as a neural contribution for the beneficial effects of mirror training has not yet verified (37, 49).

Mirror training exerts a strong influence on the motor network, mainly through the increased activation of areas involved in the allocation of attention and cognitive control (13). There is evidence that mirror-viewing of hand and finger movements performed at a fraction of the maximal voluntary force can facilitate ipsilateral corticospinal excitability (23) and

corticomotor activity (61) compared with a no-vision condition. The increased activation of the ipsilateral M1 (48, 60) and the increased excitability of the corticospinal path targeting the resting hand (21, 27, 28, 30, 45, 52, 53) are also observerd for forceful unilateral contractions without a mirror, however, it is unknown if the visual illusion of a slowly moving, forcefully contracting wrist in the mirror can additionally affect corticospinal excitability and motor cortical activity in the hemisphere ipsilateral to the moving hand. Such information is needed as a first step to explain how mirror-viewing could augment interlimb strength transfer, a viable treatment option for patients with unilateral orthopaedic and neurlogical impairments (19).

The purpose of the present study was to determine the effects of mirror-viewing of the resting and contracting right wrist on corticospinal excitability and short-interval intracortical inhibition (SICI), assessed with transcranial magnetic stimulation (TMS) in the resting left flexor carpi radialis (FCR) and extensor carpi radialis (ECR). The ECR was measured to determine if the observed responses to TMS would provide evidence for a directional specificity of excitability related to the mirror illusion. We suspect that mirror-viewing of the right wrist's movement (however monotonic, slow, and low-skill) creates the illusion in the ipsilateral M1 that the resting left wrist is actually moving and this illusion, a surrogate for actual movement, triggers the increase in ipsilateral M1 excitability. If this assumption is correct then we predict a mirror effect to increase neuronal excitability during a contraction that is caused by the illusion of the left hand moving but no mirror effect at rest because the trigger, i.e., movement illusion, for modulating excitability, is absent.

Materials and Methods

Participants

Twenty-seven right-handed (average handedness score 95%, (50)) healthy volunteers (22 men, 5 women) with a mean (± SD) age, height, mass and body mass index of 27 years (± 7), 1.76 m (± 0.07), 76.0 kg (± 13.0), and 24.4 kg/m^2 (± 2.9), respectively, participated in the study. Prior to testing, participants completed a comprehensive screening questionnaire to determine medical (screening standard questionnaire for TMS (57)) and experimental (i.e., previous fracture in arm or hand, pain in arm or hand) contraindications to the protocol. All participants provided written informed consent to the experimental procedure, which was approved by the University's Research Ethics Committee and in accordance with the Declaration of Helsinki.

Experimental setup

One week before the main experiment, participants visited the laboratory for a 30-minute familiarization trial to be accustomed with the laboratory setting and TMS. During the experiment, which lasted approximately 1.5 h, the participant sat comfortably in a chair with both forearms resting on a custom-built table. The lever arm of an isokinetic dynamometer (Biodex Medical Systems, Shirley, NY, USA) was aligned and configured so that the participant was able to perform shortening contractions of the right wrist flexors in the transversal plane over the table surface. Contractions were performed at 20°/s and started with the wrist at 20° extension and ended with the wrist at 20° flexion (ensuring a total range of motion of 40°). The participant touched the lever arm in the sagittal plane with the thumb upper most and the fingers extended to avoid finger flexion during wrist flexion. Participants performed shortening wrist flexion contractions with the right hand by pressing at the metacarpophalangeal joint on a plastic covered manipulandum that projected vertically

downward toward the table surface. The distance between the axis of rotation and the metacarpophalangeal joint position on the manipulandum was held at a constant length between conditions for each participant, but was adjusted between participants to account for anatomical differences. For the resting conditions, the participant touched the lever arm in neutral position, meaning that the right wrist was in anatomical zero (0°) position.

The experiment started with recording the torque produced during a shortening maximal voluntary contraction (MVC) of the right wrist flexors. Thereafter, participants placed the left and right forearms inside two different boxes. The right box was open on the left side, but was positioned in a way that prevented the participant from seeing the right hand directly. Depending on the experimental condition, a cardboard wall (no-mirror condition) or a mirror (mirror condition) was mounted on the central vertical wall of the left box and aligned in the sagittal plane in front of the participant. The cardboard and the mirror were used to either prevent seeing, or to create a mirror image of the right hand, thereby giving the illusion that the left hand was being observed (Fig. 1). To maintain a constant position of the head, participants focused on a dot placed on the cardboard wall at a position that equated to the gaze of the participant when viewing the mirror image of their right hand.

Approximately 20 minutes after the MVCs, TMS was delivered to measure corticospinal excitability and short-interval intracortical inhibition (SICI) of the right M1 in four different conditions namely, the mirror and no-mirror condition at rest and during a forceful shortening contraction of the dominant-right wrist flexors at 60% MVC. TMS was delivered when the right wrist was in anatomical zero (0°) position (no-mirror and mirror resting condition) or when the right wrist passed anatomical zero position (no-mirror and mirror contraction condition). The left arm was placed in the same anatomical position as the right arm during

all conditions, and any adornments (e.g., jewellery, watches) were removed for the duration of the experiment. The order of conditions was randomized between participants. Participants received verbal feedback from one of the researchers to reach the target torque that appeared on the dynamometer's monitor, but visual feedback was not provided at any point. Data acquisition was initiated 30 ms before the TMS stimulus was delivered. The TMS protocol was in adherence to current safety and ethical guidelines (57) and all items on the methodology checklist that pertain to paired pulse TMS have been reported and controlled (9). It remains unclear if corticospinal excitability and SICI are affected by associated activity (i.e., the electromyogram [EMG] activity of the contralateral resting muscles during a unilateral muscle contraction) and because participants were less able to prevent associated activity at higher force levels (74), we used 60% MVC as the target contraction intensity to minimize the influence of associated activity on corticospinal excitability and SICI. During the experimental conditions, participants were frequently reminded to completely relax the left arm when performing shortening right wrist flexion movements. Trials in which the associated left FCR or left ECR activity exceeded the background noise level of 25 μV were excluded from the analyses (28, 45, 53). Thereafter and for all variables, outliers were identified with a modified and more stringent version of the interquartile range method, marking values below Q1 – 1.5 * (Q2 - Q1) and values above Q3 + 1.5 * (Q3 - Q2) as outliers. All outliers were excluded from further analysis.

Maximum voluntary contraction

After a warm-up consisting of one set of 10 shortening muscle contractions at individually estimated 50% MVC, participants performed a further three shortening right wrist flexion MVCs followed by three shortening left wrist flexion MVCs. MVCs were recorded at the same movement speed (20°/s) and range of motion (20° wrist extension to 20° wrist flexion)

as during the task. The torque was recorded when the wrist passed anatomical zero for each MVC; the highest of the three contractions was recorded as the MVC. After completion of the experiment we measured shortening right wrist flexion MVC in a subsample of participants ($N = 5$) to examine the potential existence of fatigue.

Magnetic stimulation of the primary motor cortex

To evoke motor-evoked potentials (MEPs), TMS was delivered from a magnetic stimulator (Magstim 200^2; Magstim Company Ltd, Carmarthenshire, UK) through a figure-of-eight remote control coil (loop diameter 9 cm; Magstim, Spring Gardens, Wales, UK) with a monophasic current waveform. Paired pulses were produced with the addition of a second Magstim 200^2 stimulator equipped with a BiStim^2 timing module, and pulses were delivered through the same figure-of-eight coil. The coil was placed over M1 and was moved in 0.5-cm steps over the M1 to identify the optimal scalp position, i.e., hotspot, for activation of the left FCR overlying right M1. The hotspot targeting the left FCR is also able to produce stable MEPs in the left ECR (6, 38). The hotspot correlates well with the stimulation of Brodmann's area 4 (43). The coil was held with the handle pointing backwards and 45º away from the midline so the direction of the current induced in the brain was from posterior to anterior. Initially the "hotspot" was located on each participant. The hotspot was defined as the optimal position of the coil on the scalp where the lowest threshold is capable of evoking the biggest potential in the targeted muscle (58). The hotspot was marked with a marker pen to ensure constant positioning throughout the experiment. After the hotspot had been identified, resting motor threshold (rMT) was determined as the lowest stimulator intensity to produce an MEP of ≥ 50 μV in the target muscle in 5 out of 10 trials (58).

201 *Corticospinal excitability and SICI right M1*

202 To determine the effect of mirror-viewing on corticospinal excitability and SICI of the right
203 M1 during rest and shortening right wrist flexion, single pulse (to measure corticospinal
204 excitability) and paired pulse (to measure SICI) TMS was presented in random order for the
205 mirror and no-mirror conditions. During all conditions, the MEP amplitude determining
206 corticospinal excitability and SICI was measured in the resting left FCR and ECR. We
207 measured corticospinal excitability by a single TMS pulse delivered at a supra-threshold
208 intensity of 120% rMT, as part of the SICI measurement. For measuring SICI a sub-
209 threshold conditioning pulse at 80% rMT, an intensity sufficient to produce intracortical
210 inhibition (28, 53), preceded the supra-threshold test pulse of 120% rMT with an
211 interstimulus interval of 2 ms (36). The 2 ms interstimulus interval was used to create a deep
212 amount of inhibition (36) and to avoid a mixture of the two distinct phases of inhibition (20).
213 A total of 20 MEPs were evoked in each condition, 10 MEPs for measuring corticospinal
214 excitability and 10 MEPs for measuring SICI, with an interval of ~5 s between stimuli. For
215 determining SICI the conditioned MEPs were expressed relative to the MEPs from the
216 unconditioned test pulse.

217

218 *Surface EMG*

219 Surface EMG was recorded from the left and right FCR and ECR to quantify voluntary
220 muscle activity during the experimental conditions and evoked responses (MEPs) from TMS.
221 After the skin surface was shaved and cleaned with an alcohol wipe, electrodes (model
222 1041PTS; Kendall, Tyco Healthcare Group, Mansfield, MA, USA) were placed on the
223 muscle belly (inter-electrode distance, 2 cm) with the ground electrode fixed on the distal
224 styloid process of the left radius. Surface EMG was band-passed filtered at 20-2000 Hz,
225 amplified ×1000 (CED 1902, Cambridge Electronic Design, Cambridge, UK Digitimer,

226 Hertfordshire, UK), sampled at 5 kHz (CED Power 1401; Cambridge Electronic Design,
227 Cambridge, UK) and recorded on a personal computer. MEPs were analyzed off-line for
228 peak-to-peak amplitude (Signal, v.5.04; Cambridge Electronic Design). The mean surface
229 EMG, expressed relative to the EMG activity during shortening wrist flexion MVC, was
230 rectified and computed over a 30 ms period prior to the stimulation artifact.
231
232 *Statistical analyses*
233 Data in the text and figures are presented as mean ± SD. The normal distribution for each
234 variable was tested with the Kolmogorov-Smirnov test. For all variables except for torque, a
235 log transformation was applied to correct for a positively skewed distribution of the data.
236
237 The main analysis addressing the hypothesis that mirror-viewing of a moving and forcefully
238 contracting hand increases ipsilateral M1 excitability, was a State (rest, contraction) by
239 Condition (no-mirror, mirror) ANOVA with repeated measures on both factors. We
240 performed this main analysis for each of the following variables: corticospinal excitability,
241 SICI, surface EMG activity in the left and right FCR and ECR, respectively. We also used a
242 one-way repeated measures ANOVA with five levels to determine if wrist flexion torque of
243 60% MVC was similar during the mirror and no-mirror condition in which we measured CSE
244 and SICI. We performed Tukey HSD *post hoc* pairwise comparison to determine the means
245 that were different.
246
247 To verify that fatigue did not affect the results, a paired-samples t-test was used to determine
248 if the maximal torque was similar at the start and end of the experiment. For the mirror and
249 no-mirror condition, a Pearson's correlation analysis was used to determine if the change in
250 corticospinal excitability and SICI relative to rest was correlated with the associated activity

measured in the left ('resting') FCR. For all four conditions, an additional Pearson's correlation analysis was performed to test if surface EMG recorded from the right and left wrist were correlated. For Pearson's product correlations we used the non-transformed data. Significance was accepted as $P < 0.05$. For main effects partial eta squared was calculated as a measure of effect size with cut-offs ≥ 0.01 small, ≥ 0.06 medium, and ≥ 0.14 large (11).

Results

Table 1 shows the descriptive data for the four conditions. The main results were that viewing the mirror at rest did not affect TMS metrics but viewing the mirror while contracting the right wrist flexors reduced SICI in the left wrist flexors but not in the antagonist wrist extensors. These results were obtained under experimental conditions that were well controlled for muscle EMG activity and the level of torque subjects generated.

Torque. The torque produced during right wrist shortening contractions successfully attained the 60% MVC target torque and was similar for corticospinal excitability and SICI measured with and without the mirror ($F_{3,26} = 0.8$; $P = 0.513$). Also, the maximal torque production at the start (12.6 ± 3.9 Nm) was not different from the maximal torque produced at the end of the experiment (13.1 ± 4.5 Nm; $t_{(4)} = -0.845$; $P = 0.446$) indicating the protocol did not induce fatigue.

Corticospinal excitability. Figure 2A shows a representative trace of MEPs for a single participant and Fig. 2B shows the group data illustrating corticospinal excitability of the right M1 recorded from the left FCR for the mirror and no-mirror condition when both hands were at rest and during contraction. The State (rest, contraction) by Condition (no-mirror, mirror) repeated measures ANOVA showed that corticospinal excitability was higher in both FCR

($F_{1,26}$ = 77.5; P < 0.001; η_P^2= 0.749) and ECR ($F_{1,26}$ = 27.0; P < 0.001; η_P^2= 0.510) during contraction compared to rest (FCR +105%, ECR +47%), but there was no effect of mirror for either muscle (FCR: $F_{1,26}$ = 0.1; P = 0.734; η_P^2= 0.005, ECR: $F_{1,26}$ = 0.1; P = 0.712; η_P^2= 0.005).

SICI. Figure 3A illustrates a representative trace of MEPs illustrating SICI for a single participant, and Fig. 3B and 3C show the SICI group data, evoked in the right M1 and recorded from the left FCR, for the four different conditions. There was no State ($F_{1,26}$ = 3.6; P = 0.070; η_P^2= 0.120) nor Condition ($F_{1,26}$ = 2.9; P = 0.101; η_P^2= 0.100) main effect but there was State by Condition interaction ($F_{1,26}$ = 6.9; P = 0.014; η_P^2= 0.209) for SICI recorded from the left FCR. Post-hoc analysis revealed that there was ~9% less SICI only when subjects contracted the right wrist flexors while viewing the wrist flexion movement in the mirror (P < 0.05; $d \geq 0.62$). No State ($F_{1,26}$ = 0.9; P = 0.347; η_P^2= 0.034), Condition ($F_{1,26}$ = 0.1; P = 0.782; η_P^2= 0.003), nor State by Condition interaction ($F_{1,26}$ = 0.2; P = 0.676; η_P^2= 0.007) was observed for SICI recorded from the left ECR.

EMG responses in the resting left limb. The ongoing EMG activity in the "resting" left FCR and ECR prior to stimulation was 43% higher during contraction of the contralateral limb compared to at rest (FCR: $F_{1,26}$ = 32.4; P < 0.001; η_P^2= 0.555, ECR: $F_{1,26}$ = 15.1; P = 0.001; η_P^2= 0.368, Fig. 4A). No effect of viewing the limb in the mirror (FCR: $F_{1,26}$ = 1.4; P = 0.255; η_P^2= 0.049; ECR: $F_{1,26}$ = 0.9; P = 0.343; η_P^2= 0.035) nor state by condition interaction (FCR: $F_{1,26}$ = 0.4; P = 0.521; η_P^2= 0.016; ECR: $F_{1,26}$ = 0.9; P = 0.343; η_P^2= 0.035) was observed.

EMG responses in the right limb. The EMG activity present in the right FCR (0.119 ± 0.055 mV) was substantially greater than the EMG activity in the right ECR (0.026 ± 0.013 mV) during shortening right wrist flexion contractions. Mean surface EMG of the right FCR was higher during contractions compared to rest ($F_{1,26}$ = 1030.9; P < 0.001; η_P^2= 0.975) but was not affected by the mirror ($F_{1,26}$ = 0.290; P = 0.595; η_P^2= 0.011). For the mean surface EMG of the right ECR, a State ($F_{1,26}$ = 440.6; P < 0.001; η_P^2= 0.944), Condition ($F_{1,26}$ = 13.4; P = 0.001; η_P^2= 0.341), and State by Feedback interaction effect ($F_{1,26}$ = 23.4; P < 0.001; η_P^2= 0.473) was observed. Post hoc analysis revealed that EMG activity of the right ECR was not different for the mirror and no-mirror contraction condition (P > 0.05), but was 80% higher for the mirror compared with the no-mirror condition at rest (P < 0.05, Fig. 4B).

Relationships between TMS responses and EMG activity in the resting left limb. Figure 5 shows the relationship for the mirror and no-mirror viewing condition between the change in corticospinal excitability relative to rest and the change in surface EMG of the left (non-contracting) FCR relative to rest. The change in corticospinal excitability was positively correlated to the change in surface EMG activity for the mirror but not for the no-mirror condition (mirror: r = 0.496, P = 0.009; no-mirror: r = 0.297, P = 0.132). No correlation was found between the change in SICI relative to rest and the change in surface EMG activity relative to rest for the mirror and no-mirror condition (mirror: r = 0.042, P = 0.833; no-mirror: r = 0.175, P = 0.383).

Relationships between EMG activity in the left and right limb. The amount of EMG activity of the resting left limb was unrelated to the amount of surface EMG of the right limb for both FCR (no-mirror, rest: r = -0.075, P = 0.711; mirror, rest: r = 0.135, P = 0.501; no-mirror, contraction: r = 0.121, P = 0.548; mirror, contraction: r = 0.378, P = 0.052) and ECR

(no-mirror, rest: $r = 0.070$, $P = 0.728$; mirror, rest: $r = 0.318$, $P = 0.106$; no-mirror, contraction: $r = -0.061$, $P = 0.762$; mirror, contraction: $r = 0.291$, $P = 0.140$).

Discussion

We tested the hypothesis that mirror-viewing of the right wrist's flexion movement creates the illusion in the ipsilateral M1 that the resting left wrist is actually moving, and this illusion changes neuronal excitability in healthy young adults. We demonstrate for the first time that performing slow, monotonic, and effortful wrist flexion while looking at the mirror image of the moving right hand reduced inhibition in the left FCR, but not ECR, when compared with the no-mirror contraction and resting conditions with and without a mirror. The data are consistent with the idea that the illusion of the left hand moving and not the mirror image of the resting hand triggered the reduction in motor cortical excitability in the right-ipsilateral M1. The absence of an effect in the ECR indicates that the mirror seems to affect only the homologous agonist but not the antagonist projections. Mirror-viewing did not affect corticospinal excitability during contraction and at rest.

The results of the present study are consistent with the preponderance of data showing that mirror-viewing has little or no effect on corticospinal excitability during motor activity (6, 22, 56). For example, the use of a mirror does not seem to interact with contraction intensity or the nature of the contraction (static: (56); dynamic: (6, 22)). However, there is also evidence for a ~25% increase in ipsilateral M1 corticospinal excitability in conjunction with viewing the isometrically contracting index finger (~20% MVC) in a mirror (23). The cause of the discrepant data is unclear, considering that the experimental and recording conditions were similar in two studies, one showing an increase (Garry et al (23)) the other showing no effect (Reissig et al (56)). The insensitivity of corticospinal excitability to mirror-viewing in

the present study may be related to a saturation effect. Conceivably, the strong (60% MVC) muscle contraction produced peri-maximal level of excitation in the ipsilateral corticospinal path so that mirror-viewing of the contracting hand could not further increase excitability compared with the no-mirror condition.

The present data are the first to document that SICI in the right-ipsilateral M1 is modulated when a forceful right-handed unilateral contraction is performed whilst viewing the slowly moving wrist in the mirror. Previous studies have shown that SICI in the right-ipsilateral M1 decreased with increasing isometric right wrist flexion force (53), and decreased during shortening wrist flexion contractions compared to rest (28), and decreased during forceful lengthening compared to shortening wrist flexion contractions (28). SICI in the no-mirror condition showed that contractions at 60% MVC did not affect SICI compared with rest. However, uniquely we demonstrate that mirror-viewing of the slowly moving and contracting hand decreased SICI in the right-ipsilateral M1, suggesting that it is not the contraction itself, but the visual illusion of a moving left hand that modulates SICI. In support of this, a previous study showed mirror-viewing of isometric index finger abductions did not change ipsilateral SICI compared with the no-vision and other visual feedback conditions (56); hence, to create a mirror illusion and modulate SICI, it would seem the viewed image must be moving.

The premotor cortex, an area engaged in the modulation of M1 interneuron activity (46), plays a significant role in the visual guidance of upper limb movements (70) and is therefore involved in mirror training (24). Thus, it is possible that the modulatory effects of the premotor cortex on M1 interneurons caused the mirror-induced effect on SICI. In addition to the increased activation of the right-ipsilateral dorsal premotor cortex, Hamzei and colleagues

375 (24) observed an increased activation of the left supplementary motor area following mirror
376 training; an area known to be important in bimanual coordination (15, 62). The present study
377 focused on the M1, an area also known to be involved in the control of bimanual coordination
378 (15). There is evidence that SICI contributes to the regulation of bimanual coordination (63,
379 64). Therefore, this collective evidence of attenuated SICI together with the increased
380 activation of the supplementary motor areas (24) following mirror training suggests that
381 mirror-viewing of the exercising hand creates the illusion of a synchronous bimanual
382 movement (i.e., wrist flexion with the right hand and an illusionary wrist flexion movement
383 observed in the left hand).
384
385 An additional cortical structure that responds to the mirror image of a moving limb, but not
386 measured in the present study, is the superior temporal gyrus. Visual information is
387 processed differently when unilateral motor practice is performed with and without viewing a
388 mirror (40, 41, 69). During mirror training with the right arm, visual input is directed
389 towards both occipital lobes with the concomitant activation of the right-ipsilateral precuneus
390 (41, 69) and superior temporal gyrus (40). The superior temporal sulcus has similar
391 coordinates to the superior temporal gyrus (40), which is a core element of the mirror-neuron
392 system involved in the processing of visual information (31, 32), whereas the precuneus
393 seems to be involved in mediating visuomotor transformations (14). The fact that visual
394 information is solely processed in the ipsilateral hemisphere corresponding to the mirror
395 image, implies that the mirror creates the visual illusion as if participants exercised the left
396 hand. Although not measured in the current experiment, there is evidence that the anterior
397 portion of the corpus callosum, involved in interhemispheric inhibition (IHI), contributes to
398 the integration of perception and action within a subcortico-cortical network creating a
399 unified experience of how we perceive the visual world and prepare our actions (59). It is

suggested that stimulus-driven activity in one hemisphere suppresses activity in the opposite hemisphere by increasing the amount of IHI (1, 8). The illusion of a moving left hand while mirror-viewing the moving right hand might cause a shift in attention to the ipsilateral hemisphere to process the visual information associated with the mirror image.

During a unilateral contraction there is normally some inadvertent, so-called associated activity in the resting contralateral muscle (60, 68, 73, 74). Viewing the mirror did not affect the magnitude of associated activity in the left FCR and antagonist ECR. Although we repeated the instruction to the participant to keep their left hand relaxed, the magnitude of EMG activity was twofold during contractions compared with rest and was higher for the ECR than FCR. The associated activity, relative to the EMG activity at rest, was slightly higher than in some previous work examining unilateral wrist contractions (60) but the absolute values were still low compared with other unilateral contraction studies (25, 73, 74). The source of this associated activity is unclear but bilateral M1 activation (73) together with the bilateral activation of the SMA and cerebellum (60) are thought to give rise to associated activity. Our data favor the idea that associated activity comes from the concomitant activation of both hemispheres, both M1s in particular. We found a strong and significant correlation ($r = 0.496$) between the associated activity and the increase in corticospinal excitability of the right-ipsilateral M1 compared with rest for the mirror and a moderate but non-significant correlation ($r = 0.297$) for the no-mirror condition (Fig. 5). This correlation implies that there is a link between the magnitude of corticospinal excitability and the amount of associated activity and that this link is strengthened when the contracting right hand is viewed in the mirror. Thereby, mirror-viewing of the contracting right hand resulted in a borderline significant correlation between EMG activity of the left (i.e., associated activity) and right agonist FCR. Altogether, mirror-viewing of the contracting right hand strengthens

the connectivity between the contracting agonist and contralateral homologous muscle, possibly via a mirror-induced modulation of the link between bilateral M1 activation and amount of associated activity.

Mirror-viewing of a unilateral muscle contraction affected SICI but not associated activity in the current study. Thus, a lack of change in associated activity strengthens the idea that the activity that modulates SICI in response to mirror-viewing arises in the ipsilateral M1. However, without measuring IHI, we cannot specifically ascertain if this modulation occurs as a process intrinsic to ipsilateral M1, through IHI, or both. Future studies will have to disentangle the effects of mirror-viewing on associated activity and IHI to better understand the mechanism of how mirror-viewing works and could be applied to clinical conditions.

Limitations. The anterior corticospinal tract, which does not cross the medulla and occupies 5-15% of the entire corticospinal tract, has been proposed as a motor recovery pathway from the unaffected M1 to the affected extremities (33). It is hypothesized that this ipsilateral motor pathway might be facilitated by mirror training (13), so for our study this would mean that mirror-viewing not only affected the right-ipsilateral but also the left-contralateral M1, an area we did not examine. Another interesting aspect that is missing is the comparison between an active vision condition, where participants directly viewed the contracting right hand, and the mirror condition where participants observed the contracting right hand in the mirror. Previous work showed that ipsilateral M1 corticospinal excitability was not different between these two conditions during a static movement (23, 56) but during a dynamic movement, ipsilateral corticospinal excitability (35) and ipsilateral M1 activity (67) were significantly higher for the mirror condition. This again underpins the notion that the observed image must be dynamic to induce a mirror effect and although we have not tested

450 the hypothesis, we expect that mirror-viewing of a wrist flexion increases corticospinal
451 excitability compared with an active vision condition.
452
453 **Implications for practice.** Mirror training is used in the treatment of chronic pain conditions
454 (3) and to improve motor function after stroke (66). Somewhat surprisingly, recent work
455 without a mirror showed that strength training of the unaffected limb is beneficial for the
456 recovery of the impaired limb after stroke (10, 16, 17), wrist fractures (39), and anterior
457 cruciate ligament reconstructive surgery (51). The performance improvement in the
458 contralateral homologous muscle of the non-trained limb following a period of effortful
459 unilateral motor practice is referred to as cross-education (18, 26, 47, 72), but there may be
460 additional clinical benefits from the hypothesis that unilateral strength training with a mirror
461 could augment the cross-education of muscle strength (29, 75). Reduction in SICI observed
462 in the present study could be one mechanism to explain how the use of mirror increases the
463 transfer effect reported in cross-education studies.
464
465 In summary, viewing one's own right hand in a mirror, appearing as the left hand, during a
466 slow but forceful muscle contraction, reduces one form of intra-cortical inhibition (SICI) in
467 the right-ipsilateral M1. This modulation of SICI was specific to the left FCR, the
468 contralateral homolog of the task muscle on the right side. The use of a mirror, however, did
469 not affect corticospinal excitability of the right M1 and the associated activity in the homolog
470 FCR and non-homolog ECR. Thus, viewing the moving hand and not just the mirror image
471 of the non-moving hand seems to affect motor cortical inhibitory networks in the hemisphere
472 associated with the mirror image. These acute mirror-induced changes support the idea that
473 mirror-aided unilateral strength training might be more effective than unilateral strength
474 training without a mirror for accelerating functional recovery from unilateral impairments.

Future studies should determine if the use of a mirror could increase inter-limb transfer produced by cross-education, especially in patients populations with unilateral orthopaedic and neurological conditions.

Acknowledgements

The authors thank Koen Huits for creating the artwork for Fig. 1.

Grants

This work was supported by a start-up fund from the University Medical Center Groningen.

Disclosures

The authors declare no conflicts of interest, financial or otherwise.

Author contributions

Author contributions: T.Z., S.G., T.H., and G.H. conception and design of research; T.Z., S.G., K.T., and G.H. performed experiments; T.Z., analyzed data: T.Z., S.G., K.T., T.H., and G.H interpreted results of experiments; T.Z. and S.G. prepared figures; T.Z., S.G., K.T., T.H., and G.H. drafted manuscript; T.Z., S.G., T.H., and G.H. edited and revised manuscript; approved final version of manuscript.

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697

698

Table

Table 1. Descriptive data for the four experimental conditions.

Condition	Torque[a] (Nm)	Torque[b] (Nm)	CSE left FCR (mV)	CSE left ECR (mV)	SICI[c] left FCR (% of control)	SICI[c] left ECR (% of control)	EMG left FCR (mV)	EMG left ECR (mV)	EMG right FCR (mV)	EMG right ECR (mV)
No-mirror, rest	N/A	N/A	0.20 (0.15)	0.40 (0.44)	39.1 (23.3)	57.0 (25.5)	0.0010 (0.0003)	0.0035 (0.0034)	0.0017 (0.0023)	0.0015 (0.0012)
Mirror, rest	N/A	N/A	0.21 (0.14)	0.37 (0.33)	38.4 (24.4)	56.2 (21.8)	0.0011 (0.0004)	0.0031 (0.00265)	0.0019 (0.0030)	0.0027 (0.0019)‡
No-mirror, contraction	7.8 (2.3)	7.8 (2.3)	0.43 (0.29)*	0.58 (0.44)*	37.8 (16.2)	58.8 (22.0)	0.0021 (0.0021)*	0.0054 (0.0040)*	0.1159 (0.0494)*	0.0270 (0.0137)*
Mirror, contraction	7.9 (2.4)	7.8 (2.3)	0.41 (0.26)*	0.55 (0.32)*	46.9 (18.9)†	58.9 (17.4)	0.0021 (0.0018)*	0.0042 (0.0025)*	0.1227 (0.0601)*	0.0245 (0.0128)*

714 Values are mean (SD). CSE, corticospinal excitability; ECR, extensor carpi radialis; EMG, electromyogram; FCR, flexor carpi radialis; MVC,
715 maximal voluntary contraction; N/A, not applicable; SICI, short-interval intracortical inhibition; [a], torque recorded at the moment of stimulation
716 for measuring corticospinal excitability; [b], torque recorded at the moment of stimulation for measuring SICI; [c], a higher value means less

717 inhibition; *, compared with the resting conditions ($P < 0.001$); †, compared with all other conditions ($P < 0.05$); ‡, compared with the no-

718 mirror resting condition ($P < 0.05$).

719 **Figure captions**

720 Figure 1. Experimental setup at rest (Panel A) and during a forceful shortening contraction of
721 the right wrist flexors (Panel B). Both forearms were rested on a built table and placed inside
722 two different boxes that blocked the view of the participant. (i) The mirror mounted on the
723 central vertical wall of the left box created the illusion of the left hand moving by mirror-
724 viewing the right hand. (ii) The no-mirror condition had a cardboard wall mounted on the
725 central vertical wall of the left box.

726

727 Figure 2. Corticospinal excitability of the right primary motor cortex recorded from the left
728 flexor carpi radialis. A representative trace (Panel A) of motor-evoked potentials (MEPs)
729 from a single participant. Mean (±SD) MEP (Panel B) size for the four different conditions.
730 NM_{rest}: both hands at rest with vision of both hands blocked; $Mirror_{rest}$: both hands at rest
731 while mirror-viewing the right hand; $NM_{contraction}$: left hand at rest while the right hand
732 performed shortening wrist flexion contractions with vision of both hands blocked;
733 $Mirror_{contraction}$: left hand at rest while mirror-viewing of shortening right wrist flexion
734 contractions. * Significantly different to corticospinal excitability in resting conditions ($P <$
735 0.001; $N = 27$).

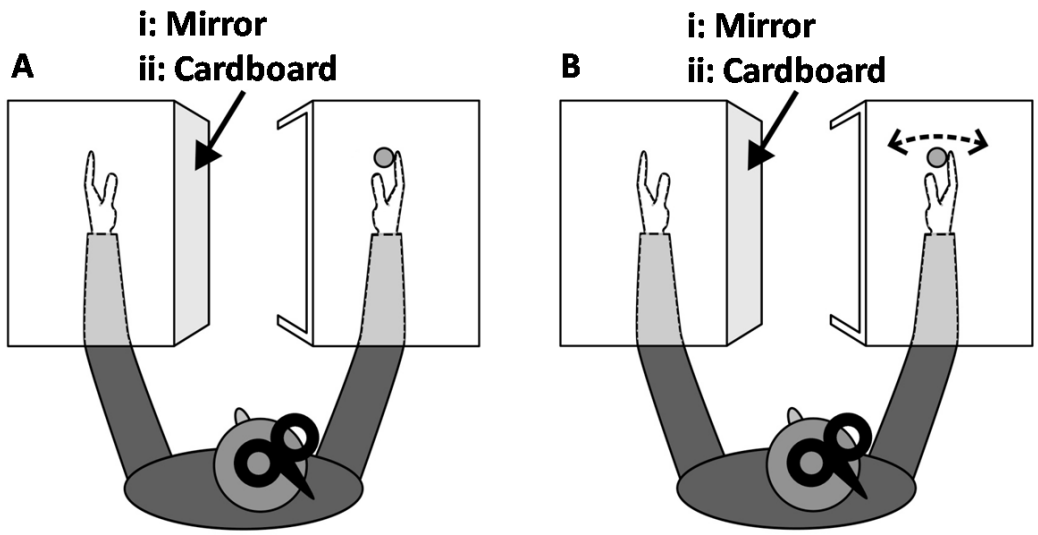
736

737 Figure 3. Short-interval intracortical inhibition (SICI) in the right primary motor cortex
738 recorded from the left flexor carpi radials. A higher value means less SICI. Representative
739 trace (Panel A) of motor-evoked potentials (MEPs) of a single participant, each tracing
740 comprises one trial; control MEP (solid line), conditioned MEP illustrating SICI (dotted line).
741 Mean (±SD) percentage of SICI relative to control (Panel B). The horizontal dashed line at
742 100% represents the control value, i.e., absence of inhibition or facilitation. Individual
743 percentage difference of SICI between the mirror and no-mirror condition (Panel C) at rest

744 (white bars) and during contraction (black bars). A positive value means a mirror image
745 induced reduction of SICI, whereas a negative value means a mirror image induced increase
746 of SICI. NM_{rest}: both hands at rest with vision of both hands blocked; $Mirror_{rest}$: both hands
747 at rest while mirror-viewing the right hand; $NM_{contraction}$: left hand at rest while the right hand
748 performed shortening wrist flexion contractions with vision of both hands blocked;
749 $Mirror_{contraction}$: left hand at rest while mirror-viewing of shortening right wrist flexion
750 contractions. * Significantly different to SICI in all other conditions ($P < 0.05$; $N = 27$).
751
752 Figure 4. Mean (±SD) surface electromyogram (EMG), expressed relative to the EMG
753 activity of a maximal shortening wrist flexion contraction. Panel A; mean surface EMG for
754 the left FCR (white bars) and left ECR (black bars) for the four different conditions ($N = 27$).
755 Panel B; surface EMG for the right FCR (white bars) and right ECR (black bars) for the four
756 different conditions ($N = 27$). NM_{rest}: both hands at rest with vision of both hands blocked;
757 $Mirror_{rest}$: both hands at rest while mirror-viewing the right hand; $NM_{contraction}$: left hand at
758 rest while the right hand performed shortening wrist flexion contractions with vision of both
759 hands blocked; $Mirror_{contraction}$: left hand at rest while mirror-viewing of shortening right wrist
760 flexion contractions. * Significantly different to surface EMG in the resting conditions ($P <$
761 0.001) and with the no-mirror resting condition ($P < 0.05$).
762
763 Figure 5. Relationship for the mirror and no-mirror condition between the change in
764 corticospinal excitability relative to rest and the change in associated activity of the left flexor
765 carpi radialis relative to rest . The change in corticospinal excitability was positively
766 correlated to the change in surface EMG activity for the mirror but not for the no-mirror
767 condition (mirror: $r = 0.496$, $P = 0.009$; no-mirror: $r = 0.297$, $P = 0.132$; $N = 27$).
768

Figures

Figure 1.



787 *Figure 2.*

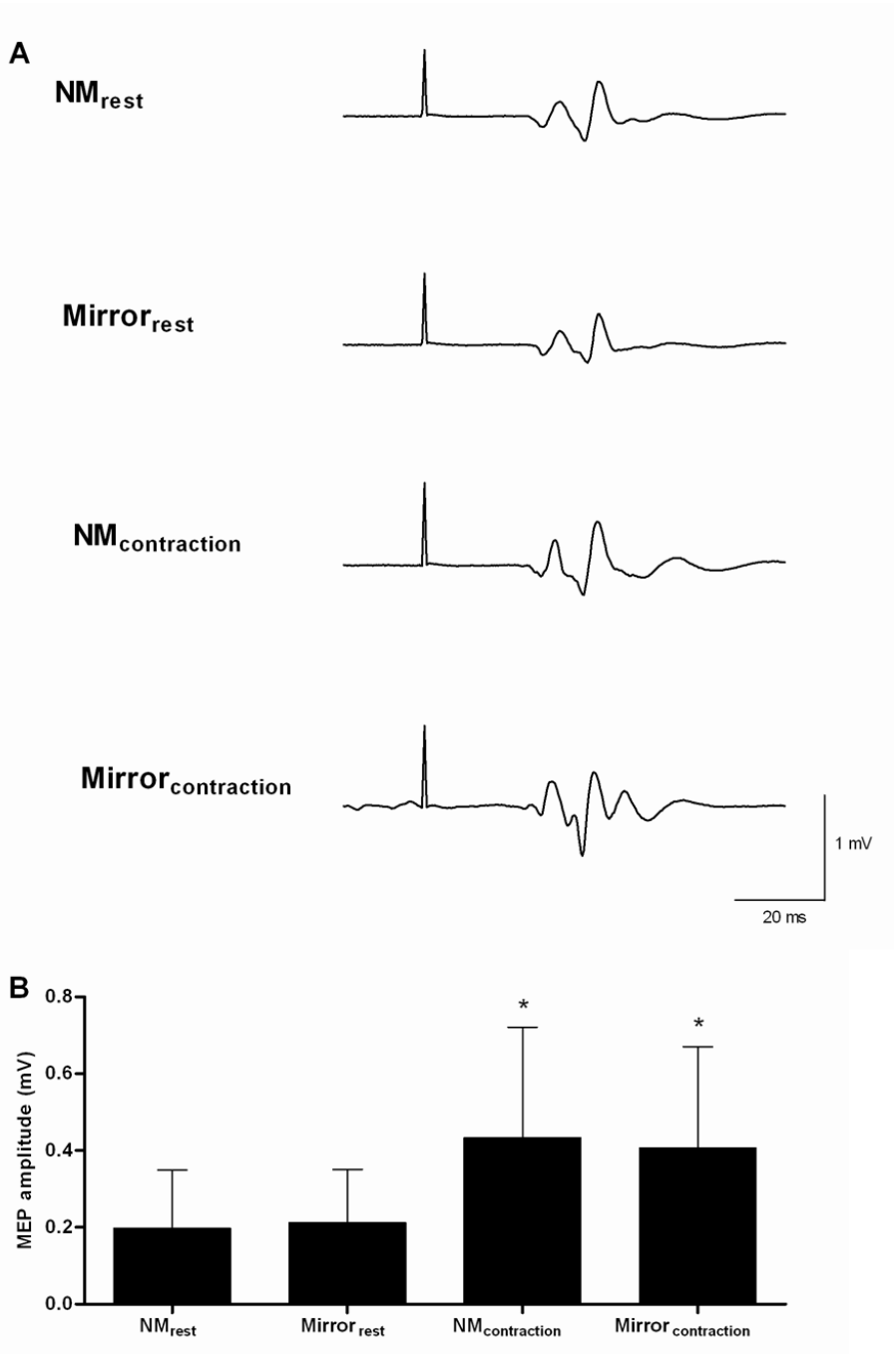


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Figure 3.

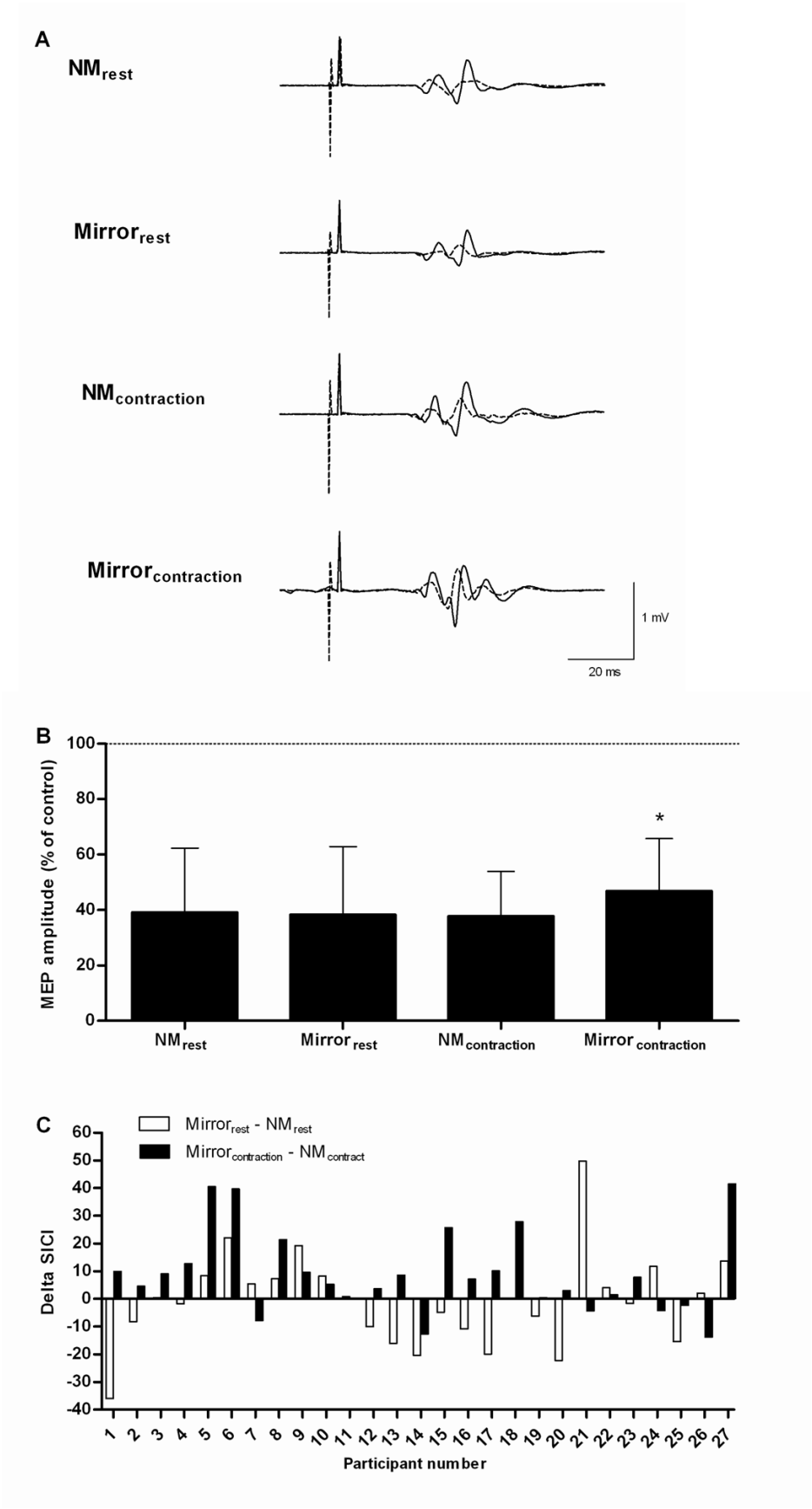

A
NMrest
Mirrorrest
NMcontraction
Mirrorcontraction
1 mV
20 ms
B
MEP amplitude (% of control)
100
80
60
40
20
0
*
NMrest
Mirror rest
NMcontraction
Mirror contraction
C
Mirrorrest - NMrest
Mirrorcontraction - NMcontract
Delta SICI
60
50
40
30
20
10
0
-10
-20
-30
-40
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27
Participant number

Figure 4.

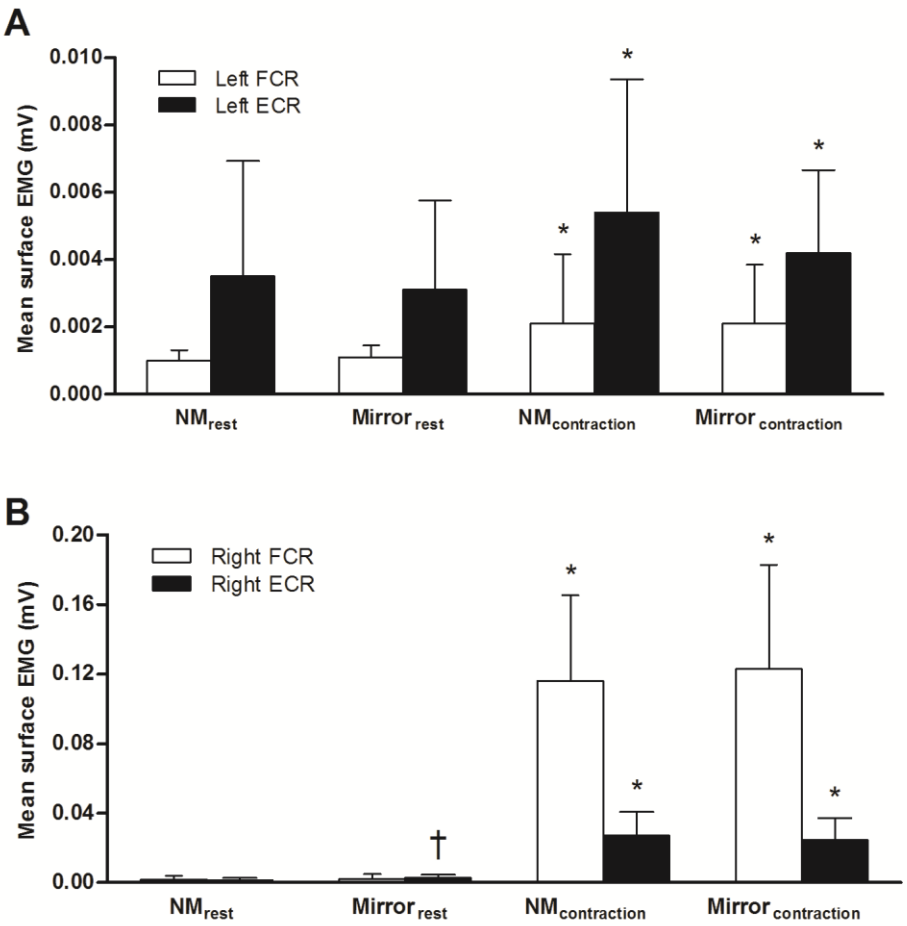
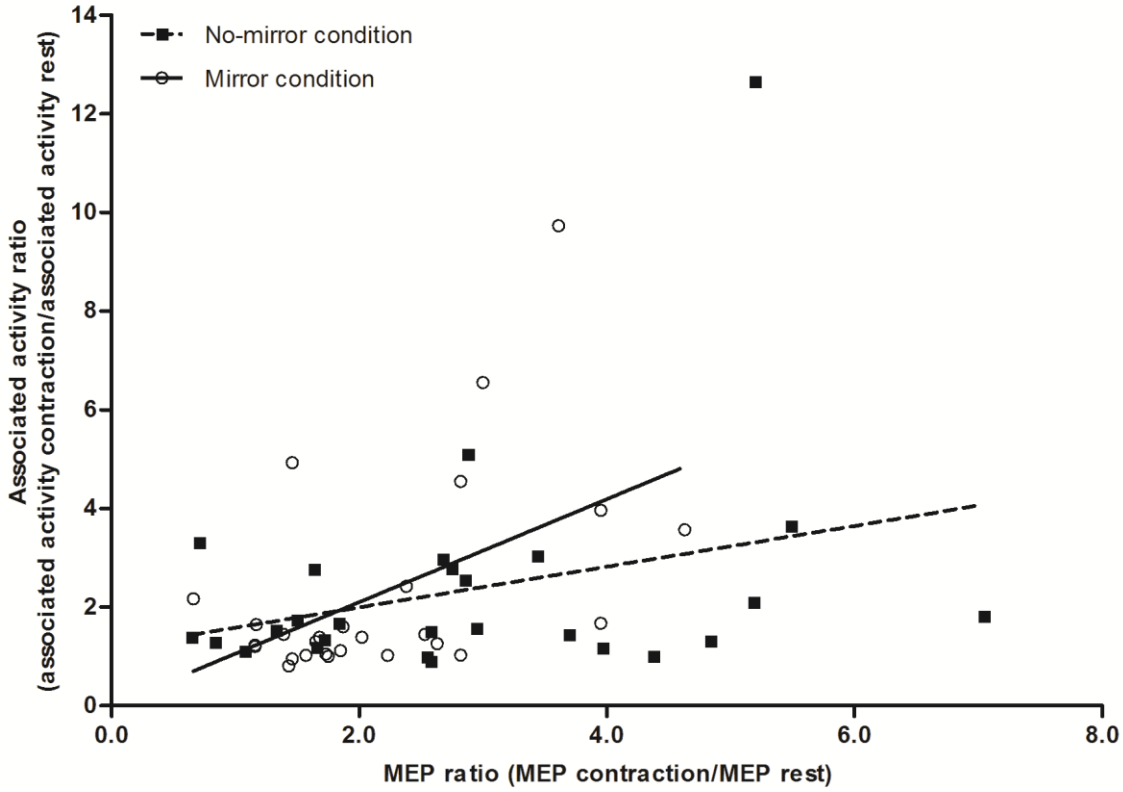

A
Mean surface EMG (mV)
0.010
0.008
0.006
0.004
0.002
0.000
Left FCR
Left ECR
*
*
*
NMrest
Mirror rest
NMcontraction
Mirror contraction
B
Mean surface EMG (mV)
0.20
0.16
0.12
0.08
0.04
0.00
Right FCR
Right ECR
*
*
*
*
†
NMrest
Mirror rest
NMcontraction
Mirror contraction

Figure 5.


No-mirror condition
Mirror condition
Associated activity ratio
(associated activity contraction/associated activity rest)
MEP ratio (MEP contraction/MEP rest)
0
2
4
6
8
10
12
14
0.0
2.0
4.0
6.0
8.0