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Lack of evidence for interhemispheric inhibition in the lower face primary motor cortex

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HIGHLIGHTS

- Interhemispheric inhibition (IHI) was absent in the facial motor cortex representation of the depressor anguli oris muscle.
- In contrast, IHI was clearly expressed in motor cortex representations of hand and axial muscles.
- Integration of facial bilateral movement may occur mainly in the brainstem.

ABSTRACT

Objective: To investigate interhemispheric inhibition (IHI) between the facial primary motor cortices (fM1s).

Methods: IHI was investigated in 10 healthy subjects using paired-pulse TMS in the depressor anguli oris (DAO), upper trapezius (UT) and first dorsal interosseous (FDI) muscles. Conditioning stimuli (CS) of 90–130% resting motor threshold (RMT) preceded test motor evoked potentials (MEPs) by 7 interstimulus intervals (ISIs) ranging 4–12 ms. In the DAO, we also examined IHI at 1–2 ms ISIs.

Results: IHI was detected in the UT (CS 130% RMT; ISI 8 ms; $p = 0.02$) and FDI (CS 120% and 130% RMT, at 8–10 ms ISIs; $p = 0.004$), but not in DAO at any ISI, instead, there was facilitation at 1–4 ms ISIs and 110–130% RMT CS. In the DAO, conditioned responses at 1–4 ms ISIs were significantly larger than both test MEPs and the response induced by the CS alone.

Conclusion: In the DAO there was no evidence of IHI even though this was clear in hand and axial muscles. Control experiments excluded a transcallosal origin of the facilitation observed at the shortest intervals.

Significance: Data suggest that integrated bilateral control of facial muscles occurs mainly at the level of brainstem circuits engaged by corticobulbar output from fM1.

1. Introduction

Co-ordination between the two hands in bimanual movements is common to many daily tasks (Wahl and Ziemann, 2008; Takeuchi et al., 2012) and has been shown to involve activity in supplementary motor area (SMA) and the lateral premotor cortex (Sadato et al., 1997; Toyokura et al., 1999), as well as the transcallosal connection between the premotor and sensorimotor areas of both hemispheres (Sperry, 1968; Preilowski, 1972; Jeeves et al., 1988; Leonard et al., 1988; Geffen et al., 1994). Indeed, many studies have shown that interhemispheric interactions are an important contributor to movements involving both body sides (Wahl and Ziemann, 2008; Perez and Cohen, 2009).

Ferbert et al. (1992) described a technique to evaluate the interhemispheric interactions between the hand primary motor cortices (M1) of the two sides in intact human subjects using double-pulse transcranial magnetic stimulation (TMS). They showed that the motor evoked potential (MEP) evoked by a supra-threshold stimulus over one M1 was suppressed by a conditioning stimulus to the contralateral M1 given between 6 and 15 ms earlier. This phenomenon was termed inter-hemispheric inhibition (IHI) and was suggested to be due to activation of transcallosal outputs by the

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conditioning pulse, since this effect was absent in patients with agenesis of the corpus callosum (Meyer et al., 1995).

IHI was described initially in hand muscles. However, later studies found that IHI between the more proximal triceps or scapula-thoracic muscles was less effective than in the FDI (Harris-Love et al., 2007; Matthews et al., 2013). The implication was that bilateral coordination between more proximal muscles was less dependent on transcallosal connections than between distal muscles. Indeed, animal studies have shown that the control of proximal muscles is less affected by callosal section, presumably due to the fact that each hemisphere has access to bilateral connections to proximal muscles via cortico-reticulospinal pathways (Brinkman and Kuypers, 1972).

There are few studies of bilateral control in facial primary motor cortex (fM1). Anatomical tracer studies in animals, demonstrated that fM1, as defined by intracortical microstimulation, is connected with its homolog in the other hemisphere through callosal fibres, at least in the owl monkey (Gould et al., 1986) and in the macaque monkey (Rouiller et al., 1994). In contrast with these findings, a neuroimaging study failed to identify callosal motor fibres connecting fM1s, in humans (Wahl et al., 2007). A previous TMS study demonstrated that fM1 sends bilaterally symmetric projections to the lower facial muscles and that the ipsilateral projections utilised a direct corticobulbar connection rather than employing a transcallosal pathway via the opposite hemisphere (Pilurzi et al., 2013). The aim of the present study was therefore to investigate the presence of IHI between the two fM1s using the depressor anguli oris muscle (DAO) as a model. Results were compared with those from the FDI and the upper trapezius muscle (UT). When interpreting the results note that it is necessary to bear in mind that DAO motoneurones receive a bilateral projection from fM1 (Pilurzi et al., 2013) that complicates interpretation of the IHI data.

2. Methods

2.1. Participants

Experiments were conducted in fifteen healthy volunteers (8 females and 7 males; mean age 28.57 ± 3.90 years), all right handed according to the Oldfield Inventory Scale (Oldfield, 1971). All subjects gave their informed written consent to participate in the study, which was approved by the local ethical committee and conducted in accordance with the declaration of Helsinki. None of the subjects had history or current signs/symptoms of neurological diseases. Subjects sat in a comfortable chair and were asked to stay relaxed but alert during the experiments.

2.2. EMG

EMG was recorded contralaterally, in different experimental sessions, from the DAO, FDI and UT muscles, using 9 mm diameter Ag-AgCl surface electrodes. For EMG recordings from the DAO, the active electrode was placed at the midpoint between the angle of the mouth and the lower border of the mandible, the reference electrode over the mandible border, 1 cm below the active electrode and the ground electrode over the right forehead (Pilurzi et al, 2013). For EMG recordings from the FDI, the active electrode was placed over the muscle belly, the reference electrode at the second finger metacarpo-phalangeal joint and the ground electrode over the forearm (Ferbert et al., 1992; Rossini et al., 2015). For the UT EMG recording, the active and reference electrode were placed 3 cm apart over UT with a distance of 3 cm between each other's and the ground on the sternum (Matthews et al., 2013). Unrectified EMG signals were recorded (D360 amplifier, Digitimer Ltd, Welwyn Garden City, UK), amplified (×1000), filtered (bandpass 3–3000 Hz), sampled (5 kHz per channel; window frame length: 250 ms) using a 1401 power analog-to-digital converter (Cambridge Electronic Design, Cambridge, UK) and Signal 6 software on a computer and stored for off-line analysis.

2.3. TMS

TMS was performed using a figure-of-eight shaped coil with external loop diameter of 7 cm connected to a Magstim 200 stimulator (Magstim Co., Whitland, and Dyfed, UK). The optimal stimulation site, for the contralateral DAO, FDI or UT muscles was carefully searched and then marked with a soft tip pen over the scalp, to maintain the same coil position throughout the experiments. The optimal coil position for eliciting MEPs in the DAO was roughly 4 cm anterior and 8 cm lateral from the Cz with the handle of the coil pointed posteriorly and laterally, at approximately 30–45 deg to the interhemispheric line (Kujirai et al., 2006; Pilurzi et al., 2013). For both FDI and UT the coil pointed backwards and laterally (postero-anterior orientation) at 45 deg away from the midline. The resting motor threshold (RMT) was taken as the lowest TMS intensity that elicited, in the relaxed muscle, MEPs of 0.05 mV in at least 5 out of 10 consecutive trials and was expressed in percentage of the maximum stimulator output (MSO) (Groppa et al., 2012; Rossini et al., 2015). Active motor threshold (AMT) was established as the minimum stimulus intensity able to evoke MEPs > 0.2 mV peak-to-peak amplitude in at least five out of ten consecutive trials during isometric contraction of the tested muscle at 10% of maximum voluntary isometric contraction (MVIC) (Rossini et al., 2015). The intensity of the TS for TMS was 120% of RMT.

2.4. Experimental design

The design of the study comprised a main experiment (experiment 1) and two control experiments (experiment 2 and 3) which took place one week apart from the main experiment.

2.4.1. Experiment 1. Interhemispheric inhibition between M1s innervating the DAO, FDI and UT muscles

In ten subjects, the IHI was performed in the M1 representation of the DAO, FDI and UT muscles. IHI was tested using 7-cm double coils and delivering a CS to the M1 of one side before the administration of a test stimulus to the contralateral M1, using a CS intensity between 90 and 130% of RMT. IHI was measured in the contralateral muscle from both left-to-right and right-to-left M1s in a randomized order. The experiment was divided up into three blocks: IHI in DAO, IHI in FDI and IHI in UT muscles. In each block, TS alone and 4, 6, 8, 10, 12 ms conditioning-test interstimulus intervals (ISIs) were tested. The three blocks and all states (TS alone and ISIs) were randomized in each subject. Ten unconditioned MEPs and ten conditioned responses for each ISI were recorded.

2.4.2. Experiment 2. Investigation of a possible direct activation of the DAO by the CS alone and by paired CS-TS at 1–4 ms ISIs

In order to investigate the origin of the early facilitation of the DAO observed at 4 ms ISI following the IHI protocol, the effects of the CS alone and of paired pulse TMS at 1, 2 and 4 ms ISIs were investigated in 6 out of 10 subjects who participated in Experiment 1 (4 females and 2 males; mean age 31.5 ± 0.38 years), using CS intensities between 110% and 130% of RMT. The effect of CS alone and of IHI was measured both from left-to-right and from right-to-left M1s in both left and right DAO. Ten unconditioned MEPs and ten conditioned responses for each ISI were recorded in a random order.

2.4.3. Experiment 3. Contribution of corticobulbar tract activation to facilitation of the conditioned DAO MEP

To assess the effect of the activation of the corticobulbar tract on the conditioned DAO MEP, in 5 out of 10 subjects who participated in Experiment 1 (3 females and 2 males; mean age 31.60 ± 0.42 years), the recruitment curve (RC) was constructed plotting peak-to-peak amplitudes of mean MEPs, recorded from both the resting (rest RC) and active (active RC) contralateral DAO, following single-pulse TMS delivered to the contralateral fM1 at intensities from 90 to 130% of RMT and AMT. MEP amplitude was measured from the left and right DAO. The following three blocks each composed of ten stimuli for each intensity were collected: (1) rest-RC and (2) active-RC with intensity of 90–130% of RMT (RMT-RC); (3) active-RC with intensity of 90–130% of AMT (AMT-RC). For the active-RC the subject was required to keep a constant contraction of the DAO at a level of at least 10% of maximal isometric voluntary contraction. The results were compared with those obtained in experiment 2.

2.4.4. Experiment 4. Contribution of I3 waves to the lack of IHI detected in the DAO

To investigate a possible contribution of I3 waves, in 5 subjects (4 females and 1 male; mean age 26.6 ± 4.27 years) IHI was investigated in the DAO using an anterior-posterior coil orientation (Sakai et al., 1997; Adank et al., 2018). More specifically, the handle of the coil pointed from anterior to posterior direction, at approximately 30–45 deg away from the interhemispheric line (Kujirai et al., 2006; Pilurzi et al., 2013). IHI was tested using a CS intensity between 90 and 130% of AMT and a TS of 120% of RMT. MEPs were recorded in the contralateral DAO following paired TMS of both left-to-right and right-to-left M1s in a randomized order. The experiment was divided up into two blocks: IHI in the left and right DAO muscles. In each block, TS alone and paired TS-CS at 4, 6, 8, 10, 12 ms ISIs were tested. The two blocks and all states (TS alone and ISIs) were randomized in each subject. Ten unconditioned and ten conditioned MEPs for each ISI were recorded.

2.4.5. Experiment 5. Interhemispheric inhibition in the active DAO

To exclude a possible floor-effect due to the small size of the DAO MEPs recorded at rest, in the 5 subjects who participated in Experiment 4, the IHI protocol was performed during a constant contraction of the DAO (10% of maximal isometric voluntary contraction), using a TS of 120% AMT and a CS of 90–130% AMT. IHI was recorded in the contralateral DAO following paired TMS of both left-to-right and right-to-left M1s in a randomized order. The experiment was divided up into two blocks: IHI in left DAO and IHI in right DAO. In each block, TS alone and paired CS-TS at 4, 6, 8, 10, 12 ms ISIs were tested. The two blocks and all states (TS alone and ISIs) were randomized in each subject. Ten unconditioned MEPs and ten conditioned responses for each ISI were recorded.

2.5. Statistical analysis

Statistical analysis was performed with SPSS 20 software (SPSS Inc, Chicago, IL, USA). Student's paired t-test, repeated measures analysis of variance (ANOVA) and planned post hoc t-test with Bonferroni correction for multiple comparison were used. Compound symmetry was evaluated with the Mauchly's test and the Greenhouse-Geisser correction was used when required. Significance was set for p value < 0.05. Unless otherwise stated, values are expressed as means ± standard error of the mean (SEM). In all experiments latency and amplitude of conditioned and unconditioned MEPs were analysed.

Experiment 1, 2, 4 and 5: A three-way repeated measure ANOVA with ISI (Experiment 1, 4 and 5: TS, 4, 6, 8, 10 and 12 ms ISIs; Experiment 2: CS, TS, 1, 2, 4 ms ISI), INTENSITY of CS (Experiment 1: 90–130% RMT; Experiment 2: 110–130% RMT; Experiment 4 and 5: 90–130% AMT) and SIDE (contralateral muscle from both right-to-left and left-to-right IHI) as within subject factors was used. In case the analysis detected a non-significant SIDE effect, left and right responses were pooled together as a single distribution. In that case a two-way ANOVA with a ISI and INTENSITY as a within factors was performed. Moreover, a two-way repeated measure mixed ANOVA, on the MEP onset latency with ISI (TS, 4, 6, 8, 10, 12 ms ISIs), INTENSITY of CS (90–130% RMT or AMT) as within subject factors, and EXPERIMENT as between subject factor (PA at rest, AP at rest and PA active) was performed.

Experiment 3: A preliminary three-way repeated measure ANOVA with SIDE (left and right muscle contralateral to TS), INTENSITY (90–130% RMT or AMT, according to the resting or active condition) and CONDITION (rest-RC, active-RMT-RC and active-AMT-RC) as a within subject factors was performed. In case the analysis detected a non-significant SIDE effect, left and right responses were pooled together as a single distribution. To compare MEPs obtained in the RC with those obtained in experiment 2, a two-way repeated measure ANOVA with INTENSITY (110–130% RMT or AMT, according to the resting or active condition) and TYPE OF MEP (TS, CS, conditioned-MEP at 1, 2, 4 ms ISIs, rest-RC, active-RMT-RC and active-AMT-RC) as a within subject factors was used.

3. Results

3.1. Experiment 1. Interhemispheric inhibition between M1s innervating the DAO, FDI and UT muscles

No significant effect of SIDE for all muscles (DAO: $F_{1,7} = 0.007$ $p = 0.937$, FDI: $F_{1,7} = 0.323$ $p = 0.590$, UT: $F_{1,7} = 0.020$ $p = 0.901$) was detected, thus right and left MEPs were pooled together.

In the DAO, the mean RMT was 51.34 ± 3.73% MSO. No clear IHI was detected at any stimulation intensity and ISI; a significant facilitation was rather found at 4 ms ISI (Fig. 1). Indeed ANOVA showed a non-significant main effect of INTENSITY ($F_{5,13} = 1.021$, $p = 0.378$) on MEP amplitude, but a significant effect of ISI ($F_{5,13} = 4.756$, $p = 0.013$) and a significant interaction among factors ($F_{5,13} = 2.945$, $p = 0.011$). Post-hoc analysis showed that the conditioned MEP was significantly bigger than the test MEP at 4 ms ISI at intensities of 110% ($p = 0.007$), 120% ($p = 0.04$) and 130% ($p = 0.005$) of RMT.

In the FDI, the mean RMT was 40.54 ± 2.12% of MSO. A clear IHI at ISIs of 8 and 10 ms with high intensity stimuli (120 and 130% of RMT) was detected (Fig. 1). ANOVA showed a non-significant effect of INTENSITY ($F_{5,13} = 1.391$, $p = 0.258$) on MEP amplitude, but a significant main effect of ISI ($F_{5,13} = 8.232$, $p < 0.001$) and a significant interaction among the factors ($F_{5,13} = 1.990$, $p = 0.051$). Bonferroni test showed a clear inhibition at ISIs of 8 ms ($p = 0.026$) and of 10 ms ($p = 0.011$) at 120% RMT intensity and only at 8 ms ISI with 130% RMT Intensity ($p = 0.005$).

In the resting state, the high threshold of UT M1 allowed to complete the experiment in only in 6 of the 10 subjects, in whom mean RMT was 52.85 ± 2.58% MSO. A clear inhibition of the conditioned MEP was detected at an ISI of 8 ms with an intensity of 130% RMT (Fig. 1). Statistical analysis showed a non-significant effect of INTENSITY ($F_{5,13} = 1.265$, $p = 0.304$) on MEP amplitude, but a significant effect of ISI ($F_{5,13} = 7.040$, $p = 0.004$) and interaction among the factors ($F_{5,13} = 1.660$, $p = 0.045$).Post-hoc analysis showed a clear MEP inhibition at 8 ms with a 130% RMT intensity ($p < 0.001$). Fig. 2 illustrates recordings from a representative subject.

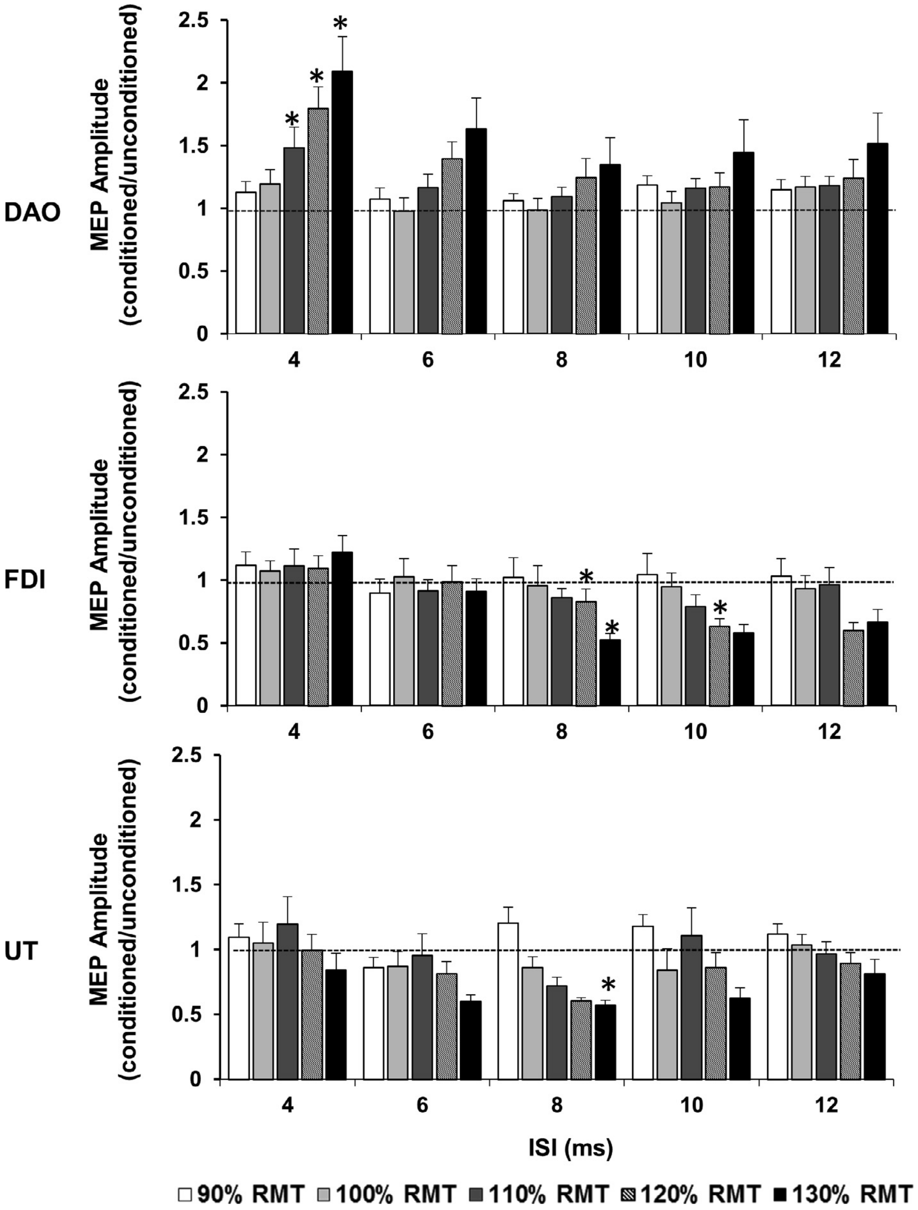

Fig. 1. Effect of the IHI protocol on the M1 representation of the depressor anguli oris (DAO), first dorsal interosseus (FDI) and upper trapezius (UT) muscles. IHI was clearly detected in the UT and FDI muscles at the expected ($\geq$8 ms) interstimulus time intervals (ISIs). In the DAO, no IHI was found, an early significant facilitation was instead observed at 4 ms ISI. Graph reporting mean ± SEM conditioned MEP amplitudes ($N = 10$ subjects for the DAO and FDI; $N = 6$ subjects for the UT), which are expressed, as a percentage of the unconditioned MEP induced by the TS alone The graphs show the IHI protocol for each interstimulus interval (ISIs; 4, 6, 8, 10, 12 ms) at different conditioning stimulus intensities raging 90–130% of the resting motor threshold (RMT). $^*p < 0.05$.

3.2. Experiment 2. Investigation of a possible direct activation of the DAO by the CS alone and by paired CS-TS at the shortest ISIs

The 6 subjects who participated in this experiment had a mean RMT of 52.5 ± 3.60% of MSO, which was not statistically different from that detected in experiment 1 ($p = 0.40$).

No significant effect of SIDE for both amplitude ($F_{1,5} = 2.808$ $p = 0.169$) and latency ($F_{1,5} = 5971$ $p = 0.07$) was detected, thus right and left MEPs were pooled together.

Within subject ANOVA showed a significant effect of the INTENSITY ($F_{2,9} = 10.836$, $p = 0.001$) and ISI ($F_{2,9} = 26.964$, $p < 0.001$) on MEP amplitude, but a non-significant interaction among factors ($F_{2,9} = 1.523$, $p = 0.212$). Post-Hoc analysis showed that the test MEP was not significantly different from the response induced by the CS alone ($p = 0.9$) but both MEPs were smaller than the conditioned MEP at ISIs of 1, 2 and 4 ms (all $p < 0.01$) (Fig. 3A).

The mean latency of the conditioned MEP at 1, 2 and 4 ms ISIs was significantly shorter than that of the test MEP and of the MEP induced by the CS alone (Fig. 3B). ANOVA detected a significant effect of ISI ($F_{2,9} = 41.101$, $p < 0.001$) but a non-significant effect of INTENSITY ($F_{2,9} = 1.073$, $p = 0.360$) nor interaction among the factors ($F_{2,9} = 0.890$, $p = 0.492$). Bonferroni analysis showed that the latencies of the test MEP and of the response induced by the CS alone were not significantly different ($p = 0.99$), but significantly longer than the latency of the conditioned MEP ($p < 0.001$).

3.3. Experiment 3. Contribution of corticobulbar tract activation to facilitation of the conditioned DAO MEP

No significant effect of SIDE for both MEP amplitude ($F_{1,4} = 1.842$, $p = 0.246$) and latency ($F_{1,4} = 2.167$, $p = 0.237$) was detected, thus right and left MEPs were pooled together (Fig. 4).

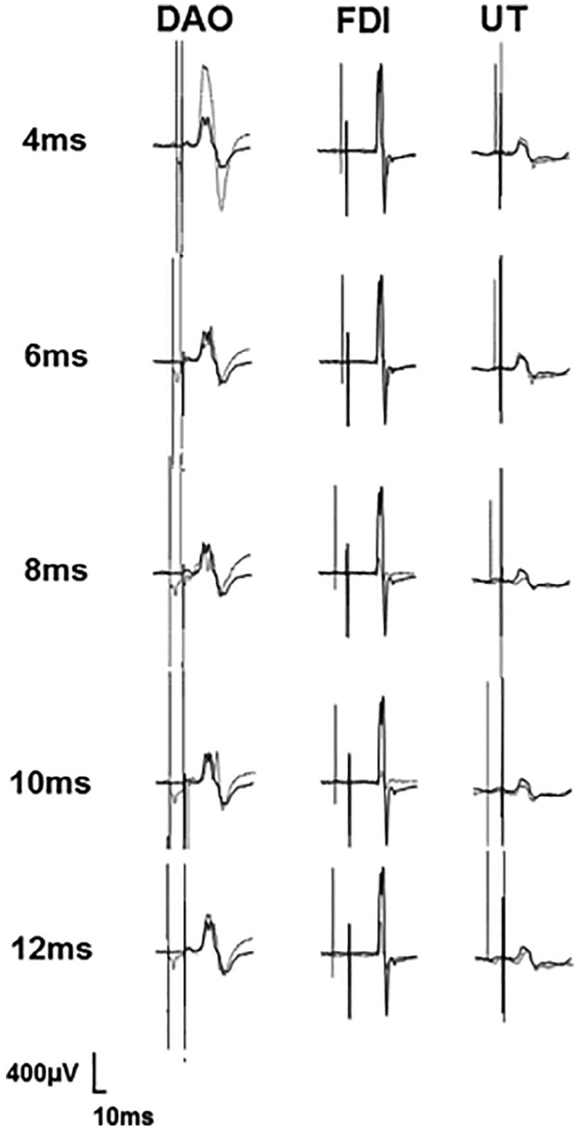

Fig. 2. Effect of IHI protocol on the M1 of DAO, FDI and UT muscles at high conditioning stimuli intensity. Recordings of unconditioned MEP (continuous line) and superimposed conditioned MEPs (dashed lines) at ISIs of 4, 6, 8 10 and 12 ms from a representative subject are reported for each muscle with 130% RMT conditioning stimuli intensity.

Statistical analysis of MEP amplitude revealed a significant effect of INTENSITY ($F_{1,9} = 59.969$, $p < 0.001$), TYPE OF MEP ($F_{1,9} = 20.142$, $p < 0.001$) and a significant interaction among factors ($F_{1,9} = 4.717$, $p < 0.001$) (Fig. 3). ANOVA of latency showed a significant effect of TYPE OF MEP ($F_{1,9} = 22.508$, $p < 0.001$) but a non-significant effect of INTENSITY ($F_{1,9} = 1.933$, $p = 0.171$) nor interaction among factors ($F_{1,9} = 1.463$, $p = 0.211$).

Bonferroni post Hoc test showed that amplitude and latency of the conditioned MEPs were significantly different from those of both the test MEP and MEP induced by CS alone ($p < 0.01$), but non-significantly different from the MEP obtained in active-RMT-RC ($p > 0.8$) and active-AMT-RC with intensity of 120–130% RMT and AMT, respectively.

3.4. Experiment 4. Contribution of I3 waves to the lack of IHI detected in the DAO

Mean RMT was 57.20 ± 5.41% MSO. No significant effect of SIDE ($F_{1,4} = 1.800 p = 0.272$) was detected, so that we pooled together right and left MEPs as a single distribution. No clear IHI was detected at any stimulation intensity and ISI (Fig. 5). The two-way ANOVA showed no significant main effect of INTENSITY ($F_{1,9} = 1.728$, $p = 0.196$), ISI ($F_{1,9} = 3.388$, $p = 0.073$) and no interaction among factors ($F_{1,9} = 1.383$, $p = 0.265$).

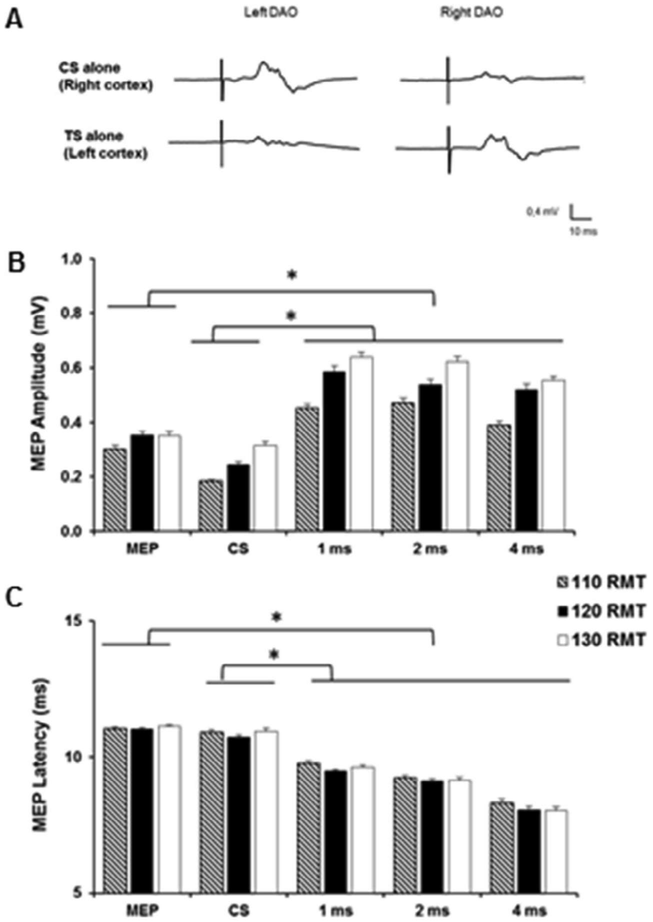

Fig. 3. Effect of the conditioning stimulus alone and of paired TS-CS at the shortest ISIs on the DAO MEP. Responses of the right and left DAO to TS alone (120% RMT) delivered to the left cortex and to the CS alone (120% RMT) delivered to the right cortex are reported for a representative subject (A). The effects of the CS alone and of the paired pulse TMS at 1, 2, and 4 ms ISIs on amplitude (B) and latency (C) of the DAO MEP are shown. The conditioned MEPs were significantly bigger and faster than both test MEP (induced by test stimulation, TS, of the contralateral face primary motor cortex, fM1) and conditioned MEPs (CS, obtained following stimulation of the ipsilateral fM1 with the CS alone). The graphs report means + SEM ($N = 6$ subjects). Post hoc results $^{*}p < 0.05$.

3.5. Experiment 5. Interhemispheric inhibition in the active DAO

Mean AMT was 43.80 ± 6.27% MSO. The three-way RM-ANOVA showed a no significant effect of SIDE ($F_{1,4} = 0.376$ $p = 0.573$), and therefore right and left MEPs were pooled together. Two-way ANOVA on MEP amplitude showed a non-significant main effect of INTENSITY ($F_{1,9} = 1.954$, $p = 0.171$), ISI ($F_{1,9} = 1.716$, $p = 0.199$) but no significant interaction among factors ($F_{1,9} = 1.560$, $p = 0.204$), (Fig. 6).

Finally, MEP latencies at rest (with PA and AP coil orientation) and active (Table 1) were compared. Mixed factor ANOVA showed a non-significant main effect of INTENSITY ($F_{2,35} = 2.473$, $p = 0.065$), but a significant effect of ISI ($F_{2,35} = 6.782$, $p = 0.001$) and EXPERIMENT ($F_{2,35} = 25.365$, $p < 0.001$). The analysis showed no significant effect of any interactions among the factors except for the ISI × EXPERIMENT ($F_{2,35} = 6.782$, $p = 0.001$). MEPs in PA and AP rest conditions were not different but they were always different from

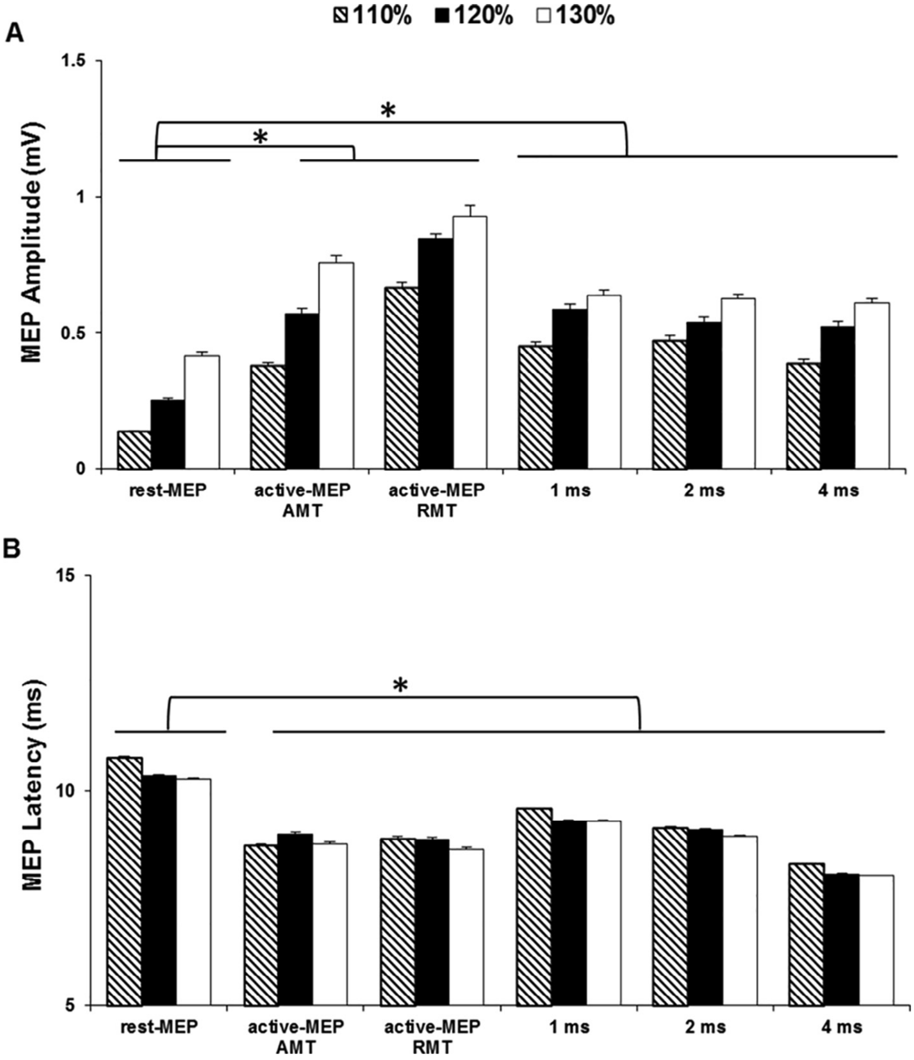

Fig. 4. Mean amplitude and latency of resting and active unconditioned DAO MEPs at increasing TMS intensities and of conditioned DAO MEPs at 1, 2 and 4 ms ISIs. The amplitude (A) and the latency (B) of the conditioned MEPs were significantly larger and faster than that of the test MEPs obtained in resting condition with 110–130% of RMT, but non-significantly different from the active test MEP obtained with both 110–130% RMT and 110–130% AMT. Error bars represent standard mean error. Post hoc results $^*p < 0.05$.

those obtained in the PA active condition (all $p < 0.001$) except for the conditioned MEP at 4 ms ISI in the PA rest condition which was significantly different from both AP rest ($p = 0.026$) and PA active conditions ($p = 0.001$).

4. Discussion

The main finding of the present study was the absence of IHI in the DAO muscle, even though it was clearly present at 8–10 ms in FDI and, with slightly reduced effectiveness, at 8 ms in UT (Matthews et al., 2013). In fact, rather than inhibition, we observed facilitation in the DAO at shorter ISIs (1–4 ms). This is unlikely to be the result of "interhemispheric facilitation" described in hand muscles (Hanajima et al., 2001). First, in the hand muscles, facilitation is only seen with subthreshold CS (Hanajima et al., 2001), while the DAO facilitation occurred only with suprathreshold CS. Second, facilitation in DAO was found at ISIs = 1–4 ms, which are shorter than the 5–10 ms conduction delay across the human corpus callosum required for interhemispheric interactions (Meyer et al., 1995). We hypothesise that IHI is absent in DAO and that the early facilitation is the result of convergence at the brainstem level between ipsilateral projections, activated by the CS, and contralateral projections from the TS. Finally, given the lack of difference in the results from muscles in the left and right sides of the body we conclude that there are no asymmetries in either IHI or early facilitation in these muscles.

For long time it was thought that projections from motor cortex to muscles of the lower half of the face emanate exclusively from the contralateral cortex while upper facial muscles receive bilateral projections from both hemispheres (Cattaneo and Pavesi, 2014; Müri, 2016). However, many TMS studies in healthy individuals seem at odds with this. Although some found no ipsilateral response in the lower facial muscles (Cruccu et al., 1990; Kobayashi et al., 2001; Paradiso et al., 2005), many others have described bilateral projections, although with a contralateral predominance (Benecke et al., 1988; Meyer et al., 1994; Werhahn et al., 1995; Urban et al., 1997, 2001; Liscić and Zidar, 1998; Rödel et al., 2000; Yildiz et al., 2004, 2007; Triggs et al., 2005, Pilurzi et al., 2013). In particular in a previous study (Pilurzi et al., 2013) we found an ipsilateral response in DAO with an onset latency that was 1–2 ms longer and a higher threshold than the contralateral response. A similar difference of latency of around 2.0–2.5 ms between ipsi- and contralateral responses has been

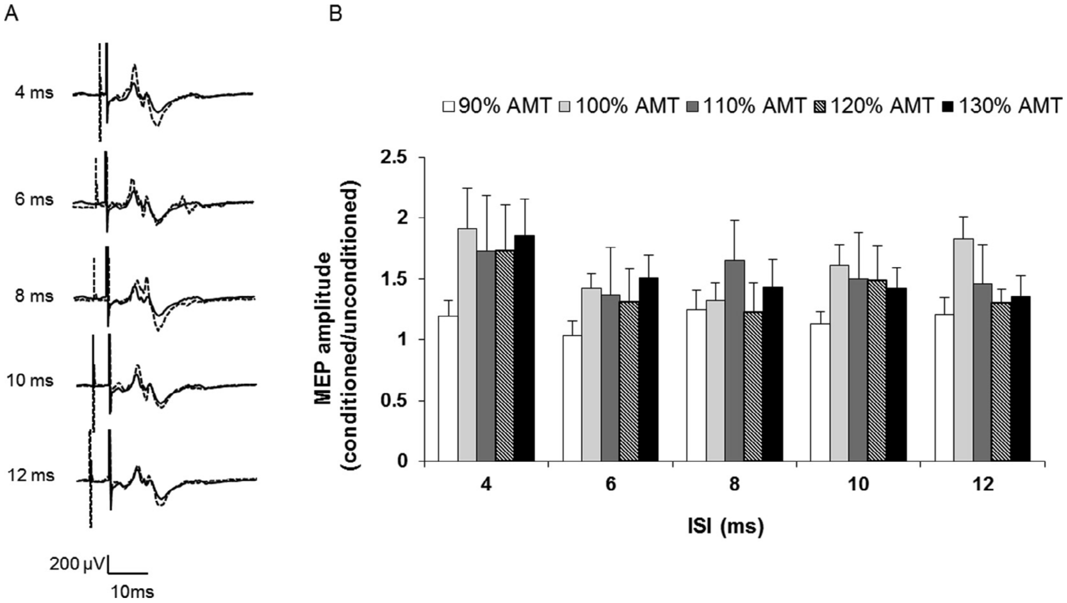

Fig. 5. IHI protocol in the DAO muscle with an antero-posterior orientation of the coil. Recordings of unconditioned MEP (continuous line) and superimposed conditioned MEPs (dashed lines) at ISIs of 4, 6, 8 10 and 12 ms from a representative subject (A) are reported for each muscle with a conditioning stimulus of 120% of active motor threshold (AMT). The histogram reports results from 5 subjects (expressed as mean ± SEM). The conditioned MEP amplitude is expressed as a ratio of the unconditioned MEP induced by the TS alone. Results are reported for each ISI (4, 6, 8, 10, 12 ms) at conditioning stimulus intensities raging 90–130% of AMT (B).

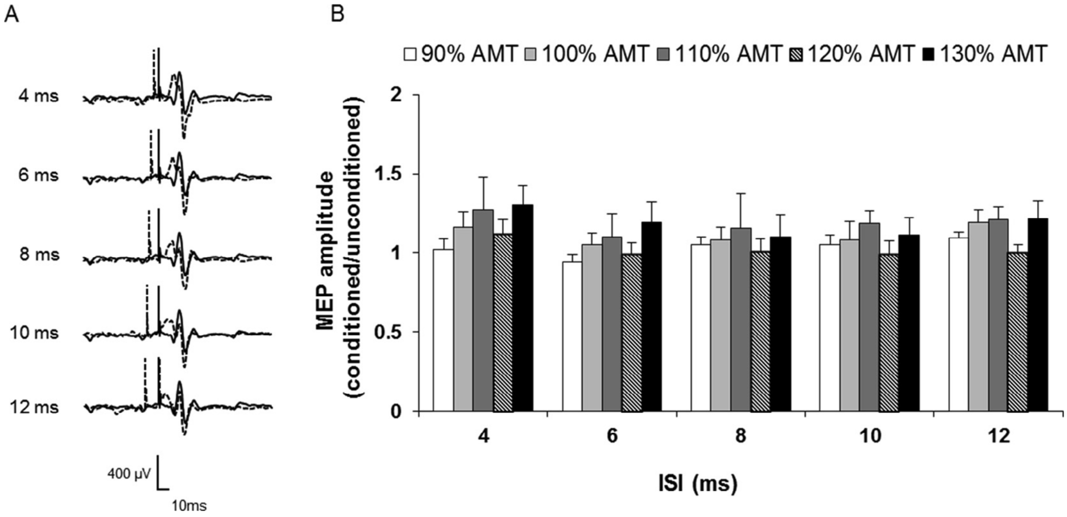

Fig. 6. Interhemispheric inhibition in the active DAO. Recordings of unconditioned MEP (continuous line) and superimposed conditioned MEPs (dashed lines) at ISIs of 4, 6, 8 10 and 12 ms from a representative subject (A) are reported for each muscle with a conditioning stimulus of 120% of active motor threshold (AMT). The histogram reports results from 5 subjects (expressed as mean ± SEM). The conditioned MEP amplitude is expressed as a ratio of the unconditioned MEP induced by the TS alone. Results are reported for each ISI (4, 6, 8, 10, 12 ms) at conditioning stimulus intensities raging 90–130% of AMT (B).

Table 1
Latency of unconditioned (TS) and conditioned MEPs at a different interstimulus intervals (ISIs).

Condition	MEP latency (ms)		
	Experiment 1 (PA, rest)	Experiment 4 (AP, rest)	Experiment 5 (PA, active)
TS	11.12 ± 0.17	10.85 ± 0.24	8.72 ± 0.23
4 ms ISI	9.79 ± 0.23	10.93 ± 0.34	8.17 ± 0.32
6 ms ISI	10.48 ± 0.24	10.86 ± 0.35	8.69 ± 0.33
8 ms ISI	10.64 ± 0.20	10.94 ± 0.29	8.86 ± 0.28
10 ms ISI	10.68 ± 0.20	10.95 ± 0.29	8.73 ± 0.27
12 ms ISI	10.69 ± 0.18	10.85 ± 0.26	8.83 ± 0.25

Latency values are reported as Mean ± SEM. PA, postero-anterior coil orientation; AP, antero-posterior coil orientation.

reported in several upper and lower facial muscles (Benecke et al., 1988; Cruccu et al., 1990; Liscić and Zidar, 1998; Triggs et al., 2005).

The pathway responsible for this ipsilateral response is uncertain. Corticobulbar pathways to the facial nucleus are of two types: direct and indirect (Noback and Demarest, 1975; Brodal, 1981). Direct pathways to the facial nucleus are only thought to arise from contralateral cortex. However, there also exist indirect pathways to interneurons in the brainstem that secondarily innervate the facial nuclei bilaterally (Courville, 1966; Holstege et al., 1977; Rinn, 1984). Such indirect pathways may be responsible for the ipsilateral response in DAO. Involvement of the corpus callosum seems unlikely in view of the longer (5–10 ms) conduction time between the hemispheres that it would involve. Indeed, transection of the corpus callosum has been reported to have no effect on ipsilateral facial responses to intracortical stimulation in the cat (Guandalini et al., 1990).

The results of experiments 2 and 3 are compatible with the idea that the early (1–4 ms) facilitation in DAO was due to interaction at the brainstem of corticobulbar projections activated by the CS and TS. The CS facilitates brainstem interneurons or facial motoneurons and increases their response to the subsequent TS. Thus the conditioned MEP was never larger than the expected sum of the MEP evoked by CS alone plus TS alone (Fig. 3) and similar in size to MEPs evoked by the same intensity of TS in active rather than relaxed muscle (Fig. 4). These results suggest that at rest, the facilitation of the conditioned MEP at shortest intervals (1–4 ms) might be due to temporal summation of excitatory input from CS and TS stimuli at the level of DAO motoneurones and interneurons in the brainstem. Similarly, during active contraction, voluntary commands increase the excitability of the interneurons and motoneurones in the brainstem which then increases the amplitude of the MEP to a similar degree as with paired pulse testing.

Brainstem interactions also account for the fact that the latency of the conditioned MEP at ISI = 1–4 ms, was shorter than the latency to the TS alone (Fig. 4). The probable reason is that the onset of the conditioned MEP was due to a small response to the CS, so that as the ISI between CS and TS increased, the latency of the conditioned MEP, which was measured from the onset of the TS, decreased.

It is possible that the apparent lack of IHI in DAO at later intervals is due to the presence of continuing facilitation at the brainstem level that cancels out the effects of later-developing IHI at the cortical level. It is difficult to discount this explanation completely since the CS could activate corticobulbar fibres with a range of conduction velocities that could continue to facilitate brainstem neurones for many ms after the initial, fast-conducted excitation at 1–4 ms ISIs. However, if this were the case, facilitation should gradually fade over time: specifically, we might expect to see less facilitation 10 ms after CS than at 8 ms. Taken together with the fact that IHI is greater at 10 ms than at 8 ms, this means that the conditioned MEP at 10 ms should be smaller than at 8 ms. But Fig. 1 shows that this is not the case. It therefore seems more plausible to conclude that IHI is absent or very small in the DAO.

It is possible that we failed to detect IHI because we used a test TMS pulse with a posterior-anterior orientation. This preferentially recruits early I-waves (Sakai et al., 1997) whereas IHI preferentially suppresses later I-waves. However, experiment 4 suggests this was not the case since we failed to detect IHI even when we used an anterio-posterior coil orientation which preferentially recruits later I-waves (Sakai et al., 1997; Adank et al., 2018). The possibility that IHI could have been overlooked due to the small size of the MEP in the relaxed DAO (which may lead to a "floor-effect"), was excluded by experiment 5. In fact, IHI was not detectable in the active MEP, which is 30–50% larger in amplitude than the resting MEP.

The absence of IHI in DAO is consistent with a previous study using a combined functional magnetic resonance imaging/diffusion tensor imaging fiber-tracking procedure that failed to track lip callosal motor fibres in humans (Wahl et al., 2007). Interestingly, this differs from data in animal studies which shows that fM1, as defined by intracortical microsimulation, is connected with its homolog in the other hemisphere through callosal fibres, at least in the owl monkey (Gould et al., 1986) and in the macaque monkey (Rouiller et al., 1994). The difference between animal and human data may have an evolutionary explanation. Facial muscles are involved in the emotional expressiveness and their motor control in humans has changed differently from other animals, to allow an evolutionary advantage in social behaviour (Darwin, 1872). In line with this, Sherwood (2005) studied the evolution of the brainstem orofacial motor system in 47 species of primates and found that hominids presented significantly larger volumes of the facial nucleus.

The facial nucleus receives cortical projections not only from fM1, but also from the ventral lateral premotor cortex, the supplementary motor area, the rostral cingulate motor cortex and the caudal area of the anterior midcingulate cortex (Morecraft et al., 2001; Cattaneo and Pavesi, 2014; Müri, 2016). As a consequence, the facial motor nucleus may have undergone phylogenetic specialization in humans to be able to integrate descending inputs from multiple neocortical areas to allow increased control of facial muscles (Sherwood, 2005) while at the same time, the transcallosal pathway may have progressively lost its importance.

4.1. Conclusions

Compared with the important role of interhemispheric transcallosal connections in coordination of asymmetric bilateral upper limb movements (Wahl and Ziemann, 2008; Takeuchi et al., 2012), our data suggest that the corpus callosum is barely involved in bilateral control of facial muscles. It seems likely that this is because facial muscles are rarely activated asymmetrically, especially during voluntary movements to produce a facial posture (Cattaneo and Pavesi, 2014). We suggest that symmetrical activation is facilitated by the fact that the two sides of the face tend to be represented with overlapping contralateral and ipsilateral representations in regions of M1 devoted to face (Pilurzi et al., 2013), jaw (Clark and Luschei, 1974) and tongue (Gould et al., 1986), thereby reducing the need for transcallosal connectivity and favouring interaction at the level of the brainstem.

However, some limitations of interpretation have to be acknowledged. The facial motor system has a number of anatomical and physiological peculiarities that make it technically difficult to explore transcallosal connections with other protocols used in the hand, such as the ipsilateral silent period. In addition, we cannot exclude the possibility that although the overall MEP showed no evidence of facilitation, there is still some inhibition of some component of the I-waves. For example, some I waves could be facilitated by ipsilateral effects whereas others could be suppressed by IHI. However, it is difficult to interpret the behaviour of I-waves by inspecting the shape of the MEP. This is because the supra-threshold CS on its own may produce an MEP in the ipsilateral DAO, making it impossible to separate motor units recruited by the CS and those recruited by the TS (Fig. 3A).

In conclusion, data from the present work add a new piece of information into the physiology of the facial system and thus may provide further insight into pathologies affecting the facial motor system.

Declaration of Competing Interest

The authors declare no conflicts of interest.

References

Adank P, Kennedy-Higgins D, Maegherman G, Hannah R, Nuttall HE. Effects of coil orientation on motor evoked potentials from orbicularis oris. Front Neurosci 2018;12:683.

Benecke R, Meyer BU, Schönle P, Conrad B. Transcranial magnetic stimulation of the human brain: responses in muscles supplied by cranial nerves. Exp Brain Res 1988;71(3):623–32.

Brinkman J, Kuypers HG. Splitbrain monkeys: cerebral control of ipsilateral and contralateral arm, hand, and finger movements. Science 1972;176(4034):536–9.

Brodal A. Neurological anatomy: in relation to clinical medicine. New York: Oxford University Press; 1981.

Cattaneo L, Pavesi G. The facial motor system. Neurosci Biobehav Rev 2014;38:135–59. https://doi.org/10.1016/j.neubiorev.2013.11.002.

Clark RW, Luschei ES. Short latency jaw movement produced by low intensity intracortical microstimulation of the precentral face area in monkeys. Brain Res 1974;70(1):144–7.

Courville J. Rubrobulbar fibres to the facial nucleus and the lateral reticular nucleus (nucleus of the lateral funiculus). An experimental study in the cat with silver impregnation methods. Brain Res 1966;1(4):317–37.

Cruccu G, Berardelli A, Inghilleri M, Manfredi M. Corticobulbar projections to upper and lower facial motoneurons. A study by magnetic transcranial stimulation in man. Neurosci Lett 1990a;117(1–2):68–73.

Cruccu G, Inghilleri M, Berardelli A, Pauletti G, Manfredi M. Cortico-facial and cortico-trigeminal projections. A comparison by magnetic brain stimulation in man. Electroencephalogr Clin Neurophysiol Suppl 1990b;41:140–4.

Darwin C. The expression of the emotions in man and animals. London: Fontana Press; 1872.

Ferbert A, Priori A, Rothwell JC, Day BL, Colebatch JG, Marsden CD. Interhemispheric inhibition of the human motor cortex. J Physiol 1992;453:525–46.

Geffen GM, Jones DL, Geffen LB. Interhemispheric control of manual motor activity. Behav Brain Res 1994;64(1–2):131–40.

Gould 3rd HJ, Cusick CG, Pons TP, Kaas JH. The relationship of corpus callosum connections to electrical stimulation maps of motor, supplementary motor, and the frontal eye fields in owl monkeys. J Comp Neurol 1986;247(3):297–325.

Groppa S, Oliviero A, Eisen A, Quartarone A, Cohen LG, Mall V, Kaelin-Lang A, Mima T, Rossi S, Thickbroom GW, Rossini PM, Ziemann U, Valls-Solé J, Siebner HR. A practical guide to diagnostic transcranial magnetic stimulation: report of an IFCN committee. Clin Neurophysiol 2012;123(5):858–82. https://doi.org/10.1016/j.clinph.2012.01.010.

Guandalini P, Franchi G, Spidalieri G. Low threshold unilateral and bilateral facial movements evoked by motor cortex stimulation in cats. Brain Res 1990;508(2):273–82.

Hanajima R, Ugawa Y, Machii K, Mochizuki H, Terao Y, Enomoto H, Furubayashi T, Shiio Y, Uesugi H, Kanazawa I. Interhemispheric facilitation of the hand motor area in humans. J Physiol 2001;531:849–59.

Harris-Love ML, Perez MA, Chen R, Cohen LG. Interhemispheric inhibition in distal and proximal arm representations in the primary motor cortex. J Neurophysiol 2007;97(3):2511–5.

Holstege G, Kuypers HG, Dekker JJ. The organization of the bulbar fibre connections to the trigeminal, facial and hypoglossal motor nuclei. II. An autoradiographic tracing study in cat. Brain. 1977;100(2):264–86.

Jeeves MA, Silver PH, Jacobson I. Bimanual co-ordination in callosal agenesis and partial commissurotomy. Neuropsychologia 1988;26(6):833–50.

Kujirai K, Kujirai T, Sinkjaer T, Rothwell JC. Associative plasticity in human motor cortex during voluntary muscle contraction. J Neurophysiol 2006;96(3):1337–46.

Kobayashi M, Théoret H, Mottaghy FM, Gangitano M, Pascual-Leone A. Intracortical inhibition and facilitation in human facial motor area: difference between upper and lower facial area. Clin Neurophysiol 2001;112(9):1604–11.

Leonard G, Milner B, Jones L. Performance on unimanual and bimanual tapping tasks by patients with lesions of the frontal or temporal lobe. Neuropsychologia 1988;26(1):79–91.

Liscić RM, Zidar J. Functional organisation of the facial motor system in man. Coll Antropol 1998;22(2):545–50.

Matthews D, Murtagh P, Risso A, Jones G, Alexander CM. Does interhemispheric communication relate to the bilateral function of muscles? A study of scapulothoracic muscles using transcranial magnetic stimulation. J Electromyogr Kinesiol 2013;23(6):1370–4. https://doi.org/10.1016/j.jelekin.2013.06.007.

Meyer BU, Röricht S, Gräfin von Einsiedel H, Kruggel F, Weindl A. Inhibitory and excitatory interhemispheric transfers between motor cortical areas in normal humans and patients with abnormalities of the corpus callosum. Brain 1995;118:429–40.

Meyer BU, Werhahn K, Rothwell JC, Roericht S, Fauth C. Functional organization of corticonuclear pathways to motoneurones of lower facial muscles in man. Exp Brain Res 1994;101(3):465–72.

Morecraft RJ, Louie JL, Herrick JL, Stilwell-Morecraft KS. Cortical innervation of the facial nucleus in the non-human primate: a new interpretation of the effects of stroke and related subtotal brain trauma on the muscles of facial expression. Brain 2001;124:176–208.

Müri RM. Cortical control of facial expression. J Comp Neurol 2016;524(8):1578–85. https://doi.org/10.1002/cne.23908.

Noback CR, Demarest RJ. The human nervous system: Basic principles of neurobiology. 2nd ed. New York: McGraw-Hill; 1975.

Oldfield RC. The assessment and analysis of handedness: the Edinburgh inventory. Neuropsychologia 1971;9(1):97–113.

Paradiso GO, Cunic DI, Gunraj CA, Chen R. Representation of facial muscles in human motor cortex. J Physiol 2005;567:323–36.

Perez MA, Cohen LG. Interhemispheric inhibition between primary motor cortices: what have we learned? J Physiol 2009;587:725–6. https://doi.org/10.1113/jphysiol.2008.166926.

Pilurzi G, Hasan A, Saifee TA, Tolu E, Rothwell JC, Deriu F. Intracortical circuits, sensorimotor integration and plasticity in human motor cortical projections to muscles of the lower face. J Physiol 2013;591(7):1889–906. https://doi.org/10.1113/jphysiol.2012.245746.

Preilowski BF. Possible contribution of the anterior forebrain commissures to bilateral motor coordination. Neuropsychologia 1972;10(3):267–77.

Rinn WE. The neuropsychology of facial expression: a review of the neurological and psychological mechanisms for producing facial expressions. Psychol Bull 1984;95(1):52–77.

Rödel R, Laskawi R, Markus H. Motor potentials of lower-lip mimetic muscles and distal arm muscles to cortical transcranial magnetic stimulation: the possibility of one-dimensional separation of two cortical representation areas. ORL J Otorhinolaryngol Relat Spec 2000;62(2):96–9.

Rossini PM, Burke D, Chen R, Cohen LG, Daskalakis Z, Di Iorio R, et al. Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. Clin Neurophysiol. 2015;126(6):1071–107. https://doi.org/10.1016/j.clinph.2015.02.001.

Rouiller EM, Babalian A, Kazennikov O, Moret V, Yu XH, Wiesendanger M. Transcallosal connections of the distal forelimb representations of the primary and supplementary motor cortical areas in macaque monkeys. Exp Brain Res 1994;102(2):227–43.

Sadato N, Yonekura Y, Waki A, Yamada H, Ishii Y. Role of the supplementary motor area and the right premotor cortex in the coordination of bimanual finger movements. J Neurosci 1997;17(24):9667–74.

Sakai K, Ugawa Y, Terao Y, Hanajima R, Furubayashi T, Kanazawa I. Preferential activation of different I waves by transcranial magnetic stimulation with a figure-of-eight-shaped coil. Exp Brain Res 1997;113(1):24–32.

Sherwood CC. Comparative anatomy of the facial motor nucleus in mammals, with an analysis of neuron numbers in primates. Anat Rec A Discov Mol Cell Evol Biol 2005;287(1):1067–79.

Sperry RW. Hemisphere deconnection and unity in conscious awareness. Am Psychol 1968;23(10):723–33.

Takeuchi N, Oouchida Y, Izumi S. Motor control and neural plasticity through interhemispheric interactions. Neural Plast. 2012;2012. https://doi.org/10.1155/2012/823285 823285.

Toyokura M, Muro I, Komiya T, Obara M. Relation of bimanual coordination to activation in the sensorimotor cortex and supplementary motor area: analysis using functional magnetic resonance imaging. Brain Res Bull 1999;48(2):211–7.

Triggs WJ, Ghacibeh G, Springer U, Bowers D. Lateralized asymmetry of facial motor evoked potentials. Neurology 2005;65(4):541–4.

Urban PP, Beer S, Hopf HC. Cortico-bulbar fibers to orofacial muscles: recordings with enoral surface electrodes. Electroencephalogr Clin Neurophysiol 1997;105(1):8–14.

Urban PP, Wicht S, Hopf HC. Sensitivity of transcranial magnetic stimulation of cortico-bulbar vs. cortico-spinal tract involvement in Amyotrophic Lateral Sclerosis (ALS). J Neurol 2001;248(10):850–5.

Wahl M, Lauterbach-Soon B, Hattingen E, Jung P, Singer O, Volz S, Klein JC, Steinmetz H, Ziemann U. Human motor corpus callosum: topography, somatotopy, and link between microstructure and function. J Neurosci 2007;27(45):12132–8.

Wahl M, Ziemann U. The human motor corpus callosum. Rev Neurosci 2008;19(6):451–66.

Werhahn KJ, Classen J, Benecke R. The silent period induced by transcranial magnetic stimulation in muscles supplied by cranial nerves: normal data and changes in patients. J Neurol Neurosurg Psychiatry 1995;59(6):586–96.

Yildiz N, Yildiz S, Ertekin C, Aydoğdu I, Uludag B. Changes in the perioral muscle responses to cortical TMS induced by decrease of sensory input and electrical stimulation to lower facial region. Clin Neurophysiol 2004;115(10):2343–9.

Yildiz S, Bademkiran F, Yildiz N, Aydogdu I, Uludag B, Ertekin C. Facial motor cortex plasticity in patients with unilateral peripheral facial paralysis. NeuroRehabilitation 2007;22(2):133–40.