

Modulation of interhemispheric inhibition by volitional motor activity: an ipsilateral silent period study

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Brief interruption of voluntary EMG in a hand muscle by focal transcranial magnetic stimulation (TMS) of the ipsilateral primary motor cortex (M1), the so-called ipsilateral silent period (ISP), is a measure of interhemispheric motor inhibition. However, little is known about how volitional motor activity would modulate the ISP. Here we tested in 30 healthy adults to what extent and under what conditions voluntary activation of the stimulated right M1 by moving the left hand strengthens interhemispheric inhibition as indexed by an enhancement of the ISP area in the maximally contracting right first dorsal interosseous (FDI). Left index finger abduction, already at low levels of contraction, significantly enhanced the ISP compared to left hand at rest. Even imagination of left index finger movement enhanced the ISP compared to rest or mental calculation. This enhancement occurred in the absence of motor-evoked potential amplitude modulation in the left FDI, thus excluding a non-specific contribution from an increase in right M1 corticospinal excitability. Contraction of the left extensor indicis, but not contraction of more proximal left upper limb or left or right lower limb muscles also enhanced the ISP. A reaction time experiment showed that the ISP enhancement developed at a late stage of movement preparation just before or at movement onset. Interhemispheric inhibition of the motor-evoked potential as tested by a bifocal paired-pulse TMS protocol and thought to be mediated via a neuronal circuit different to the ISP was not enhanced when tested under identical motor task conditions. Finally, ISP enhancement by contraction of the left FDI correlated inversely with EMG mirror activity in the right FDI during phasic abductions of the left index finger. Our findings strongly suggest that voluntary M1 activation by real or imagined movement of the contralateral hand increases interhemispheric motor inhibition of the opposite M1. This phenomenon shows substantial topographical, temporal and neuronal circuit specificity, and has functional significance as it probably plays a pivotal role in suppressing mirror activity.

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Abbreviations BB, biceps brachii; CSP, cortical silent period; ECR, extensor carpi radialis; EIP, extensor indicis proprius; EMG, electromyographic; FDI, first dorsal interosseous; IHI, interhemispheric inhibition; ISP, ipsilateral silent period; L-IHI, long-interval interhemispheric inhibition; M1, primary motor cortex; MEP, motor-evoked potential; MSO, maximum stimulator output; RMS, root mean squares; RMT, resting motor threshold; RT, reaction time; S-IHI, short-interval interhemispheric inhibition; TA, tibialis anterior; TMS, transcranial magnetic stimulation.

Introduction

In humans, intricate and independent finger movements are enabled by a largely crossed system of fast-conducting axons that provides mono-synaptic connections between primary motor cortex (M1) and contralateral spinal motoneurons (Porter & Lemon, 1993). Execution of unimanual or asymmetric bilateral movements relies on a

neural network that is capable of lateralising motor cortical output (Carson, 2005; Cincotta & Ziemann, 2008). While a full characterisation of this distributed network is still lacking, data from lesioned monkeys (Brinkman, 1984) and human patients (Chan & Ross, 1988) are in keeping with the view that it probably includes the supplementary motor area and the cingulate gyrus. Positron emission tomography (PET) findings (Sadato *et al.* 1997) and

transcranial magnetic stimulation (TMS) data in healthy human subjects (Meyer-Lindenberg *et al.* 2002; Cincotta *et al.* 2004; Giovannelli *et al.* 2006) suggest that the dorsal premotor cortex is also involved.

This notion of a neuronal network for movement lateralisation upstream of M1 by no means rules out the possibility that movement lateralisation is supported, in addition, by active inhibition from the voluntarily active M1 to the opposite M1. TMS studies that examined inter-hemispheric inhibition (IHI) by a paired-pulse protocol with the conditioning stimulus delivered to one M1 and the test stimulus delivered to the other M1 support this hypothesis (Ferber *et al.* 1992; Mochizuki *et al.* 2004; Duque *et al.* 2007; Hübers *et al.* 2008). In particular, volitional activity in the M1 receiving the conditioning pulse, e.g. slight unilateral contraction of the contralateral hand, facilitates inhibition of the motor-evoked potential (MEP) elicited by a test stimulus delivered 10 ms later to the opposite M1 (interhemispheric inhibition at short-interstimulus interval, S-IHI) when compared to the rest condition (Ferber *et al.* 1992; Mochizuki *et al.* 2004; Talelli *et al.* 2008).

Besides S-IHI of the MEP, interhemispheric inhibition can also be studied by a short attenuation or interruption of ongoing voluntary electromyographic (EMG) activity in hand muscles induced by focal TMS of the ipsilateral M1 (Wassermann *et al.* 1991; Ferber *et al.* 1992; Meyer *et al.* 1995; Trompetto *et al.* 2004; Cincotta *et al.* 2006). This ipsilateral silent period (ISP) begins 30–40 ms after a single magnetic pulse and lasts, on average, 25 ms (Meyer *et al.* 1995). Studies in patients with callosal lesions indicate that the ISP reflects interhemispheric cortico-cortical inhibitory mechanisms mediated by fibres passing through the posterior half of the trunk of the corpus callosum (Meyer *et al.* 1995, 1998). By examining the effects of different stimulus intensities and current directions, Chen *et al.* (2003) suggested that the neural mechanisms underlying the ISP differ from those responsible for the S-IHI of the MEP. The ISP is currently considered as a measure that provides complementary but not identical information on interhemispheric inhibition compared to S-IHI measurements (Perez & Cohen, 2009). As the ISP measures inhibition of volitional motor activity rather than inhibition of MEPs, the ISP appears to be an original and particularly suited tool to investigate interhemispheric control of voluntary cortical motor output.

The aim of the present study was to investigate to what extent and under what conditions the ISP is modulated when the stimulated M1 is engaged in a voluntary motor task. Hence, we examined the effects of different real or imagined movements of the contralateral hand and of other body segments on the ISP in healthy humans. Furthermore, we tested whether motor task-related modulation of the ISP could be dissociated from modulation of the S-IHI. Finally, the functional

significance of motor task-related modulation of the ISP was examined by testing its relation to the occurrence of EMG mirror activity, i.e. the failure to lateralise intended unilateral hand movements.

Methods

Subjects

Thirty right-handed healthy volunteers (sixteen women, mean age 32.1 years, range 19–56 years) were included in the study. Handedness was confirmed by the Edinburgh inventory (Oldfield, 1971) (mean laterality index 86.7, range 60–100). The study was performed according to the latest version of the *Declaration of Helsinki* and approved by the local ethics committee in Florence. Participants gave their written informed consent. During the experiments, subjects were seated in a comfortable chair with their arms fully supported.

EMG recording

Surface EMG activity was recorded simultaneously from the right and left first dorsal interosseous (FDI) muscles ('target' and 'task' muscle, respectively). Moreover, in control experiments aiming to explore the topographical specificity of motor task-related modulation of the ISP, EMG recordings were also made from the left extensor indicis proprius (EIP), the left extensor carpi radialis (ECR), the left biceps brachii (BB), and the right or left tibialis anterior (TA) muscles, which acted as task muscles in specific experimental conditions (see below). Signals were amplified, analog filtered (100–2000 Hz), digitised (A/D rate, 5 kHz) by a micro 1401 unit and Signal 2 software (Cambridge Electronic Design, UK) and stored on a personal computer for off-line analysis. Analysis time was 1.5 s with 1 s preceding the TMS pulse. Except for the experiment specifically aiming to test the effects of unintended, slight contraction ('physiological' mirroring) of the left FDI on the ISP in the right FDI (see below), appropriate relaxation or activation of the task muscles in different experimental conditions was monitored throughout the experiments by an acoustic feedback from high gain EMG and was a precondition for delivery of TMS. In this way, in all the other experiments, off-line analysis revealed very slight unwanted EMG activity in the left FDI in less than 5% of the trials. These traces were discarded from further analysis. Moreover, in all conditions where full relaxation of the task muscle (left FDI) was requested, the root mean squares (RMS) of the pre-stimulus EMG activity were calculated off-line to confirm full relaxation. Group RMS values (mean \pm s.d.) ranged from 0.008 ± 0.007 mV to 0.011 ± 0.006 mV throughout experiments. These very

low values were well within the range of previously reported RMS data in resting muscles (Möller *et al.* 2009).

TMS

Single-pulse TMS was delivered using a Magstim 200 stimulator (Magstim Co., UK) with a monophasic current waveform, connected to a figure-of-eight-shaped coil (external diameter of each loop, 9 cm) held tangentially to the scalp. The centre of the junction of the coil was placed over the hand area of the right or left M1 at the optimal position (hot spot) to elicit MEPs in the contralateral FDI, with the handle pointing backwards and 45 deg away from the midline. With this coil orientation, the induced current flowed in an anterior–medial direction, approximately perpendicular to the central sulcus. In most experiments, the right M1 was stimulated. In one of the two experiments addressing the circuit specificity of task-related modulation of the ISP, focal TMS was delivered to the left M1 to study the cortical silent period (CSP) in the right FDI (see below). In the other experiment, S-IHI of the MEP was tested using two Magstim 200 stimulators, each connected to a figure-of-eight-shaped coil. The coils were placed over the FDI hot spot of the M1 of either hemisphere (Ferber *et al.* 1992).

General experimental design

We performed a series of experiments designed to thoroughly examine to what extent the ISP in the right FDI is modulated by tonic or phasic voluntary contractions of the left FDI, by different levels of voluntary contraction of the left FDI, by unintended, slight contraction ('physiological' mirroring) of the left FDI, by imagined movements involving the left index finger, by voluntary contraction of muscles other than the left FDI (addressing topographical specificity of ISP modulation) or during preparation of left FDI contraction (addressing temporal specificity). Further experiments explored the effects of left FDI contraction on other measures of cortical inhibition in the right FDI (circuit specificity) and the relationship between the motor task-related modulation of the ISP and the ability to lateralise voluntary movements of the left hand (addressing behavioural significance of ISP modulation). Single experiments were carried out on separate days. For all ISP experiments, subjects were always asked to perform an isometric contraction of the right FDI at the maximum strength level that they could steadily maintain (Trompetto *et al.* 2004; Cincotta *et al.* 2006), whereas the task of the left hand (or other body parts) differed across experimental conditions. A large background EMG level in the right FDI and relatively low TMS intensity (see below) minimised the possibility of ceiling or floor effects in task-related

ISP modulation. Within each experimental session, the order of task conditions was varied pseudorandomly in a counterbalanced manner across subjects. The interval between consecutive TMS trials varied between 5 and 10 s to minimise anticipation of the next trial. Pauses were inserted whenever necessary to avoid muscle fatigue. These precautions resulted in a close match of background EMG levels across conditions (see Results).

ISP modulation by different tonic and phasic contractions of the left FDI ('main experiment')

It is important to note that in this and all other ISP experiments the basic requirement throughout was maximum isometric contraction of the right FDI. Therefore, the term 'full relaxation' means that all other body parts were relaxed except the right FDI. Twelve subjects participated in the main experiment. The ISP in the right FDI was recorded whilst the left FDI was engaged in four different tasks: (i) full relaxation; (ii) maximal isometric contraction; (iii) repetitive thumb-to-index tapping with the left hand; and (iv) sequential left index tapping movements on a flat surface with the palm immobile and facing downward, with subjects instructed to touch with the tip of the left index finger three target points placed about 1.3 cm apart corresponding to three different levels of index abduction. Prior to the ISP measurements, the resting motor threshold (RMT) was determined in the left FDI according to the recommendations of the IFCN Committee (Rossini *et al.* 1994). RMT was measured in 1% increments of maximum stimulator output (MSO), and defined as the minimum stimulus intensity, which produced MEPs of $>50 \mu\text{V}$ in at least half of 10 consecutive trials in the relaxed FDI. RMT (mean \pm S.D.) was $39.4 \pm 7.0\%$ MSO. For the ISP measurements, TMS of the right M1 was delivered at an intensity of 120% RMT. Twenty stimuli were delivered in each experimental condition. When the tasks consisted of phasic movements of the left index, TMS was delivered as soon as the operator recognised by visual inspection the index abduction in the repetitive task and the maximal of the three levels of index abduction in the sequential task. Off-line evaluation of recordings confirmed that TMS pulses were delivered during the voluntary EMG bursts in the left FDI.

ISP modulation by isometric left FDI contraction at different strength levels

Seven subjects participated in this experiment. TMS procedures were identical to the main experiment and were performed whilst the left FDI was engaged in three different tasks: (i) full relaxation; (ii) isometric contraction at the minimum strength level that they could

steadily maintain against resistance (approximately 5% of maximum voluntary contraction); and (iii) maximum isometric contraction.

ISP modulation by unintended, slight contraction ('physiological' mirroring) of the left FDI

In eight subjects, TMS pulses identical to the main experiment were delivered during maximal voluntary isometric contraction of the right FDI alone, irrespective of whether or not high gain EMG showed unintended activation of the left FDI ('physiological' mirroring). This experiment addresses the question as to whether voluntary suppression of mirror EMG activity in the left FDI has affected the ISP in the right FDI. Traces were recorded until at least 20 trials without and 20 trials with unwanted EMG activity in the left FDI were collected. As the percentage of traces with mirroring ranged between 16% and 41% of the trials, the number of recordings ranged between 49 and 128 across subjects. The first 20 'fully relaxed' trials and the 20 trials with unintended EMG activity in the left FDI were used for analysis. In the 'fully relaxed' and 'physiological mirroring' conditions, the group RMS values (mean \pm s.d.) of the pre-stimulus EMG activity in the left FDI were 0.008 ± 0.002 mV and 0.016 ± 0.009 mV, respectively. Although the unintended EMG activity in the left FDI was slight and non-persistent, this difference was significant (paired *t* test: $P = 0.02$).

ISP modulation by imagined movements involving the left FDI

Eleven subjects participated in this experiment. TMS procedures were identical to the main experiment and were performed in three different experimental conditions of the left hand: (i) full relaxation; (ii) imagination of tonic thumb-to-index opposition; and (iii) full relaxation whilst performing a mental calculation task. Mental calculation consisted of counting backwards by 7 beginning with 100. In each condition, TMS to the right M1 was delivered only when the acoustic feedback from high gain EMG indicated complete absence of muscle activity in the left FDI.

ISP modulation by isometric contraction of muscles other than the left FDI (topographical specificity)

A set of three sub-experiments was performed to explore the topographical specificity of the ISP enhancement observed in the main experiment when contracting the left FDI (see Results). The sub-experiments were performed on separate days and designed to obtain a progressive refinement of the topographical specificity of the ISP modulation. Sub-experiment 1 (10 subjects) consisted of four experimental conditions: (i) full relaxation; (ii) maximum isometric voluntary contraction of the left

FDI (to confirm data in the main experiment); (iii) maximum isometric voluntary contraction of the right TA; and (iv) maximum isometric voluntary contraction of the left EIP. In sub-experiment 2 (eight subjects), two different experimental conditions were compared: (i) full relaxation; and (ii) maximum isometric voluntary contraction of the left TA. Finally, sub-experiment 3 (eight subjects) consisted of three different conditions: (i) full relaxation; (ii) maximum isometric voluntary contraction of the left ECR; and (iii) maximum isometric voluntary contraction of the left BB.

ISP modulation by preparation of left FDI contraction (temporal specificity)

A simple visual reaction time (RT) task was used to investigate whether the ISP enhancement in the right FDI observed during phasic movements of the left index (see Results of the main experiment), was expressed already *before* the onset of left FDI contraction. Seven subjects participated in this experiment. The visual go-signal (duration, 100 ms) consisted of a 5 cm yellow circle displayed at eye level on a blank computer screen 1 m in front of the subject. Subjects were instructed to perform a single, rapid thumb-to-index tap with the left hand as soon as possible after the go-signal whilst they maintained maximum isometric voluntary contraction of the right FDI. TMS pulses were delivered in three different experimental conditions: (i) without the go-signal (baseline); (ii) 100 ms after the go-signal; and (iii) 200 ms after the go-signal. These intervals were chosen to deliver TMS before and during the EMG burst in the left FDI, respectively (Ziemann *et al.* 1997). When this was not the case, the trial was discarded. TMS trials of the three experimental conditions were randomly intermixed until at least 20 valid trials were obtained in each condition. To test whether the visual go-signal *per se* could modulate the ISP in the right FDI, the same protocol was applied whilst the subjects were asked to observe the computer screen but not to respond with the left hand.

Circuit specificity of task-related ISP modulation

In order to test directly whether ISP modulation was related to modification of S-IHI, both measures were tested in the right FDI during identical motor tasks: (i) unilateral isometric contraction of the right FDI; and (ii) bilateral isometric FDI contraction at the maximum strength level (i.e. conditions (i) and (ii) of the main experiment). Ten subjects participated in this experiment. During each task, three different TMS conditions were randomly performed: stimulation of the right M1 alone (20 trials) for ISP recordings, stimulation of the left M1 alone (10 trials) for unconditioned test MEP recordings, and conditioning stimulation of the right M1 followed by test stimulation

of the left M1 at a 10 ms interstimulus interval (10 trials) to evaluate S-IHI. TMS of the right M1 was delivered at an intensity of 120% RMT. For left M1 stimulation, TMS intensity was adjusted to elicit an unconditioned MEP of approximately 2 mV peak-to-peak amplitude in the maximally contracting right FDI. Stimulus intensity ranged from 20% to 32% MSO.

In order to explore if the ISP modulation involved inhibitory circuits in the left M1, we tested to what extent contraction of the left FDI modulated the CSP elicited in the right FDI by TMS of the left M1. Eight subjects participated in this experiment. Prior to the CSP measurements, the active motor threshold (AMT) of the right FDI was measured in 1% increments of MSO and was defined as the minimum stimulus intensity that produced MEPs of $>50 \mu\text{V}$ in at least half of 10 consecutive trials during a minimal isometric contraction (approximately 5% of maximum voluntary strength) of the right FDI. AMT (mean \pm S.D.) was $31.8 \pm 7.9\%$ MSO. For the CSP measurements, TMS of the left M1 was delivered at an intensity equal to the AMT. In this way, the duration and depth of the CSP were comparable to those of the ISP in the right FDI in the ISP experiments. Twenty stimuli were delivered in either one of two different experimental conditions: (i) unilateral maximum isometric voluntary contraction of the right FDI; and (ii) bilateral maximum isometric voluntary contraction of the right and left FDI (i.e. same conditions (i) and (ii) as in the main experiment).

Relationship between motor task-related ISP modulation and voluntary hand movement lateralisation (behavioural significance)

In ten subjects, the ISP was tested in two experimental conditions: (i) unilateral maximum isometric voluntary contraction of the right FDI; and (ii) bilateral maximum isometric voluntary contraction of the right and left FDI (i.e. same conditions (i) and (ii) as in the main experiment). TMS procedures were also identical to the main experiment. Voluntary movement lateralisation was measured by employing a well-established experimental protocol (Mayston *et al.* 1999; Giovannelli *et al.* 2006; Hübers *et al.* 2008): Subjects were instructed to perform a voluntary phasic ('brief and brisk') abduction of the left index finger in response to a visual go-signal, while maintaining a tonic contraction of the right FDI at the minimum strength level that they could steadily maintain against resistance, under the guidance of auditory feedback of the EMG. The go-signal was identical to the one used in the simple RT protocol (see above, 'ISP modulation by preparation of left FDI contraction'). Twenty trials were performed at intertrial intervals ≥ 10 s.

Data analysis

For each experimental condition, single trials were rectified and averaged off-line and the resulting traces were exported in ASCII format to a Microsoft Excel spreadsheet for analysis. The ISP was quantified in the right FDI by the suppression of the voluntary EMG activity between the onset and the offset of the ISP (ISP area, in mV ms). ISP onset and offset were established using a method of statistical process control (Garvey *et al.* 2001, 2005). Briefly, the variation limits of background EMG activity were calculated by the average level of the 1 s EMG epochs immediately preceding the TMS pulse and by the mean consecutive difference between data points. ISP onset was defined as the first of five consecutive data points to fall below the lower 95% variation limit (equivalent to 2 S.D.) in the appropriate time window. ISP offset was the first data point above the lower 95% limit if 4 of the 9 subsequent points were also above the limit. In addition, traces were inspected to see whether ipsilateral excitatory responses (Ziemann *et al.* 1999) preceded the ISP. An ipsilateral MEP (iMEP) was considered to be present if the averaged rectified EMG signal exceeded 120% of the mean background EMG level for at least 5 ms. The method used to quantify the area of the CSP was the same as for ISP quantification. The amplitudes of the contralateral MEPs were measured in the experiments examining the effects of movement imagination (left FDI) and in the CSP experiment (right FDI) by taking the difference from baseline (0 mV) to the peak of the major deflection in the rectified averaged EMG. In the experiment comparing task-related ISP and S-IHI modulation, the raw EMG recordings of unconditioned and conditioned test MEPs were also separately averaged off-line. For each motor task, S-IHI was calculated as the percentage of peak-to-peak amplitude of the mean conditioned test MEP over the mean unconditioned test MEP. For ISP area and S-IHI, the difference between the measures obtained during bilateral FDI contraction and the measures obtained during unilateral contraction of the right FDI was expressed as a percentage of the mean value of the two measurements (percentage difference). These percentage differences were used to test whether motor task-induced changes in ISP area and S-IHI were correlated. In the experiment addressing the 'Relationship between motor task-related ISP modulation and voluntary hand movement lateralisation', EMG mirror activity in the right FDI was measured as the average of the rectified EMG traces and expressed as the percentage difference between the mean EMG level during the 50 ms after voluntary EMG burst onset in the left FDI and the mean baseline EMG amplitude in the 800 ms before burst onset. EMG mirror activity and the percentage difference in ISP area between bilateral FDI contraction and unilateral contraction of the right FDI were correlated in a linear regression analysis to

test for any relationship between motor task-induced ISP modulation and mirror activity.

Statistics

In experiments consisting of more than two experimental conditions, EMG measures were entered in one-way repeated-measures analyses of variance (rmANOVA) with 'Experimental condition' as a within-subject factor (Sigmastat 3.1 software, Jandel Scientific, Erkrath, Germany). *Post hoc* tests were performed using Newman-Keuls multiple comparisons. In experiments consisting of two different experimental conditions, comparisons were made by using two-tailed paired *t* tests. In the experiment comparing ISP and S-IHI, ISP area, unconditioned test MEP amplitude, and S-IHI were compared between the two motor tasks by two-tailed paired *t* tests. Correlations between motor task-induced changes in ISP area and S-IHI, and between task-induced ISP modulation and EMG mirror activity were analysed by linear regression. In all tests, the level of significance was set at $P < 0.05$.

Results

None of the participants reported adverse effects during or after the experimental procedures. According to the relatively low stimulus intensity used in the present study, no iMEP preceded the ISP in any of the experimental conditions.

ISP modulation by different tonic and phasic contractions of the left FDI

The rmANOVA revealed a significant effect of 'Experimental condition' on the ISP area ($F_{3,33} = 5.80$, $P = 0.003$). *Post hoc* tests showed that each of the three tasks where the left FDI was involved in voluntary motor activity (maximal isometric contraction, repetitive thumb-to-index tapping of the left hand, and sequential tapping movements of the left index) resulted in an increase in ISP area in the right FDI when compared with the left FDI in the rest condition (Table 1, Fig. 1). The trend towards a stronger increase of ISP area with sequential movements compared to repetitive thumb-to-index tapping or tonic contraction was not significant (Table 1). In addition, there was a significant effect of 'Experimental condition' on the background EMG in the right FDI ($F_{3,33} = 3.32$, $P = 0.032$). *Post hoc* testing revealed that this effect was caused by slightly but consistently lower background EMG with repetitive thumb-to-index tapping and sequential movements of the left index than maximum isometric contraction of the left FDI (Table 1). It is unlikely that so small a difference in

background EMG level could exert a relevant influence on ISP measurements. In that case, however, the ISP enhancement in the phasic movement conditions of the left index finger would anyway have been slightly *underestimated* when compared to the maximum isometric contraction condition. Importantly, *post hoc* tests showed no significant difference in the background EMG of the right FDI between left FDI relaxation and the three experimental conditions where the left FDI was involved in voluntary motor activity (Table 1).

ISP modulation by isometric left FDI contraction at different strength levels

The rmANOVA showed a significant effect of 'Experimental condition' on the ISP area ($F_{2,12} = 4.73$, $P = 0.032$). *Post hoc* testing revealed that, during minimal as well as maximal isometric contraction of the left FDI, the ISP area in the right FDI was significantly larger than in the left FDI resting condition (Table 1, Fig. 2). In addition, there was a non-significant trend for a larger ISP area with maximal than with minimal isometric contraction of the left FDI (Table 1). The level of background voluntary EMG activity in the left FDI was significantly different between maximal and minimal isometric contraction of the task muscle (mean \pm S.D.: 0.410 ± 0.168 vs. 0.088 ± 0.053 mV, $P < 0.001$). 'Experimental condition' had no significant effect on the background EMG in the right FDI ($F_{3,12} = 0.83$, $P = 0.46$; Table 1).

ISP modulation by unintended, slight contraction ('physiological' mirroring) of the left FDI

Paired *t* tests showed that neither the ISP area ($P = 0.12$) nor the background EMG in the right FDI ($P = 0.09$) differed significantly between the conditions of 'physiological mirroring' and 'full relaxation' of the left FDI (ISP area: mean \pm S.D.: 2.13 ± 1.28 vs. 2.43 ± 1.43 mV ms; background EMG: 0.336 ± 0.118 vs. 0.383 ± 0.131 mV, respectively).

ISP modulation by imagined movements involving the left FDI

rmANOVA demonstrated a significant effect of 'Experimental condition' on the ISP area ($F_{2,20} = 4.11$, $P = 0.032$). *Post hoc* testing showed that, during imagined tonic thumb-to-index opposition of the left hand, the ISP area in the right FDI was significantly greater than during isometric contraction of the right FDI alone or during mental calculation (Table 1, Fig. 3). There was no effect of 'Experimental condition' on the background EMG in the right FDI ($F_{2,20} = 1.98$, $P = 0.16$). In addition, 'Experimental condition' had no

Table 1. ISP area and background EMG level in the right FDI (mean \pm s.d.) in the different experimental conditions

	ISP area (mV ms)	Background EMG level (mV)
Different tonic and phasic contractions of the left FDI		
Unilateral isometric contraction of the right FDI	1.83 \pm 0.85*	0.399 \pm 0.120
Bilateral isometric contraction of the FDI	3.37 \pm 1.61	0.423 \pm 0.121
Isometric contraction of the right FDI and repetitive movements of the left index	3.27 \pm 1.76	0.377 \pm 0.117* ^a
Isometric contraction of the right FDI and sequential movements of the left index	4.04 \pm 2.60	0.377 \pm 0.099* ^a
Isometric left FDI contraction at different strength levels		
Unilateral isometric contraction of the right FDI	2.72 \pm 1.77*	0.424 \pm 0.095
Isometric contraction of the right FDI and minimal contraction of the left FDI	4.27 \pm 2.50	0.448 \pm 0.077
Isometric contraction of the right FDI and maximal contraction of the left FDI	4.72 \pm 2.33	0.427 \pm 0.064
Imagined movements involving the left FDI		
Unilateral isometric contraction of the right FDI	2.88 \pm 1.92	0.435 \pm 0.133
Isometric contraction of the right FDI and imagined left thumb-to-index opposition	3.81 \pm 2.59*	0.425 \pm 0.126
Isometric contraction of the right FDI and mental calculation	2.95 \pm 2.35	0.418 \pm 0.131
Isometric contraction of other muscles (topographical specificity)		
<i>Sub-experiment 1</i>		
Unilateral isometric contraction of the right FDI	2.66 \pm 1.90	0.423 \pm 0.063
Bilateral isometric FDI contraction	4.07 \pm 3.00* ^b	0.415 \pm 0.072
Isometric contraction of the right FDI and the right TA	2.24 \pm 1.16	0.420 \pm 0.080
Isometric contraction of the right FDI and the left EIP	4.72 \pm 2.54* ^b	0.422 \pm 0.074
<i>Sub-experiment 2</i>		
Unilateral isometric contraction of the right FDI	3.26 \pm 2.23	0.434 \pm 0.127
Isometric contraction of the right FDI and the left TA	3.52 \pm 2.14	0.435 \pm 0.129
<i>Sub-experiment 3</i>		
Unilateral isometric contraction of the right FDI	3.21 \pm 1.72	0.433 \pm 0.146
Isometric contraction of the right FDI and the left ECR	3.98 \pm 1.79	0.427 \pm 0.106
Isometric contraction of the right FDI and the left BB	2.57 \pm 0.90	0.425 \pm 0.090
Preparation of left FDI contraction (temporal specificity)		
Unilateral isometric contraction of the right FDI and no RT (baseline)	2.47 \pm 2.07	0.376 \pm 0.173
Isometric contraction of the right FDI during RT (TMS before the EMG onset in the left FDI)	2.95 \pm 2.42	0.380 \pm 0.147
Isometric contraction of the right FDI during RT (TMS during the EMG burst in the left FDI)	4.48 \pm 2.45* ^c	0.372 \pm 0.140

ISP, ipsilateral silent period; EMG, electromyographic; FDI, first dorsal interosseous muscle; TA, tibialis anterior muscle; EIP, extensor indicis proprius muscle; ECR, extensor carpi radialis; BB, biceps brachii. * $P < 0.05$, repeated-measures ANOVA, and *post hoc* Newman–Keuls multiple comparison tests. ^aWith respect to bilateral isometric contraction of the FDI. ^bWith respect to contraction of the right FDI alone or simultaneous isometric contraction of the right FDI and right TA. ^cWith respect to unilateral isometric contraction of the right FDI and no RT (baseline).

significant effect on the MEP amplitude in left FDI ($F_{2,20} = 0.61$, $P = 0.56$). MEP amplitude (mean \pm s.d.) was slightly larger during motor imagery (3.6 ± 1.6 mV) than during left FDI rest (3.3 ± 1.7 mV) or mental calculation (3.4 ± 1.7 mV), but these differences were not significant. RMS (mean \pm s.d.) of the pre-stimulus EMG level in the left FDI was 0.008 ± 0.007 mV in the resting condition, 0.009 ± 0.005 mV during motor imagery, and 0.011 ± 0.008 mV during mental calculation. The rmANOVA did not reveal an effect of 'Experimental condition' on these very low RMS values ($F_{2,20} = 1.08$, $P = 0.36$). These findings confirm full relaxation of the left FDI in the motor imagination condition. Therefore, unwanted activation of the left FDI cannot be attributed

as a possible cause of the observed enhancement of the ISP area by motor imagery.

ISP modulation by isometric contraction of other muscles (topographical specificity)

In sub-experiment 1, rmANOVA showed a significant effect of 'Experimental condition' on the ISP area ($F_{3,27} = 8.35$, $P < 0.001$). *Post hoc* testing indicated that maximum isometric voluntary contraction of the left FDI or the left EIP led to an increase in ISP area in the right FDI when compared to the conditions of left FDI at rest and maximum voluntary contraction of the right TA (Table 1,

Fig. 4). ‘Experimental condition’ had no significant effect on the background EMG in the right FDI ($F_{3,27} = 0.08$, $P = 0.97$; Table 1).

In sub-experiment 2, paired t tests showed that neither the ISP area ($P = 0.36$) nor the background EMG in the right FDI ($P = 0.87$), differed significantly between conditions of relaxation and maximum isometric voluntary contraction of the left TA (Table 1, Fig. 4).

In sub-experiment 3, rmANOVA showed that ‘Experimental condition’ had no significant effect on the ISP area ($F_{2,14} = 2.88$, $P = 0.09$) or the background EMG in the right FDI ($F_{2,14} = 0.04$, $P = 0.96$). The trend toward a larger ISP area in the condition of maximal isometric voluntary contraction of the left ECR compared to the other two experimental conditions was not significant (Table 1, Fig. 4).

ISP modulation by preparation of left FDI contraction (temporal specificity)

When the stimulation protocol used in the RT protocol was applied whilst the subjects were requested to observe

the visual go-signal but not to react with the left hand, the rmANOVA showed no significant effect of ‘Experimental condition’ on the ISP area ($F_{2,12} = 0.76$, $P = 0.49$). This indicates that the visual go-signal *per se* did not modulate the ISP in the right FDI.

During the RT protocol, rmANOVA revealed a significant effect of ‘Experimental condition’ on the ISP area ($F_{2,12} = 4.67$, $P = 0.032$). *Post hoc* tests showed that the ISP area in the right FDI was significantly larger when the TMS pulse was delivered 200 ms after the go-signal (i.e. during the EMG burst in the left FDI) than when the left FDI was not engaged in the RT (baseline) or when TMS was delivered 100 ms after the go-signal (i.e. before the EMG bursts in the left FDI, Table 1, Fig. 5). There was a non-significant trend in the 100 ms condition for a slightly larger ISP area than in the baseline condition (Table 1, Fig. 5). There was no effect of ‘Experimental condition’ on the background EMG in the right FDI ($F_{2,12} = 0.19$, $P = 0.83$; Table 1). The RT values (mean \pm s.d.) were 122 ± 18 ms (range 88–147 ms) when the TMS pulse was delivered during the EMG burst and 209 ± 30 ms (range 181–273) when TMS was delivered prior to the EMG bursts in the left FDI. The average EMG

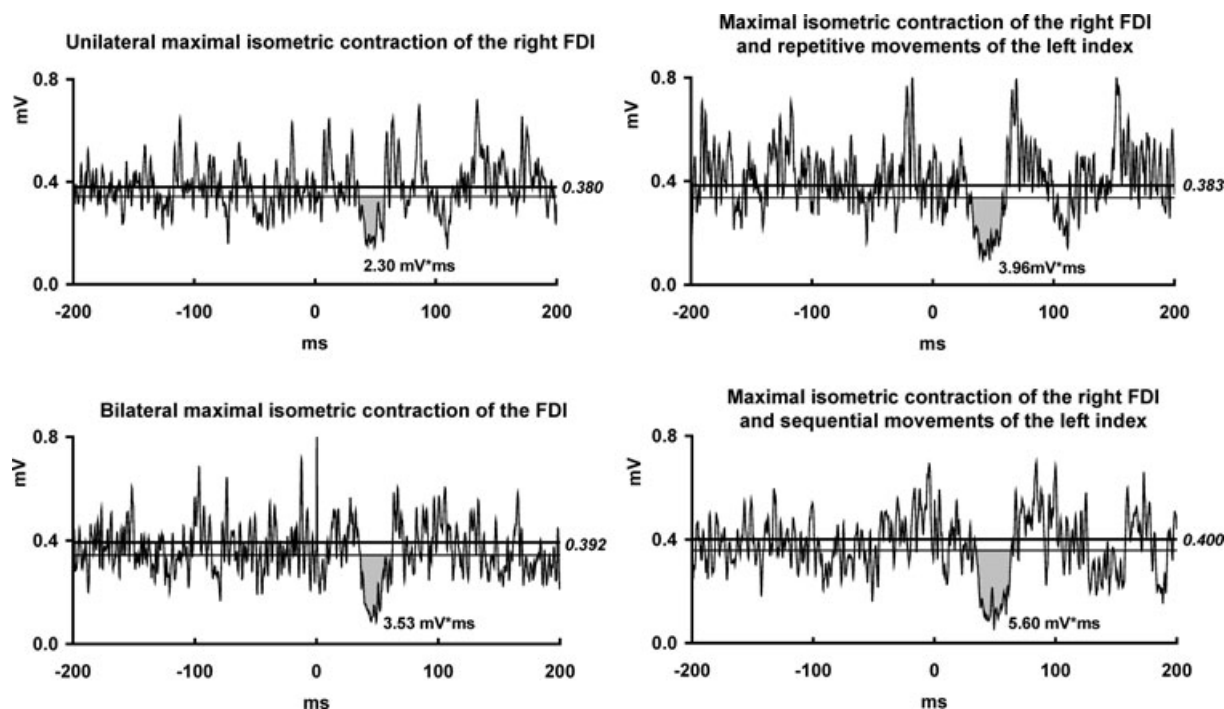


Figure 1. ISP modulation by different tonic and phasic contractions of the left FDI

EMG recordings from the maximally contracting right first dorsal interosseous (FDI) muscle of one representative subject in four experimental conditions: (i) left FDI at rest; (ii) left FDI maximally contracting; (iii) repetitive left thumb-to-index tapping; (iv) sequential movements of the left index finger. The waveforms are averages of 20 rectified trials. Grey regions represent ipsilateral silent period (ISP) areas (in mV ms). ISP onset and offset were determined by using statistical process control (see Methods). The upper horizontal line represents the mean background EMG level and the lower line represents the lower 95% variation limit of the mean background EMG. All motor tasks of the left hand enhanced the ISP area in the right FDI when compared to the left hand at rest.

level in the left FDI during the phasic movement of the left index (mean \pm S.D.) was 0.313 ± 0.119 mV. These values corresponded to $85 \pm 27\%$ of the individual EMG level during maximal isometric contraction of the left FDI.

Circuit specificity of task-related ISP modulation

The comparison of task-related ISP vs. S-IHI modulation showed that the ISP area in the right FDI was significantly larger during bilateral maximum isometric FDI contraction than during unilateral right

FDI contraction ($P = 0.025$, Table 2). In contrast, no significant task-related differences in unconditioned test MEP amplitude ($P = 0.84$) or S-IHI of the MEP in the right FDI ($P = 0.94$) were seen when evaluated under identical motor task conditions as used for ISP assessment (Table 2). MEP amplitude in the left FDI during unilateral right FDI contraction and bilateral FDI contraction was 3.4 ± 2.7 and 6.8 ± 2.1 mV, respectively. rmANOVA revealed no difference in the background EMG in the right FDI across

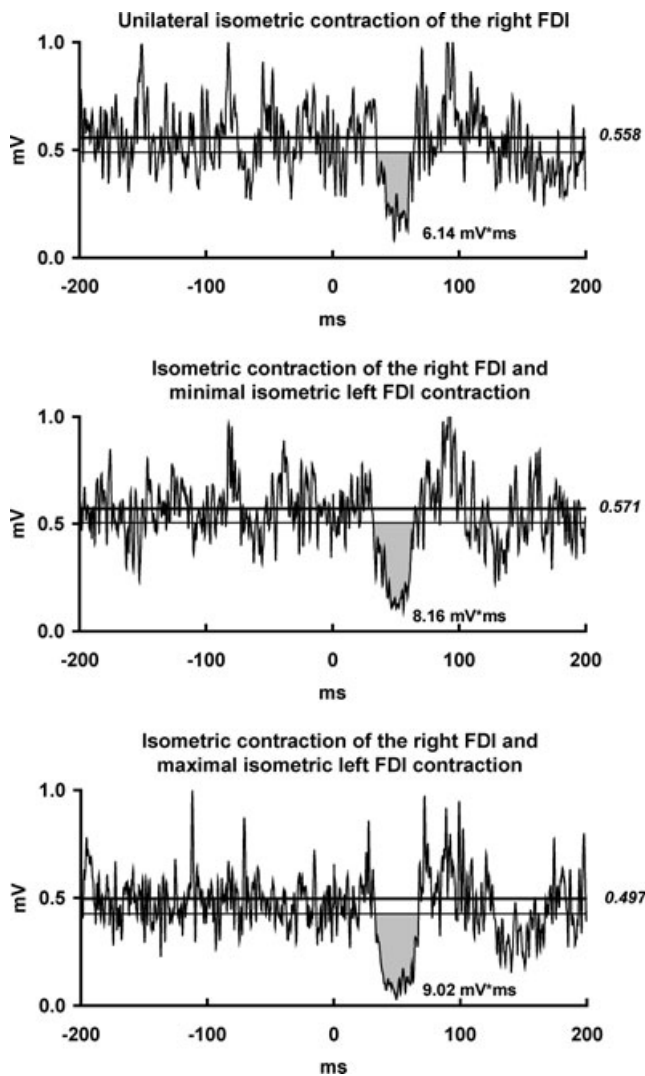


Figure 2. ISP modulation by isometric left FDI contraction at different strength levels

EMG recordings from the maximally contracting right FDI muscle of one representative subject in three experimental conditions: (i) left FDI at rest; (ii) minimal isometric contraction of the left FDI; (iii) maximal isometric contraction of the left FDI. For further arrangements and conventions, see Fig. 1. Isometric contraction of the left FDI at either minimal or maximal strength level enhanced the ISP area in the right FDI when compared to the left FDI at rest.

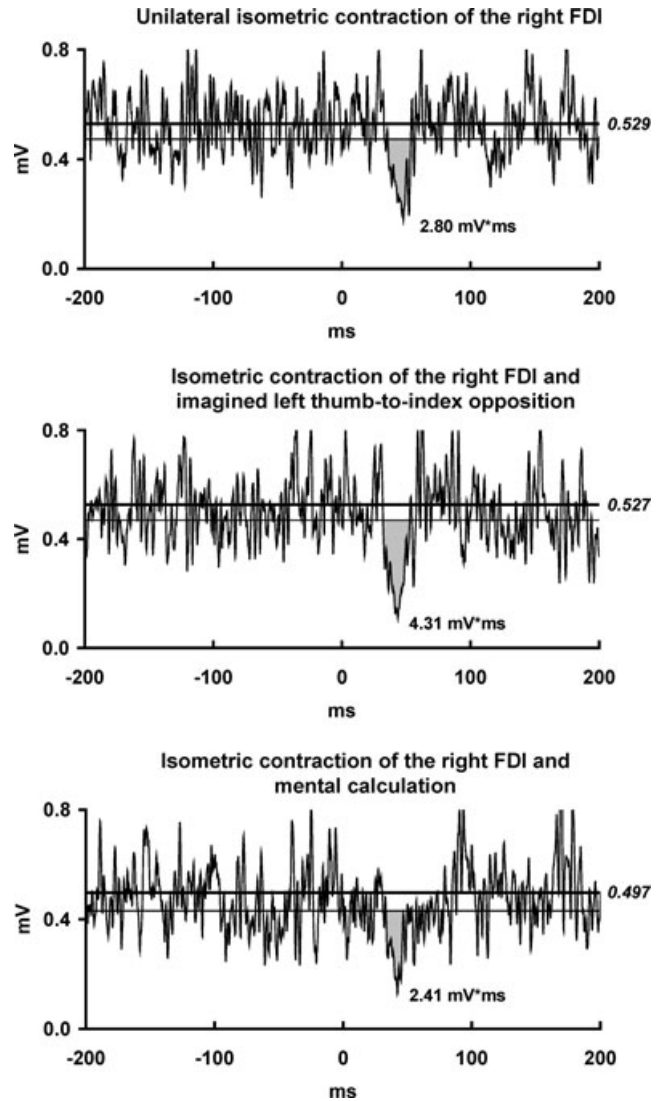


Figure 3. ISP modulation by imagined movements involving the left FDI

EMG recordings from the maximally contracting right FDI muscle of one representative subject in three experimental conditions: (i) left hand at rest, no mental calculation; (ii) imagined tonic left thumb-to-index opposition; (iii) left hand at rest and mental calculation. For further arrangements and conventions, see Fig. 1. Motor imagery of tonic thumb-to-index opposition of the left hand enhanced the ISP area in the right FDI when compared to relaxation or mental calculation.

motor tasks and TMS conditions (Table 2). The percentage differences of ISP area *vs.* S-IHI in the right FDI as calculated from the comparison between bilateral FDI contraction and unilateral right FDI contraction did not correlate with each other ($r = 0.15$, $P = 0.68$).

The CSP area was not different when comparing unilateral (mean \pm s.d.: 0.65 ± 0.68 mV ms) and bilateral maximal isometric contraction of the FDI (0.79 ± 0.80 mV ms, $P = 0.41$) (Fig. 6). Likewise, neither the amplitude of the contralateral MEP preceding

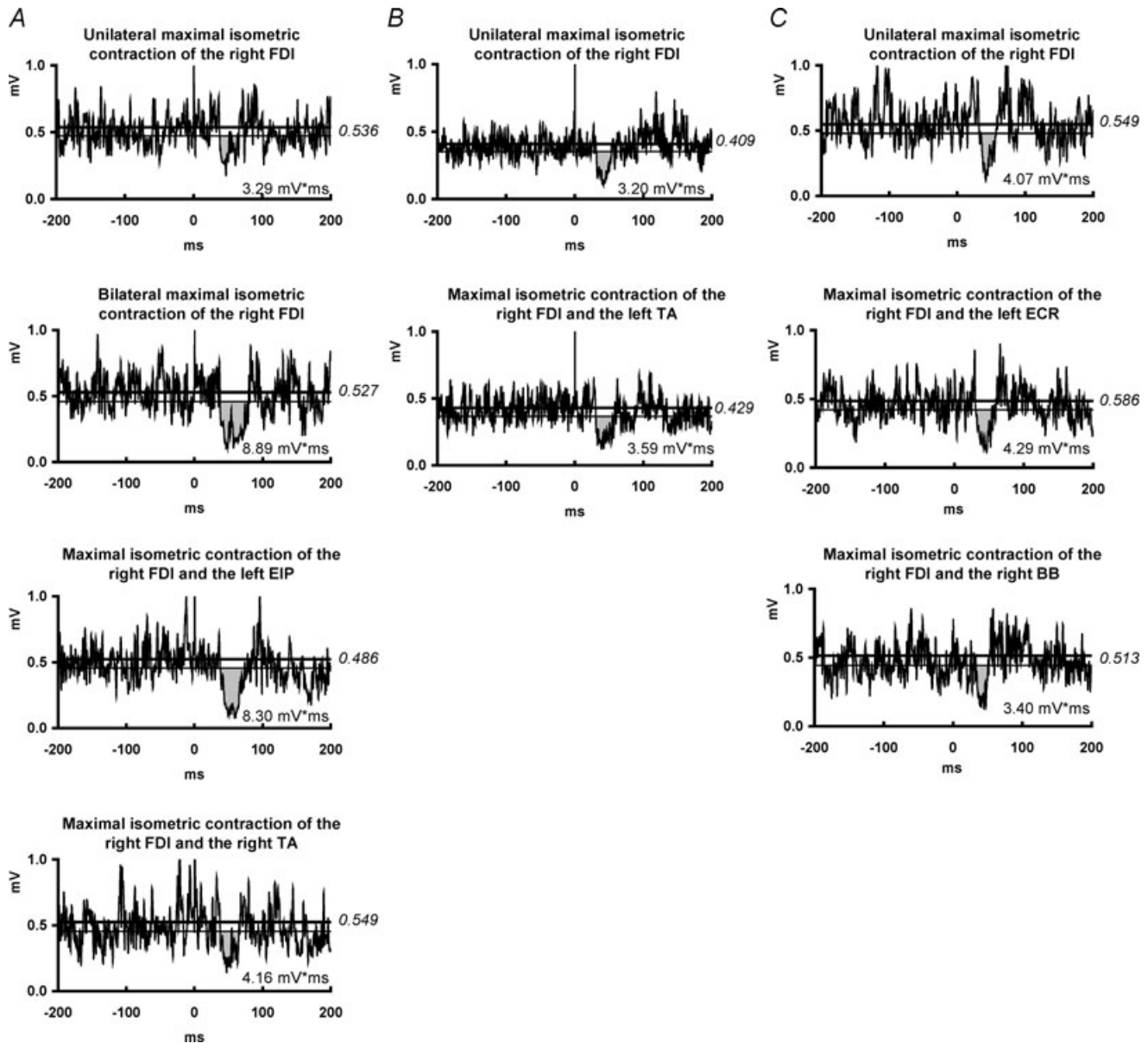


Figure 4. ISP modulation by isometric contraction of other muscles (topographical specificity)

A, sub-experiment 1: EMG recordings from the maximally contracting right FDI muscle of one representative subject in four experimental conditions: (i) no other muscle contracting; (ii) maximal isometric contraction of the left FDI; (iii) maximal isometric contraction of the left extensor indicis proprius (EIP); (iv) maximal isometric contraction of the right tibialis anterior (TA). B, sub-experiment 2: EMG recordings from the maximally contracting right FDI of one representative subject in two experimental conditions: (i) no other muscle contracting; (ii) maximal isometric contraction of the left TA. C, sub-experiment 3: EMG recordings from the maximally contracting right FDI of one representative subject in three experimental conditions: (i) no other muscle contracting; (ii) maximal isometric contraction of the left extensor carpi radialis (ECR); (iii) maximal isometric contraction of the left biceps brachii (BB). For further arrangements and conventions, see Fig. 1. Maximal isometric contraction of the left FDI or left EIP increased the ISP area in the right FDI, whereas this was not the case for maximal isometric contraction of any of the other tested muscles, suggesting topographical specificity of motor task-related ISP enhancement.

the CSP ($P=0.40$) nor the background EMG in the right FDI ($P=0.07$) differed significantly between unilateral and bilateral FDI contraction (MEP amplitude: mean \pm s.d.: 2.7 ± 1.6 vs. 2.6 ± 1.7 mV; background EMG: 0.365 ± 0.100 vs. 0.391 ± 0.116 mV, respectively). Note that, in this subset of eight subjects, the ISP area obtained in the main experiment was larger during bilateral (mean \pm s.d.: 3.43 ± 1.89 mV ms) than during unilateral FDI contraction (mean \pm s.d.: 1.56 ± 0.81 mV ms, $P=0.02$).

Relationship between motor task-related ISP modulation and voluntary hand movement lateralisation

A paired t test confirmed that the ISP area in the right FDI was larger during bilateral FDI contraction (mean \pm s.d.: 4.08 ± 2.48 mV ms) than during unilateral right FDI contraction (3.13 ± 2.26 mV ms, $P=0.019$) with non-differing levels of background EMG (0.429 ± 0.111 and 0.445 ± 0.122 mV, respectively, $P=0.13$). The percentage increment in ISP area in the right FDI with bilateral FDI contraction vs. unilateral right FDI contraction was inversely correlated ($r=-0.64$, $P=0.046$) to the amount of EMG mirror activity observed in the right FDI during phasic left index finger abduction (Fig. 7A). In contrast, no significant correlation was seen between the absolute ISP area during unilateral ($r=-0.17$, $P=0.63$) or bilateral ($r=-0.44$, $P=0.20$) FDI contraction and the amount of EMG mirror activity.

Discussion

The main novel result of the present study is that, in healthy adults, volitional motor activity of the stimulated M1 by movement of the contralateral hand produces a significant enhancement of the ISP area in the ipsilateral hand. Additional experiments characterised this phenomenon by showing its early recruitment with low levels of contraction, topographical specificity, temporal specificity, neuronal circuit specificity and functional significance. The following paragraphs provide a detailed discussion of each of these features of motor task-related ISP modulation.

Motor task-related ISP modulation

Motor task-related ISP enhancement in the right FDI is a robust finding, because it was observed with either tonic or phasic contractions of the left FDI, at different levels of isometric contraction (minimal vs. maximal), and even with just imagination of a thumb-to-index opposition of the left hand. Furthermore, maximum voluntary contraction of the left FDI does not increase

the ISP enhancement observed with minimal contraction any further. This indicates early recruitment of this motor task-related enhancement of interhemispheric inhibition, for left-hand movement, from the voluntarily active right M1 to the opposite left M1. We showed in another experiment that the ISP modulation correlated inversely

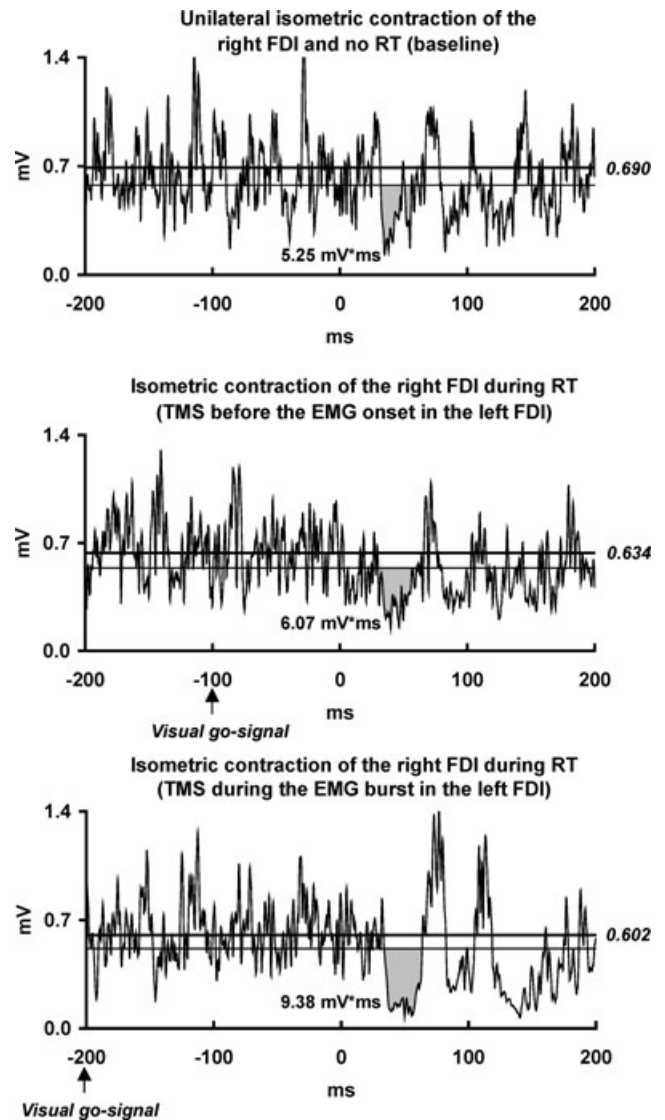


Figure 5. ISP modulation by preparation of left FDI contraction (temporal specificity)

EMG recordings from the maximally contracting right FDI muscle of one representative subject. The subject was instructed to perform a rapid thumb-to-index tap with the left hand as soon as possible after a visual go-signal whilst maintaining maximal isometric contraction of the right FDI. Recordings were made in three experimental conditions: (i) without go-signal (baseline); (ii) TMS 100 ms after the go-signal (before onset of the voluntary EMG burst in the left FDI); (iii) TMS 200 ms after the go-signal (during the EMG burst in the left FDI). For further arrangements and conventions, see Fig. 1. ISP area in the right FDI was enhanced after onset of the voluntary EMG burst in the left FDI but not during its preparation when compared with the baseline condition.

Table 2. ISP vs. S-IHI modulation: ISP area, test MEP amplitude, S-IHI of the MEP, and background EMG level in the right FDI (mean \pm s.d.) in the different motor tasks

	TMS of the right M1 alone		TMS of the left M1 alone		Paired pulse TMS at an ISI of 10 ms	
	ISP area (mV ms)	Background EMG level (mV)	Unconditioned test MEP amplitude (mV)	Background EMG level (mV)	IHI of the MEP (%) ^a	Background EMG level (mV)
Unilateral contraction of the right FDI	2.79 \pm 2.20	0.382 \pm 0.074	2.05 \pm 1.23	0.373 \pm 0.078	65 \pm 33	0.365 \pm 0.107
Bilateral contraction of the FDI	3.96 \pm 2.65*	0.372 \pm 0.093	2.10 \pm 1.21	0.363 \pm 0.068	66 \pm 28	0.351 \pm 0.114

* $P < 0.05$, two-tailed paired t test. ^aUnconditioned/conditioned test MEP amplitude (%). ISP, ipsilateral silent period; S-IHI, inter-hemispheric inhibition at short interstimulus interval; MEP, motor-evoked potential; EMG, electromyographic; FDI, first dorsal interosseous muscle.

with the amount of EMG mirror activity in the target FDI (see Fig. 7). Therefore, it can be concluded that, in terms of functional significance, early recruitment of ISP enhancement is probably particularly effective in warding off unwanted mirror activity in the case

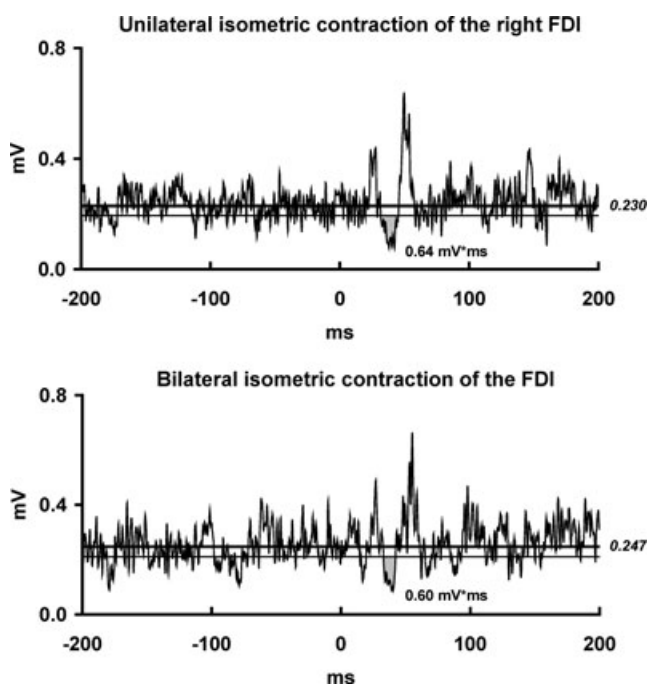


Figure 6. Circuit specificity of motor task-related ISP modulation EMG recordings from the maximally contracting right FDI muscle of one representative subject in two experimental conditions: (i) left FDI at rest; (ii) maximal isometric contraction of the left FDI. The waveforms are averages of 20 rectified trials. Grey region represents the cortical silent period (CSP) area (in mV ms). CSP onset and offset were determined by using statistical process control (see Methods). The upper line represents the mean background EMG amplitude and lower line represents the lower 95% variation limit of the mean background EMG amplitude. Note that there was no difference in the CSP area between the two experimental conditions.

of low contraction levels. This prediction is consistent with several observations that show occurrence of mirror activity mainly with high forces or demanding motor tasks (Cernacek, 1961; Zijdwind & Kernell, 2001; Bodwell *et al.* 2003; Post *et al.* 2008, 2009). In addition, we showed that the presence of slight, unintended EMG activity in the left FDI (physiological mirroring) during intended maximal unilateral isometric contraction of the right FDI did not modify the ISP in the right FDI when compared with full voluntary relaxation of the left FDI. Hence, it can be safely concluded that any intention to suppress physiological mirroring in the task FDI did not influence the ISP in the ipsilateral target FDI. Therefore, it was appropriate to use the condition 'full left FDI relaxation' as a control condition in all the other experiments.

These results could not be explained by changes in background EMG activity in the ISP target muscle (right FDI), as the background EMG did not change across experimental conditions within a given experimental session (cf. Tables 1 and 2). In addition, the observed ISP enhancement was not accounted for by a non-specific increase in motor cortical excitability in the stimulated right M1 for the following reasons. It is well known that imagery of upper limb movements enhances the excitability of the corticomotoneuronal system in the contralateral M1, as revealed by facilitation of the size of the TMS-induced MEP (Izumi *et al.* 1995; Rossi *et al.* 1998; Fadiga *et al.* 1999; Rossini *et al.* 1999). On the other hand, the slight increase of MEP amplitude we observed in the left FDI involved in the imagined motor task was not significant. However, it should be noted that the strong isometric contraction of the right FDI necessary for the ISP measurements goes along with an increase in the excitability of the crossed corticospinal system in the right M1 (Rossini *et al.* 1994; Muellbacher *et al.* 2000; Cincotta *et al.* 2004). It is possible that an interaction of these two different mechanisms produced a ceiling effect that made any MEP facilitation due to motor imagery in

the right M1 unappreciable. An alternative explanation is that, in right-handed subjects, increased excitability of the corticospinal neurones during motor imagery may predominate in the left M1, and occurs to a lesser extent or not at all in the right M1 (Stinear *et al.* 2006). Whatever the explanation, it can be concluded that the ISP enhancement during motor imagery of the contralateral hand cannot be accounted for by a non-specific increase in excitability of the stimulated right M1. This proposition is supported further by the lack of task-related S-IHI enhancement from the right to left M1 in the presence of ISP enhancement (see below). Other experiments showed that short-interval intracortical inhibition and intracortical facilitation are reduced rather than increased when the stimulated M1 is engaged in volitional

motor activity, further indicating that different neuronal circuits in M1 can show enhancement, depression or no change under the conditions of an identical motor task (Ridding *et al.* 1995; Zoghi & Nordstrom, 2007). Finally, somatosensory re-afferent feedback may cause changes in interhemispheric inhibition. In patients after a cerebral stroke, transient ischaemic anaesthesia of the intact hand resulted in a decrease of S-IHI from the lesional to the contralesional M1 (Floel *et al.* 2008). Modulation of the ISP area by somatosensory re-afferent feedback has never been investigated, but the present experiments suggest that it does not play a major role, as the ISP enhancement can occur during motor imagery, i.e. independent of changes in the proprioceptive feedback as it would be produced by real movements of the contralateral hand.

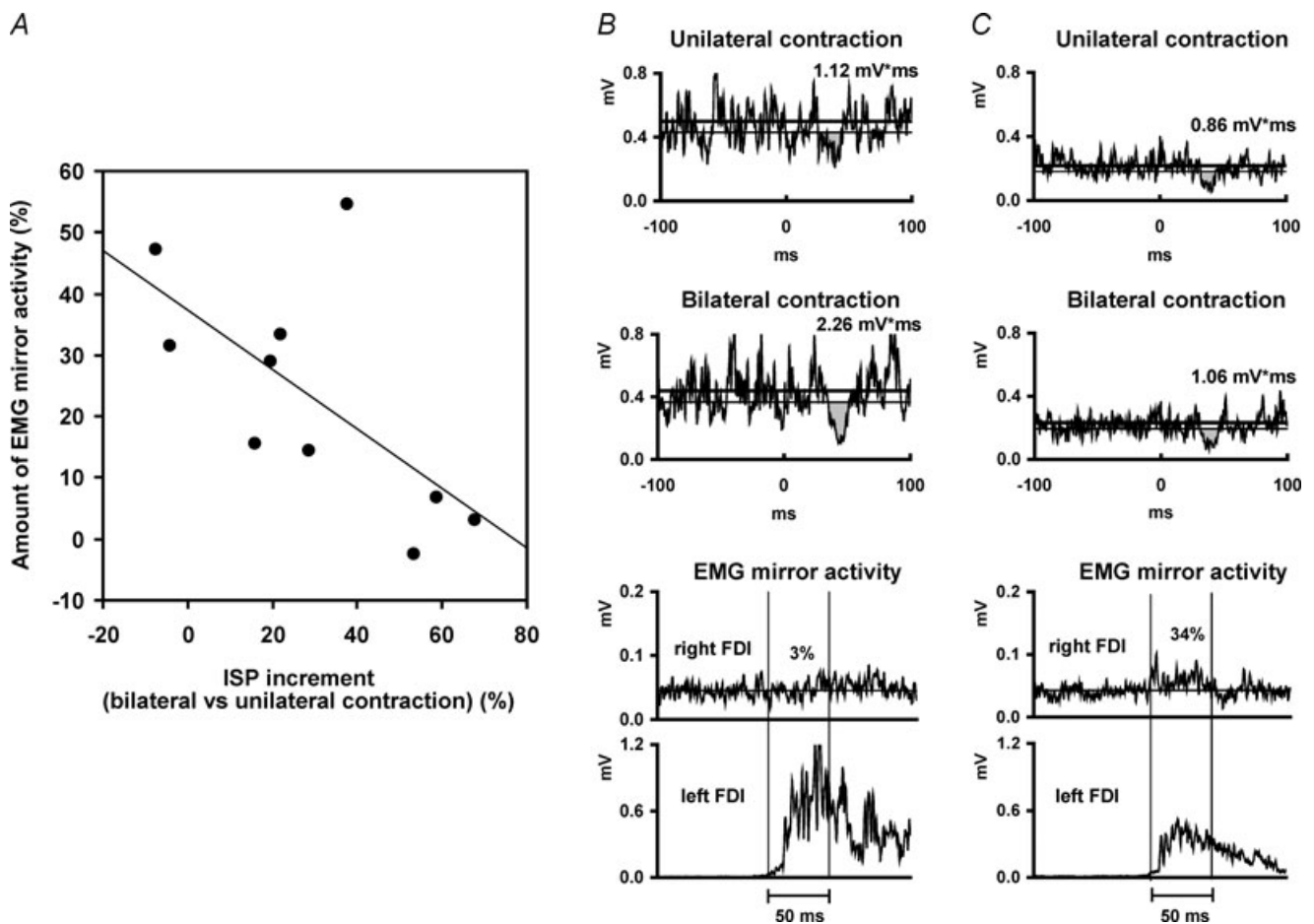


Figure 7. Relation between motor task-related ISP modulation and EMG mirror activity

A, data from all 10 subjects. The increment of ISP area in the right FDI observed when comparing bilateral maximal isometric FDI contraction with unilateral maximal isometric contraction of the right FDI is expressed as the percentage difference between the two measurements (x-axis). Positive and negative values indicate increases vs. decreases in ISP area, respectively. The amount of EMG mirror activity in the right FDI is given on the y-axis (for details, see Methods). ISP area increment and amount of EMG mirror activity correlated inversely ($r = -0.64$, $P = 0.046$). B, recordings from one representative subject with large motor task-related ISP area increment and low EMG mirror activity. C, recordings from one other representative subject with low motor task-related ISP area increment but a large amount of EMG mirror activity.

Topographical and temporal specificity of motor task-related ISP modulation

Enhancement of the ISP does not necessarily require voluntary activation of the left hand muscle that is homologous to the ISP target muscle (right FDI), as it also occurred with contraction of the left EIP, another distal upper limb muscle. However, lacking ISP enhancement with more proximal left upper limb muscles (ECR, BB), or a left or right lower limb muscle (TA) clearly indicates that the motor task-related ISP enhancement is substantially topographically specific. This is in good accord with anatomical data in monkeys (Rouiller *et al.* 1994) and magnetic resonance imaging (MRI) data in humans (Wahl *et al.* 2007), which supported the notion that callosal fibres connect largely homologous M1 body representations in the two hemispheres.

Furthermore, the RT experiment suggests that enhancement of the ISP occurs relatively late in the neuronal processes related to contralateral hand movements, as only a non-significant trend toward ISP facilitation was seen during preparation of a finger movement of the opposite hand 100 ms after the go-signal. A higher temporal resolution was excluded by the experimental design. However, the time course of ISP modulation seems to differ from the time course of S-IHI modulation from the active to non-active M1 in preparation for a unimanual movement, where a maximal inhibition was found ~100 ms after the go-signal, compared to less inhibition in a control resting condition and very shortly before movement onset (Duque *et al.* 2007). This supports the notion of dissociable processes underlying the ISP and the S-IHI (see further below). Another bifocal paired-pulse TMS study investigated the interhemispheric interactions between the left dorsal premotor cortex, an area important for movement selection and preparation, and the right M1, and showed an early inhibition 100 ms after the go-signal, if the right hand had to be moved (Koch *et al.* 2006). This is another example pointing to the rather late-stage operation of the motor task-related ISP modulation in the present study.

Circuit specificity of motor task-related ISP modulation

The ISP induced by focal single-pulse TMS of the M1 ipsilateral to the target hand muscle measures transcallosal inhibition of voluntary motor output from the non-stimulated contralateral M1 (Wassermann *et al.* 1991; Ferbert *et al.* 1992; Meyer *et al.* 1995; Trompetto *et al.* 2004; Cincotta *et al.* 2006). The ISP is absent or delayed in patients with agenesis or surgical lesions of the corpus callosum (Meyer *et al.* 1995, 1998). A transcallosal route of the ISP is further supported by ISP abnormalities in several neurological and psychiatric

disorders with frequent structural alteration of the callosal interhemispheric connections, such as cerebral stroke (Boroojerdi *et al.* 1996), multiple sclerosis (Boroojerdi *et al.* 1998; Höppner *et al.* 1999; Schmierer *et al.* 2000; Lenzi *et al.* 2007), movement disorders (Niehaus *et al.* 2001; Trompetto *et al.* 2003; Wolters *et al.* 2004), schizophrenia (Höppner *et al.* 2001; Fitzgerald *et al.* 2002; Bajbouj *et al.* 2004), and attention deficit hyperactivity disorder (Buchmann *et al.* 2006).

S-IHI reflects the suppressive effect of a conditioning TMS pulse applied to one M1 on the MEP elicited by a test TMS pulse given some 10 ms later to the M1 of the other hemisphere (Ferber *et al.* 1992). Epidural cervical spinal cord recordings of the corticospinal volley induced by the test stimulus (Di Lazzaro *et al.* 1999) and a significant correlation between magnitude of S-IHI and fractional anisotropy, an MRI-based measure of microstructural integrity, of callosal motor fibres (Wahl *et al.* 2007) strongly suggest that S-IHI is also mediated through the corpus callosum. A number of paired-pulse studies demonstrated motor task-related S-IHI modulation. Weak unilateral isometric contraction of the FDI slightly enhances S-IHI from the voluntarily active to the non-active M1 (Ferber *et al.* 1992; Mochizuki *et al.* 2004; Talelli *et al.* 2008).

It can be argued that the ISP enhancement observed in the present study by imagined or real motor activity of the hand contralateral to the stimulated M1 does not merely confirm those findings of the previous S-IHI studies. First, the increase of S-IHI observed during slight unilateral contraction of the FDI contralateral to the conditioning stimulus disappeared when the ipsilateral FDI was contracted in addition (Ferber *et al.* 1992). In contrast, ISP enhancement produced by imagined or real movements of the contralateral hand is observed during isometric contraction of the ISP target muscle (right FDI ipsilateral to the stimulated M1), a mandatory prerequisite for ISP recordings. In addition, by matching conditioned and test MEP amplitudes across different force levels, Perez & Cohen (2008) recently found that S-IHI *decreased* rather than increased when the hand contralateral to the conditioning pulse was engaged in generating high levels of force when compared to rest and slight muscle contraction. Finally, Chen *et al.* (2003) did not find any correlation between S-IHI and ISP duration or ISP area across different stimulus intensities (45, 60, 75 and 90% of MSO) and different current directions induced in the brain. Taken together, these results provide substantial evidence that the neuronal circuits responsible for the ISP differ from those underlying the S-IHI. Here we put this notion a step further by demonstrating that motor task-induced modulations of the ISP and the S-IHI were not correlated with each other. On the other hand, Chen *et al.* (2003) and Avanzino *et al.* (2007) found similarities between ISP and IHI tested at a long interstimulus interval of 40 ms (L-IHI). Several other recent studies support

the notion that S-IHI and L-IHI constitute physiologically distinct forms of interhemispheric inhibition (Kukaswadia *et al.* 2005; Irlbacher *et al.* 2007; Ni *et al.* 2008). Whether or not motor task-related modulation of the ISP and L-IHI is similar as well as the question of whether ISP and S-IHI modulation can also be dissociated using lower levels of target FDI contraction is beyond the scope of this study and may be tested in further experiments.

Hence, the present ISP findings expand the current knowledge on the interhemispheric control of hand movements because ISP measurements deal more directly than S-IHI with interhemispheric inhibition of *voluntary* motor cortical output.

At present, however, it is not entirely clear in which of the two hemispheres the motor task-related ISP enhancement originates. The CSP elicited in the right FDI by stimulation of the left M1, which was carefully matched to the duration of the ISP, was not modulated by left FDI contraction. This dissociation between ISP and CSP suggests that the motor task-related ISP enhancement occurs separately from the inhibitory circuits responsible for the CSP in the left M1, and this may be taken as supportive although not conclusive evidence for an origin of the ISP modulation in the stimulated right M1.

Finally, a possible hemispheric asymmetry of the task-related ISP modulation was not assessed in this study, as only the modulation from the non-dominant right M1 (voluntarily active for left hand movements) to the dominant left M1 (non-active for left hand movements) was tested. This may constitute a slight limitation of the present study. However, one previous study by Duque *et al.* (2007) showed that task modulation of S-IHI in right-handed subjects did not show hemispheric asymmetry if tested from the voluntarily active to the non-active M1. Whether this holds true for the motor task-related ISP modulation needs to be tested in further experiments.

Functional significance of motor task-related ISP modulation

The enhancement of the ISP seen in our experiments reflects a task-specific facilitation of interhemispheric inhibitory influences from the stimulated M1 to the opposite M1. Numerous studies showed that bimanual coordination is more stable in motor tasks requiring mirror-symmetrical rather than non-symmetrical contraction of homologous muscles (for review, see Swinnen, 2002; Swinnen & Wenderoth, 2004). Spatial and temporal constraints limiting the execution of non-symmetrical bimanual movements have been identified (Swinnen, 2002; Swinnen & Wenderoth, 2004). In addition, although healthy adults are usually able to perform unilateral movements in daily life (Schott & Wyke, 1981), a subtle, involuntary mirror activation

is often present (Cernacek, 1961; Armatas *et al.* 1994; Zijdwind & Kernell, 2001; Bodwell *et al.* 2003; Baliz *et al.* 2005; Ottaviani *et al.* 2008; Post *et al.* 2008, 2009). This physiological mirroring, which increases with more demanding motor tasks, fatigue, cognitive distraction, decrease in attentional capacity, and age, depends on unintended motor output along the crossed corticospinal projection from the M1 ipsilateral to the voluntary task ('mirror' M1) and suggests a tendency for movements of the upper extremities to be drawn towards one another (for review, see Cincotta & Ziemann, 2008). Therefore, performance of symmetrical bimanual voluntary movements is considered a basic coordinative behaviour of the nervous system, whereas asymmetrical bimanual motor tasks as well as unimanual movements require complex mechanisms of motor control (Cincotta & Ziemann, 2008) in order to set into action the so-called 'non-mirror transformation' of default 'symmetrical' motor programmes (Chan & Ross, 1988). In the present study, we demonstrated that the ISP enhancement observed when the stimulated M1 is generating motor output to the contralateral hand inversely correlated to the amount of EMG mirror activity in the tonically contracted target hand muscle by intended unilateral phasic contraction of the homologous contralateral muscle. These data provide novel evidence as to the functional significance of the ISP by directly linking its motor task-related modulation to neural mechanisms involved in lateralisation of voluntary movements. This strongly support the hypothesis that the M1 contralateral to a given voluntary movement contributes to this process by a late-stage inhibition of unwanted 'mirror' motor output in the opposite M1. This view is in keeping with the absence of the ISP in childhood (Heinen *et al.* 1998; Koerte *et al.* 2009), when physiological mirroring is far greater than in adults.

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Author contributions

F.G., A.B., M.C., and U.Z. conceived and designed the experiments; F.G., A.B., F.B., and M.C. carried out the experiments; F.G. and M.P.V. performed data analysis; F.G., A.B., and F.B. drafted the paper; G.Z., M.P.V., M.C., and U.Z. revised the paper. All authors approved the final version of the article. The experiments were done in the TMS laboratory at the Unit of Neurology, Florence Health Authority, Florence, Italy.

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