ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/323266992

Spatial and Temporal Characteristics of Set-Related Inhibitory and Excitatory Inputs from the Dorsal Premotor Cortex to the Ipsilateral Motor Cortex Assessed by Dual-Coil Transcran...

Article *in* Brain Topography · February 2018
DOI: 10.1007/s10548-018-0635-x

CITATIONS
3

READS
145

4 authors:

Sara Parmigiani
University of Milan
10 PUBLICATIONS **10** CITATIONS
SEE PROFILE

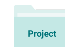
Benedetta Zattera
Università degli Studi di Torino
4 PUBLICATIONS **3** CITATIONS
SEE PROFILE

Guido Barchiesi
University of Salzburg
22 PUBLICATIONS **321** CITATIONS
SEE PROFILE

Luigi Cattaneo
Università degli Studi di Trento
116 PUBLICATIONS **4,183** CITATIONS
SEE PROFILE

Some of the authors of this publication are also working on these related projects:

Dense spatial mapping of the cerebral cortex with TMS View project


Who does what to whom: the role of the l-IPS in the comprehension of reversible and irreversible sentences View project

All content following this page was uploaded by Luigi Cattaneo on 27 February 2018.

The user has requested enhancement of the downloaded file.

Spatial and Temporal Characteristics of Set-Related Inhibitory and Excitatory Inputs from the Dorsal Premotor Cortex to the Ipsilateral Motor Cortex Assessed by Dual-Coil Transcranial Magnetic Stimulation

Sara Parmigiani, [1]

Benedetta Zattera, [1]

Guido Barchiesi, [2]

Luigi Cattaneo, [3] ✉

Phone +39-045-8027198
Email luigi.cattaneo@univr.it

[1] University of Trento, Trento, Italy

[2] University of Salzburg, Salzburg, Austria

[3] Dipartimento di Neuroscienze, Biomedicina e Movimento, University of Verona, Strada Le Grazie, 8, 37134 Verona, Italy

Received: 22 September 2017 / Accepted: 14 February 2018

Abstract

The capacity to produce movements only at appropriate times is fundamental in successful behavior and requires a fine interplay between motor inhibition and facilitation. Evidence in humans indicates that the dorsal premotor cortex (PMCd) is involved in such preparatory and inhibitory processes, but how PMCd modulates motor output in humans is still unclear. We investigated this issue in healthy human volunteers, using a variant of the dual-coil transcranial magnetic stimulation (TMS) technique that allows testing the short-latency effects of conditioning TMS to the left

PMCd on test TMS applied to the *ipsilateral* orofacial primary motor cortex (M1). Participants performed a delayed cued simple reaction time task. They were asked to produce a lip movement cued by an imperative GO-signal presented after a predictable SET-period, during which TMS was applied at different intervals. Results showed that the area of motor evoked potentials (MEPs) to test TMS was modulated by conditioning TMS. A transient inhibition cortico-bulbar excitability by PMCd stimulation was observed around the middle of the SET-period. Conversely, a ramping excitatory effect of PMCd stimulation appeared towards the end of the SET-period, as the time of the predicted GO-signal approached. The time-course of PMCd–M1 activity scaled to the varying SET-period duration. Our data indicate that inhibition and excitation of motor output during a delayed reaction time task are two distinct neural phenomena. They both originate in PMCd and are conveyed via cortico–cortical connections to the ipsilateral M1, where they are integrated to produce harmonic fluctuations of motor output.

AQ1

Keywords

Cortico–cortical connectivity
Inhibition
Premotor
Action preparation
Voluntary movement

Introduction

Consider an orchestral flute player just few instants before starting to play her piece. Her successful performance requires the interplay between the capacity to stand still when necessary and the capacity to move at the appropriate time. Besides this paradigmatic case, our daily life is permeated with behavioral situations requiring to withhold a preloaded action to be performed only when appropriate contextual conditions occur. In these cases appropriate motor performance is thought to rely on two distinct neural processes, i.e. active inhibition of motor output coupled with active facilitation of motor output (Aron 2007; Aron et al. 2007; Chambers et al. 2009; Boy et al. 2010; Duque et al. 2017). Evidence suggests that the dorsal premotor cortex (PMCd) of human and nonhuman primates might be involved in this interplay. In

monkeys PMCd is densely interconnected with the primary motor cortex (M1) (Muakkassa and Strick 1979; Tokuno and Tanji 1993; Kiefer et al. 1998; Hatanaka et al. 2001) and it is known that PMCd can exert inhibitory as well as excitatory effects on M1. PMCd neurons exhibit a rich variety of set-related activity, being time-locked to the ongoing inhibitory behavior (Weinrich et al. 1984; Godschalk et al. 1985; Wise 1985; Kurata and Wise 1988a, b; di Pellegrino and Wise 1993; Mirabella et al. 2011). Therefore, although most set-related activity in PMCd has been interpreted as related to motor preparation (Hoshi et al. 2014), its functional features make it a possible candidate also for motor inhibition. Indeed, several neurons show preferential set-related activity with reduced or absent movement-related activity (see, for instance, Wise and Mauritz 1985). Indeed, the injection of GABA-A antagonists within PMCd reduces the ability of monkeys to withhold movements (Sawaguchi et al. 1996). Similarly, lesions PMCd may result in increased frequency of impulsive and uncontrolled reaching movements (Moll and Kuypers 1977). Furthermore, a single cell study recorded from PMCd neurons of two monkeys when performing both no-stop and stop trials in a countermanding task (Mirabella et al. 2011) showed that more than one-third of recorded PMCd neurons involved in motor planning exhibit a countermanding modulation. These neurons changed their pattern of discharge when a reaching movement were executed with respect to when it was inhibited, and this change preceded the end of the stop-signal reaction time. Furthermore, others single-neuron neurophysiology studies that been done to examine how activity in the premotor and motor cortex changes with time during delay periods found that some pyramidal neurons in PMCd synapse primarily on inhibitory cells in M1, producing feedforward inhibition (see, among the others, Ghosh and Porter 1988; Keller 1993; Tokuno and Nambu 2000; Riehle et al. 2006; Kaufman et al. 2010).

In humans, transcranial magnetic stimulation (TMS) studies investigating premotor–motor cortico–cortical connections provide evidence for an involvement of PMCd in facilitatory or inhibitory processes. Using a dual coil TMS, O'Shea et al. (2007) found facilitatory effects of the left PMCd on the contralateral M1 with TMS delivered just after the GO-signal presentation, when action releasing was required. Similar results were obtained by Bestmann et al. (2008). In the same vein, Koch et al. (2006) stimulated the left PMCd and the contralateral M1 during a choice reaction time task. The results showed not only facilitatory but also inhibitory PMCd–M1 interactions. In a similar vein, Kroeger et al. (2010) using dual-site TMS in

both hemisphere during a go/no-go task found that the left PMCd exerts an inhibitory influence on corticospinal excitability in the right M1 at the early stage of the preparatory delay period, which turned into facilitation when the go signal appeared. More recently, Duque et al. (2012) combined repetitive TMS (rTMS) over PMCd and a single pulse TMS over M1 in order to evaluate the putative role of PMCd in action control. rTMS was used to produce a transient disruption of PMCd, while the single pulse TMS over M1 allowed for measuring inhibitory changes in corticospinal excitability. The results showed that rTMS over PMCd specifically attenuated inhibition before the onset of an imperative signal. This lead the authors to hypothesize a critical role of PMCd in suppressing motor impulses (see also Duque et al. 2017). Although the mentioned lines of evidence indicate an association of PMCd to both facilitatory and inhibitory processes, it is still unclear how the information in PMCd modulates the corticospinal output, particularly regarding the inhibitory aspects. The aim of the present study is to start addressing this question. We test the hypotheses that PMCd directly affects motor output by means of cortico–cortical projections to the ipsilateral M1 and that the effect of such connections is inhibitory whenever correct behavior requires immobility. We therefore adopted a simple delayed cued motor task, in which with an impeding action was to be withheld during a fixed SET-period and to be released at the presentation of an imperative GO-signal. We explicitly decided to adopt such a simple task with a fully predictable timing of the SET- and GO-components, because it allows specific evaluation of the premotor–motor functional connectivity during behavioral inhibition and facilitation of action minimizing irrelevant experimental variables and it allows precise temporal sampling of the SET-period by means of TMS.

AQ2

To sample neural signals from PMCd to M1 during the behavioral task we took advantage of dual-coil TMS. The dual coil technique employs a single TMS test pulse to M1 (testTMS) that evokes a motor evoked potential (MEP) in a muscle of interest. Prior to this, a conditioning pulse (condTMS), which does not elicit a MEP alone, is delivered in some trials to a cortical area supposedly connected to M1. If the conditioning pulse modulates the amplitude of M1 motor output at short inter-stimulus intervals (ISIs), then direct cortico–cortical connectivity between the two areas may be inferred (Cattaneo and Barchiesi 2011; Rothwell 2011). However, testing specifically PMCd–M1 direct interactions by the dual coil technique proved to be challenging because of the close spatial proximity between the PMCd and the

portion of M1 related to hand movements (handM1) (Schluter et al. 1998; Johansen-Berg et al. 2002). Most authors tackled this challenge by either placing the coil over PMCd directly on the skull, with the coil over M1 being either elevated or overlapped (Beck et al. 2009; Pirio Richardson et al. 2014) or using purpose-made coils with eccentric focality (Groppa et al. 2012). An alternative strategy is to probe the ipsilateral PMCd–M1 functional connectivity on an effector different from the hand, thus putting more distance between the two coils. In a recent study (Parmigiani et al. 2015) we delivered testTMS pulses over the left orofacial M1 (mouthM1), rather than over the left handM1. The results showed that a small region in PMCd, along the superior frontal sulcus, exerts a robust short-latency effect on the ipsilateral mouthM1 during rest.

Here, we used this strategy in three different experiments. *Experiment 1* was carried out to validate the hypothesis that short-latency connectivity between the intermediate portion of PMCd identified by Parmigiani et al. (2015) and the mouthM1 at rest actually exists. In *Experiment 2* we tested the PMCd–M1 connectivity during the SET-period of a simple delayed motor task, in which participants should produce a lip-response to a GO-signal. The SET-period was constant and therefore predictable, in all trials (900 ms). *Experiment 3* was designed to clarify the nature of the inhibitory results of *Experiment 2*, peaking at 600 ms from onset of the set period and, necessarily, 300 ms prior to the GO-signal. We wanted to investigate whether the inhibitory activity was time-locked to the set-period onset or to the GO-signal. To disentangle these two possibilities, we increased the duration of the set-period, to understand if the inhibitory peak would stay docked to the set-period onset at 600 ms or if it would be dragged forward by the GO signal.

Finally, it is important to note that physiological findings of short-latency influences from premotor to motor cortices are generally interpreted in broader functional terms. The paired-coil literature interprets the pattern of effects of condTMS over M1 as revelatory of the type of information that the premotor cortex is transferring to M1 during the task. For example, the facilitatory effect on M1 during the SET-period of a delayed task exerted by frontal and parietal cortices has been considered as revelatory of the motor information for action planning stored in the premotor cortices and ready to be transferred to M1 (Koch et al. 2008; Davare et al. 2009; Vesia et al. 2013).

Materials and Methods

Participants

Sixteen healthy individuals (11 women, mean age 25.8, ranging 18–38 years, SD 4.8) took part in *Experiment 1* and *Experiment 2*. A separate group of 12 healthy individuals (7 women, mean age 25.4 years, ranging 18–41 years, SD 6.54) participated in *Experiment 3*. They all provided informed consent. All were screened for any contraindication to TMS (Rossi and Hallett 2009).The study was approved by the local ethical committee (protocol 2031-032) and was conducted in compliance with the revised Helsinki declaration (Association 2009). Except for the brain scan acquisition, which was performed on a previous day, Experiment 1 lasted 45 min and Experiment 2 and 3 (preparatory phase and behavioural part included) lasted approximately 2 h and a half. Each block of proper task lasted 7–8 min each.

Neuronavigation

Before the experimental session, a high-resolution T1-weighted magnetization prepared rapid gradient echo sequence (176 axial slices, in-plane resolution 256 3 224, 1-mm isotropic voxels, generalized autocalibrating partially parallel acquisition with acceleration factor = 2, time repetition = 2700 ms, time echo = 4.180 ms, time to inversion = 1020 ms, flip angle = 7u) scan of the brain of each subject was obtained, using a MedSpec 4-T head scanner (Bruker BioSpin GmbH, Rheinstetten, Germany) with an 8-channel array head coil. Starting from this scan, a 3D reconstruction of the scalp and the grey matter surfaces was produced using MesH morphing tool included in the BrainVoyager software (Brain Innovation BV, The Netherlands). The BrainVoyager neuronavigation software combined with an ultrasound tracking system, CMS205S (Zebris Medical GmbH, Isny, Germany), was used to coregister the 3D scalp reconstruction with the actual participant's head, thus marking the target point for the conditioning TMS on the real head of each participant.

Localization of TMS Targets

The targets for testTMS and condTMS were the same in the three experiments. TestTMS was delivered to the mouthM1, which was localized functionally, without the aid of the neuronavigation system, as the spot on the scalp where the larger MEP from the *orbicularis oris* (OOr) muscle could be obtained with the lowest intensity. CondTMS was delivered to the mouth-related PMCd as defined in Parmigiani et al. (2015), which was localized by means of neuronavigation on individual anatomies. It was found 1.5 cm

rostral to the junction between the superior precentral sulcus (sPreCS) and the superior frontal sulcus (sFS). After their use for neuronavigation, the brain images of all participants were transformed in Talairach space so that the stimulation spot was documented in all participants in a normalized space. The average coordinates were of $x = -22$, $y = -1$ and $z = 55$, which compare favorably with the spot where PMCd is localized by meta-analyses of fMRI studies (Hardwick et al. 2013, 2015). All individual brain surfaces are available in Fig. 1. Additionally, for descriptive purposes, the stimulated PMCd spot was localized on the scalp in the 10–20 reference system. Mean distance from the Cz coordinate (vertex) are − 3.55 cm lateral, 2.45 cm anterior (SD 0.63, 0.68). Figure 2 shows the target points in all participants.
AQ3

Fig. 1

Individual surface renderings of the left hemisphere's grey-white matter border of all 28 participants that have been used for neuronavigation. The stimulation target (pink spot) is indicated together with the major anatomical landmarks that allowed the identification of the target (color coding of the different sulci is reported at the bottom of the figure)

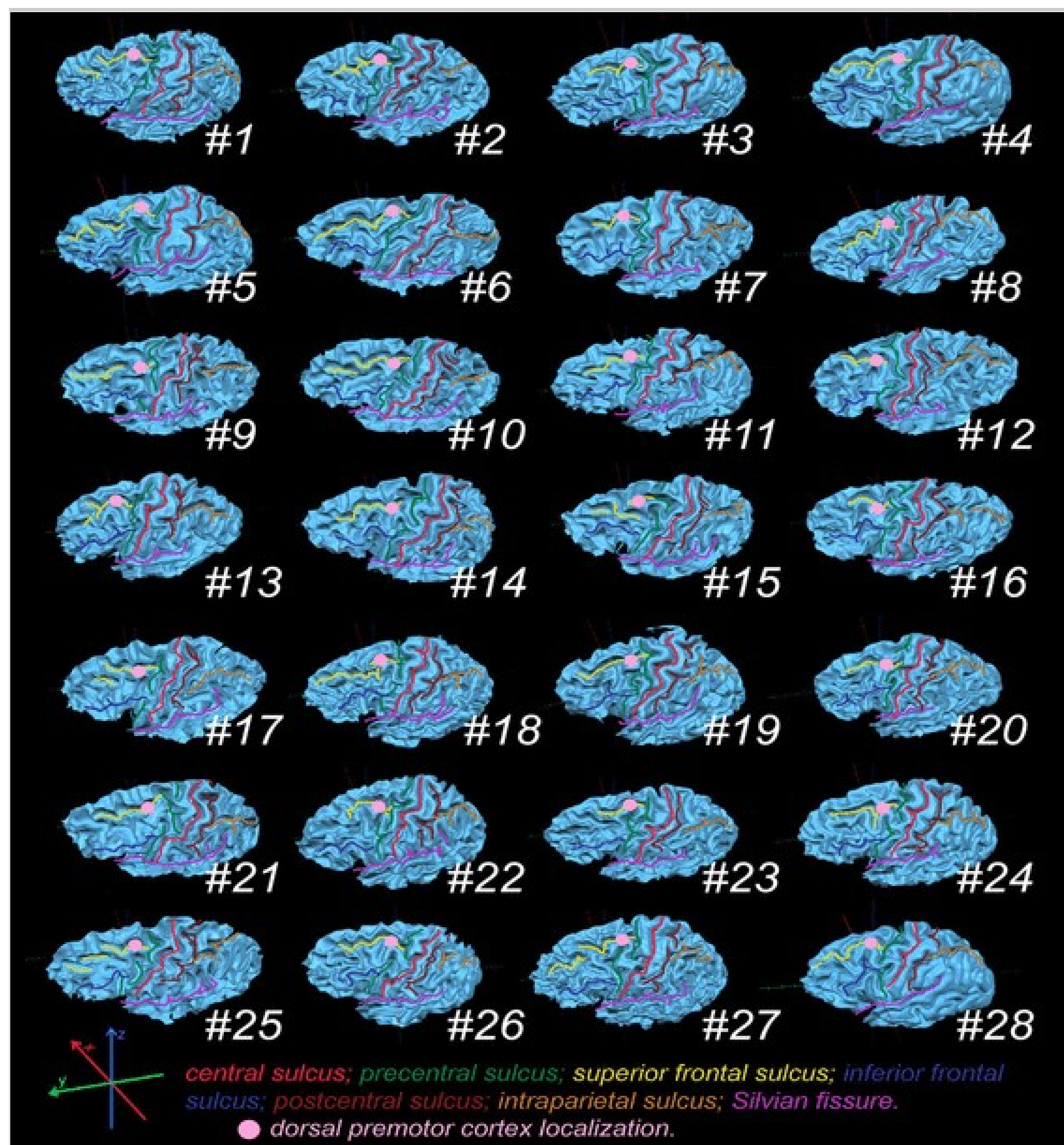
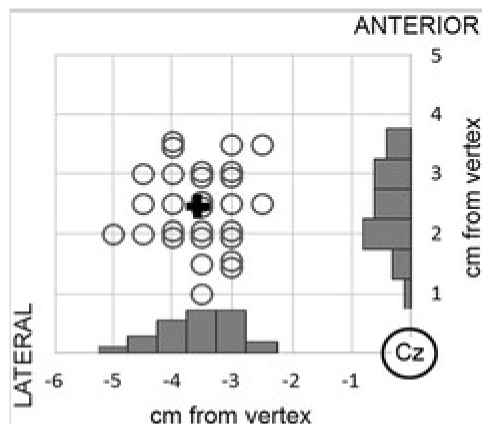


Fig. 2

The premotor target points on the scalp are shown for participants of all experiments (n = 28). Circles indicate data from single participants. For the sake of illustration clarity, a slight offset has been applied to overlapping spots so that they can be identified as multiple subjects. The black cross indicates the average coordinate. Histograms indicate the frequency of spots on the corresponding coordinates. Coordinates are given in 0.5 cm steps



Stimulating Apparatus, Muscular Pre-activation and Stimulation Intensity

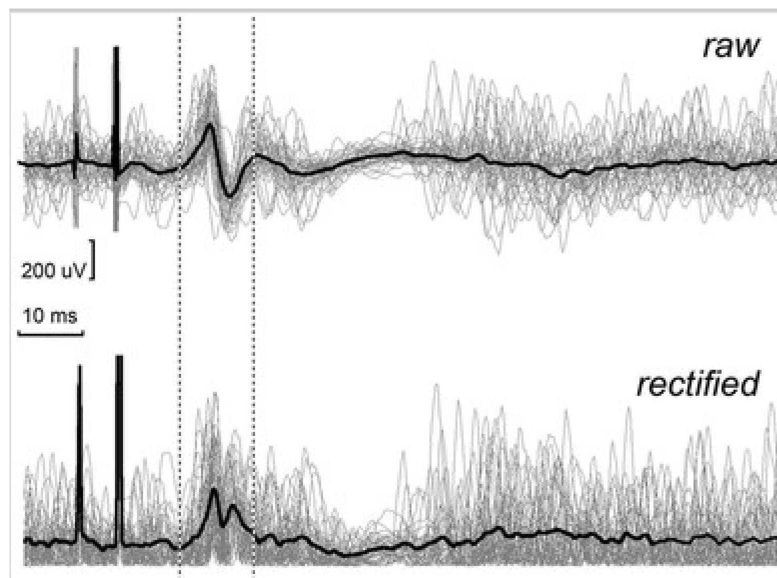
Dual-coil stimulation was achieved by means of two biphasic magnetic stimulators (MagPro and MagPro Compact) connected to two figure-of eight coils. A figure-of-eight coil with 55 mm windings (Dantec B55, Skovlunde, Denmark) oriented perpendicularly to the midline with the handle pointing laterally delivered testTMS over the motor cortex. A figure-of-eight coil with windings of 35 mm diameter (MC-B35, MagVenture, Skovlunde, Denmark) delivered condTMS, with a medio-lateral orientation of the induced current. Both the testTMS and the condTMS coils were held by an articulated mechanical arm (Manfrotto 244, VitecGroup, Italy). CondTMS and testTMS were both biphasic single stimuli. It has been reported that with certain cortical targets monophasic stimuli are more efficient in producing condTMS effects compared to biphasic pulses. However, in our experience we described the possibility to obtain dual-coil effects with biphasic stimuli (Cattaneo and Barchiesi 2011; Maule et al. 2015; Parmigiani et al. 2015). Therefore, we believe that monophasic and biphasic stimuli can be both used to apply condTMS, understanding that the two stimulus types elicit different neural activity.

Active motor threshold (AMT) was calculated. AMT was defined as the minimum stimulus intensity necessary to produce a MEP in the recorded muscle of 200 μV of average amplitude over ten consecutive trials during a mild voluntary contraction. We averaged online batches of ten consecutive trials by means of the Signal software (Cambridge Electronic Design, Cambridge, UK), which was used for all EMG acquisitions. AMT was assessed separately for the two coils. The intensity of the condTMS as well as the testTMS stimuli was set to 120% of the AMT for the OOr muscle. For

each of the two coils stimulation intensity was individually set according to the specific AMT calculated with that same coil. Since it is common practice to record facial MEPs during active contraction of the target muscle, due the high threshold of the orofacial motor cortex to TMS (Cattaneo and Pavesi 2014), participants were tested during active contraction of the lips. To achieve a stable contraction, they were asked to hold a stick in their mouth with their lips only and to generate muscular tension matching an EMG signal of around 200 µV in amplitude. The operator inspected the EMG trace online and provided feedback to the participant whenever she deviated from the desired contraction level. Obviously, this procedure allowed for small fluctuations in background EMG. This variability however did not represent a major source of noise because all MEP amplitudes were normalized to the pre-stimulus EMG to correct for such variability (see the following paragraph and Fig. 3). In each trial TMS could be delivered either as a single testTMS pulse (single-pulse trials) or as the combination of condTMS + testTMS (dual-pulse trials). The relative timing of condTMS and testTMS required a sub-millisecond temporal resolution and was therefore controlled by an input–output board, the1401 micro Mk-II unit (Cambridge Electronic Design). TMS single or dual-pulses were time-locked to the visual stimuli in Experiments 2 and 3 by means of the E-Prime 2.0 software (Psychology Software Tools Inc.).
AQ4

Fig. 3

Example of EMG recordings in a randomly selected subject (Experiment 2). The mean EMG trace is shown in thick black lines, superimposed onto the 96 raw recordings (light grey lines). The upper panel shows the raw EMG and the lower panel shows the same EMG traces after rectification. The two dashed vertical lines indicate the 10–20 ms interval that was taken as window of interest for MEP analysis



EMG Recordings, EMG Pre-processing and MEP Baseline-Correction

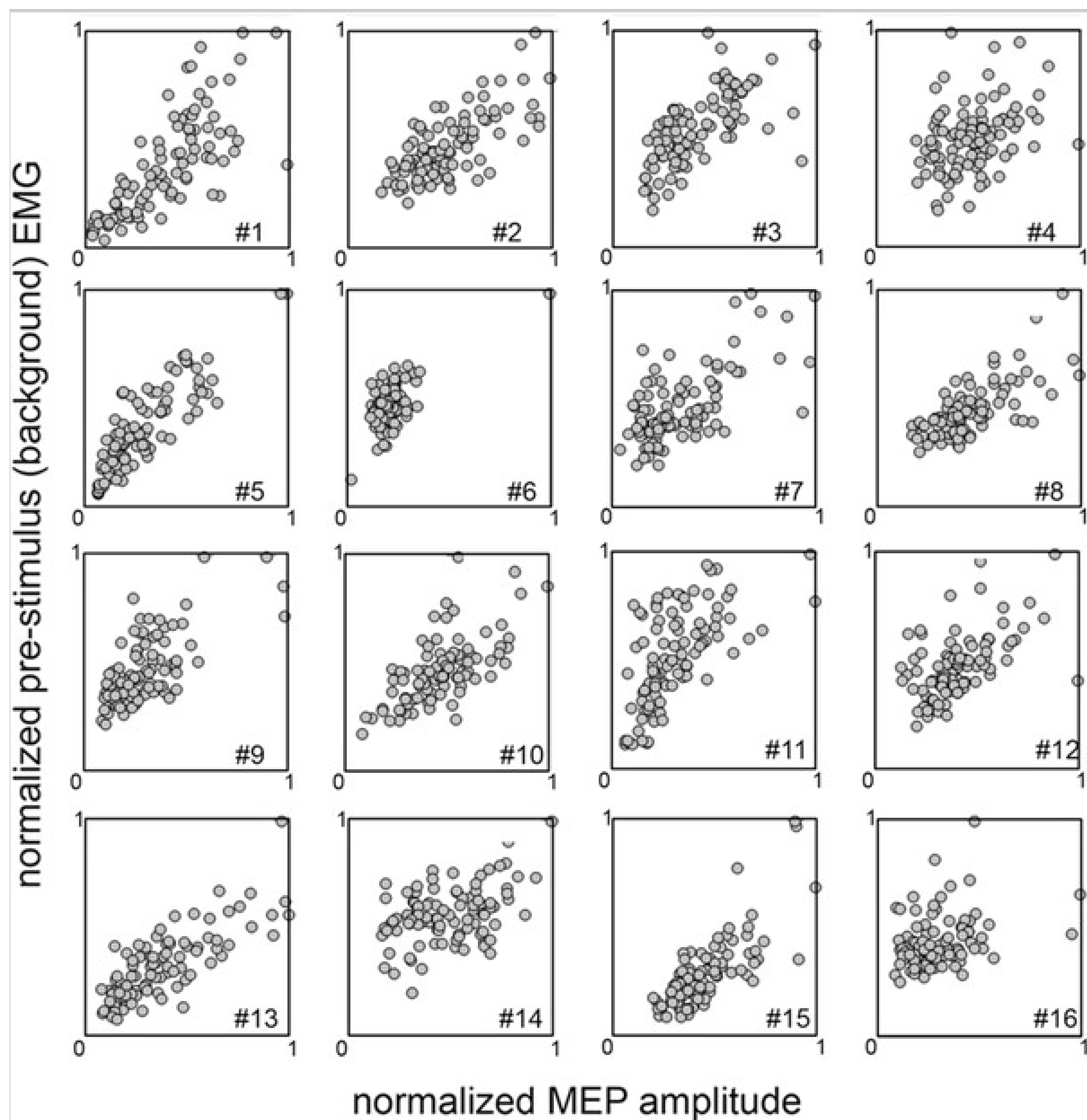
In all experiments, the right OOr and the right *interosseus dorsalis primus* muscle (1DI) were recorded with surface electrodes in a bipolar montage. Although responses were given with the lips, we decided to record also a hand muscle (the 1DI) as a control for the spatial specificity of the stimulation. We expected that neither PMCd, nor mouthM1 stimulation should spread to the hand motor area and therefore we did not expect to observe significant MEPs in the 1DI channel, in any of the experimental conditions. The analog signal was amplified 1000× by means of a 1902 two-channel amplifier (Cambridge Electronic Design, Cambridge, UK) and then digitalized with a sampling frequency of 4 kHz by means of a 1401 micro Mk-II unit (Cambridge Electronic Design, Cambridge, UK) and band-pass filtered between 20 Hz and 2 kHz. Offline pre-processing of the OOr EMG traces consisted in rectification and extraction of the area under the rectified EMG in the time window between 10 and 30 ms after testTMS (interval representative of the MEP) and of the area between 200 and 0 ms prior to condTMS (baseline, pre-stimulus EMG activity). In the 1DI channel the area between 20 and 40 ms from testTMS was considered representative of the MEPs. The choice of using areas rather than peak–peak amplitudes as a measure of MEP size is dictated by the anatomical location of motor end-plates in the OOr muscle (Cattaneo and Pavesi 2014) which are scattered throughout the muscle volume. Such spatial distribution of motor end-plates makes it impossible to use a standard “belly-tendon” montage and MEP waveforms tend to be polyphasic with no clearly defined positive or negative peaks. Participants

were keeping a stable voluntary contraction of the OOr muscle. Figure 3 shows an example of MEP recordings in a randomly selected subject. The illustration clearly indicates that the MEP signal is well-separated from the noise of voluntary contraction. However, MEP amplitudes are known to covariate strictly with the background EMG activity in the upper limb. The facilitatory effect of voluntary contraction introduces a great deal of variance in MEP amplitudes that is not related to the experimental manipulation but rather to spontaneous variations in the voluntary drive. To investigate the relation between baseline EMG and MEP amplitudes in our data we performed a simple regression analysis between non-corrected MEP areas and the relative background activity. The results showed a robust linear covariation of MEPs with the amount of voluntary activity (see Fig. 4). To reduce this source of noise we performed a baseline correction of MEP amplitudes from the OOr muscle, by dividing the MEP area by the relative pre-stimulus baseline EMG in individual trials. The window for pre-stimulus baseline EMG was of 200 ms prior to TMS. Similar approaches to the analysis of facial MEPs have been adopted previously in the literature (Watkins et al. 2003; Watkins and Paus 2004; Sato et al. 2010; Parmigiani et al. 2015). At this point of EMG pre-processing, the data from each trial was represented by a single value, i.e. that of the baseline-corrected MEP areas, obtained by the same procedure in all 3 experiments. Finally, to exclude that the pre-stimulus EMG activity could be differently represented between experimental conditions, in all 3 experiments we conducted ANOVAs on the baseline EMG data and found no significant distribution of baseline EMG between the different experimental variables (all p values > 0.24). The further step in the processing of MEPs was that to relate the conditioned-MEPs to the test-MEPs. This process was performed differently in the three Experiments and is described in detail below.

Fig. 4

Linear covariance between background EMG activity and MEP amplitudes. The data from each of the 96 trials of each single subject are shown. For the sake of comparison between-subjects, both the background EMG and the facial MEP amplitudes were normalized within each subject to the maximum value in the distribution. The normalized values were therefore comprised between 0 and 1. Single regression analysis between EMG and MEP values showed a high degree of covariance. Individual R^2 values ranged between 0.18 and 0.72. The grand average of individual R^2 values was of 0.47. This indicated that around half of the variance of MEP amplitudes was fully accounted for by variations in

voluntary EMG contraction preceding TMS. This source of variability was eliminated from the main analysis of MEP by the procedure of baseline correction described in the methods



Experiment 1—Protocol

Participants were sitting comfortably, the head on a chin rest and eyes freely open, wearing earplugs. They were asked to stay completely at rest aside from the controlled voluntary contraction of the OOr muscle. Five different inter stimulus intervals (ISIs) were used in dual-pulse trials: − 1, + 2, + 4, + 6 and + 8 ms (condTMS was delivered after testTMS in the − 1 ms ISI). Dual-pulse trials of each ISI were alternated with single-pulse trials in a fixed sequence that contained 5 dual-pulse trials (one for of the 5 ISIs) and 3 single-pulse

trials. The sequence was: single-pulse; dual-pulse; dual-pulse; single-pulse; dual-pulse; dual-pulse; single-pulse; dual pulse. The elementary sequence was repeated 12 times. The whole experimental session was therefore made of a total of 96 trials (36 single-pulse trials and 60 dual-pulse trial). The reason for adopting a fixed sequence was to be able to perform a "sliding window" normalization of conditioned-MEPs to test-MEPs (see below).

Experiment 1—MEP Normalization

Conditioned-MEPs were normalized to test-MEPs. We adopted a procedure of dividing the area of conditioned-MEPs from single trials by the average of the area of the 2 test-MEPs in the 2 adjacent (one preceding and one following) trials. Each conditioned-MEP was therefore normalized to a sliding window of test-MEPs that followed the conditioned-MEP along the series of trials (Cattaneo and Barchiesi 2011; Maule et al. 2015; Parmigiani et al. 2015). The rationale for using a sliding window to normalize conditioned-MEPs to test-MEPs is that this minimizes the very slow fluctuations in MEP amplitude that may occur during the course of experimental sessions. The resulting ratio is distributed between 0 and $+\infty$, therefore not distributed normally. To achieve normality of the data we applied a further manipulation, i.e. a base 10 logarithmic transformation, to each value (Tukey 1977). In this way, data were symmetrically distributed around 0, between $-\infty$ and $+\infty$ (the actual data were successfully tested for normality by means of Shapiro–Wilk's test). Negative values indicated amplitude of conditioned-MEPs smaller than the instantaneous value of test-MEPs, whereas positive values indicated amplitude of conditioned-MEPs larger than the instantaneous value of test-MEPs. At this step, the result of the procedure was a series of 60 MEP ratios (5 ISIs × 12 repetitions) for each participant.

Experiment 1—Statistical Analysis

We first excluded trials with MEPs that exceeded 2 standard deviations (SD) from the individual average values. The aim of statistical analysis was to assess whether, in any of the 5 ISIs, the MEP ratio was significantly deviating from the value of zero (because the data had been log-transformed) and ultimately indicating whether conditioned-MEPs were significantly different from test-MEPs. To do so we performed a series of 5 t-tests, assessing the null hypothesis that the mean of the MEP ratios was not different from zero. The p value was Bonferroni-corrected for the 5 multiple comparisons, and adjusted to $p = 0.01$.

Experiment 2—Protocol

In Experiment 2, participants performed a delayed simple reaction time task and TMS was delivered in an event-related timing, during the SET-period. Participants had an additional lateral head-constraint on the chin rest, which assured head stability and minimal movement during the execution of the orofacial action. They wore earplugs and, as in Experiment 1, they were asked to keep the contraction of the muscles constant, in a way in which they were able to hold a stick between their lips firmly also while they were waiting for the cues, assisted by the operator behind them. Stimuli were presented with the E-Prime 2.0 software, on a 75 Hz (1680 × 1050 resolution) 20″ monitor, at 45 cm of distance from the participant eyes. The experiment was organized in four similar blocks, each consisting of 96 trials. The inter trial interval was randomly jittered between 2500 and 3500 ms. Trials started with a green fixation cross, indicating the SET-period (SET-period), during which participants had to stay still and wait for the GO-signal (a circle in the middle of the screen), occurring 900 ms later. After the response (lifting a stick with their lips as fast as possible) was given, the corresponding reaction time was displayed on the screen, serving as feedback of individual performance. Any anticipation of the response prior to the GO-signal was considered as error. It should be noted that, given the fixed duration of the SET-period, the onset of the GO-signal was entirely predictable throughout the experiment.

Experiment 2—Apparatus for Lip Response Collection

All participants held a stick with their lips to collect the motor responses. To stabilize and ensure a constant OOr contraction throughout the experiment, a 15 g weight was suspended at the end of the stick. The actual motor response consisted in lifting the stick from a starting point until an end position was met, after which no more lifting was possible. At the instant when the end position was reached, a circuit was closed providing a + 5 V square-wave output. The output signal was transmitted to a PC via the serial port by the E-Prime 2 software. Every trial, such response time (RespT) was displayed on the screen to give on-line feedback to the participants. The tip of the response stick contained also a 3-axe analog accelerometer, the output of which was constantly recorded throughout the trial length, by means of the same analog–digital conversion equipment described for EMG recordings. The accelerometer's signal was analyzed offline to provide accurate estimation of the onset of the motor response, i.e. of reaction times (RTs). RespTs indicated by E-prime were used uniquely for the display of single-trial feedback, while

RTs were actually used for the statistical analysis.

Experiment 2—TMS Timing During the SET Period

The configuration and placement of the two TMS coils was the same as in Experiment 1. TMS was delivered as single-pulses or dual-pulses. The ISI for dual-pulse TMS was set to 6 ms as this had been proven to be the optimal interval to test cortico–cortical connectivity between PMCd and M1 (see Parmigiani et al. 2015, and Experiment 1 in the current work). In each trial only one single- or dual-pulse TMS was delivered. TMS was applied during the SET-period at different timings in different trials, in order to cover the whole duration of the SET-period itself (900 ms). Specifically, TMS was delivered along the duration of the SET-period at 4 different timings, corresponding to 0, 300, 600 and 900 ms after the onset of the SET-period. Dual-pulse trials were equally distributed between the 4 different TMS timings. Trial types were classified therefore in 8 possible categories, according to a STIMULATION (2 levels: single- or dual-pulse) × TIME (4 levels: 0, 300, 600 and 900 ms) factorial design, in which each trial type was repeated 48 times, for a total of 384 trials per participant. The order of trials with different TMS timings was fully randomized in the experimental session. Conversely, single-pulse trials and dual-pulse trials were alternated in a fixed order.

Experiment 2—Data Processing and Statistical Analysis

Qualitative assessment of the participants' RTs indicated that a significant amount of responses was produced prior to the GO-signal during the SET-period, while the main bulk of responses appeared to be given appropriately in response to the GO-signal. We therefore applied a procedure to exclude such trials, based on modelling the individual subjects' RTs on a Gaussian curve, to estimate the lower limit of the RT distribution. However, to optimize curve fitting, outliers should be removed. With this aim we first of all we removed trials in which responses were given prior to 100 ms after the GO-signal with the aim of excluding anticipatory responses. The 100 ms cutoff may seem arbitrary, but note that the *actual* cutoff that we applied resulted from the Gaussian fit (see below) and corresponded to some tens of ms higher than 100 ms. Additionally, consider that the lower limit of visual reaction times in humans within the cranial district is around 120 ms (Kirchner and Thorpe 2006). In the study of orofacial reflexes, it is widespread practice (see for example: Brinkworth et al. 2003; Pavesi et al. 2000 or Cattaneo et al. 2007) to

consider any response occurring above 80 ms as possibly contaminated by voluntary reactive activity, because in the masseter muscles simple RTs to tactile stimuli as fast as 80 ms have been recorded (Brodin et al. 1993). In the second step we fitted the remaining trials to a Gaussian distribution. The average number of such anticipation responses between subjects was of 10% (subject with minimum n. of anticipation responses = 3%; subject with maximum number of anticipation responses = 19%). After trimming the data, only trials with RTs falling within the mean ± 2 SD of the fitted distribution were taken into consideration for further analysis, though these were a minimal proportion (overall 2% of all trials).

MEP areas were extracted and baseline-corrected similarly to Experiment 1. In the present experiment, we decided not to follow the standard analytical approach of normalizing paired-pulse MEPs to single-pulse MEPs. This type of analysis does an excellent job in constraining the data in one single index, but has two main limitations. It does not allow, in dynamic situations to determine the relative contribution of single-pulse MEPs vs. paired-pulse-MEPs to the observed effects. Second, and most important, in the present experiment we were interested in absolute terms in inhibitory or facilitatory effects of condTMS. Analysis of variance of the paired-pulse/single-pulse MEP ratio describes variations of distribution of the index between different conditions, but is not capable of describing absolute variations of single values with respect to the ratio value of one (indicating no effect of conditioning pulse). We therefore decided to use a multivariate approach in which single-pulse and dual-pulse MEPs were not collapsed in a single ratio but rather kept separate as two levels of one factor in a bivariate ANOVA, that was therefore structured as a 2 × 4 design, with the factors STIMULATION (2 levels: single- or dual-pulse) and TIME (4 levels: 0, 300, 600 and 900 ms). Planned comparisons were the 4 paired comparisons of dual-pulse data with single-pulse data within each of the four timings of TMS. Significance threshold was adjusted for the 4 multiple comparisons to $p = 0.0125$. In this experiment we also analyzed the RTs' distribution by means of a bivariate ANOVA with the STIMULATION and TIME factors, structured identically to the ANOVA used for MEP amplitudes.

Experiment 3—Protocol and TMS Timing During the SET Period

Experiment 3 was structured similarly to Experiment 2, but explored different durations of the SET-period. Instead of a single block with a 900 ms SET-

period (as in Experiment 2), we used 3 different SET-periods, 1500, 1800 and 2100 ms, each in one of 3 different within-subjects blocks. The 3 blocks were presented in counterbalanced order between the subjects. The duration of the SET-period did not vary within each block, therefore the timing of the GO-signal was entirely predictable. Trial structure and behavioral tasks were the same as in Experiment 2, but with a varying number of TMS timings, in order to cover the whole of the SET periods in steps of 300 ms. Trials in the 1500 ms block had 6 different TMS timings: 0, 300, 600, 900, 1200 and 1500 ms. Trials in the 1800 ms block had 7 different TMS timings: 0, 300, 600, 900, 1200, 1500 and 1800 ms. Trials in the 2100 ms block had 8 different TMS timings: 0, 300, 600, 900, 1200, 1500, 1800 and 2100 ms. Consequently, the 1500 ms block was designed according to a 2 × 6 within-subjects factorial design with 2 STIMULATION types and 6 TIME. The 1800 ms block was designed according to a 2 × 7 within-subjects factorial design with 2 STIMULATION types and 7 TIME. The 2100 ms block was designed according to a 2 × 8 within-subjects factorial design with 2 STIMULATION types and 8 TIME. In all blocks, the number of repetitions per experimental condition was set to 9 trials, resulting in 108 trials in the 1500 ms block, 126 trials in the 1800 ms block and 144 trials in the 2100 block. EMG analysis for MEP extraction and the exclusion of trials with anticipatory responses were performed as in Experiment 2, but within each of the 3 blocks separately.

Experiment 3—Data Processing and Statistical Analysis

Trials were trimmed similarly to Experiment 2, by removing responses occurring prior to the GO-signal (see above). In the 1500 ms SET-period, the average number of anticipation responses between subjects was of 12% (subject with minimum n. of anticipation responses = 5%; subject with maximum number of anticipation responses = 17%). for the 1800 ms SET-period the average number of anticipation responses between subjects was of 14% (subject with minimum n. of anticipation responses = 2%; subject with maximum number of anticipation responses = 21%). for the 2100 ms SET-period the average number of anticipation responses between subjects was of 10% (subject with minimum n. of anticipation responses = 3%; subject with maximum number of anticipation responses = 24%). Following this trimming procedure, the remaining outlier RTs exceeding the mean ± 2 SD were only 1% of all trials. Statistical analysis was performed separately for each block, because the variable number of TMS timings prevented a balanced within-subjects analysis of the whole dataset. Three separate repeated-measures 2-way ANOVAs were performed, with STIMULATION (2 levels: single- or

dual-pulse) and TIME (6, 7 or 8 levels, according to the block) as factors. As in Experiment 2, planned comparisons were the paired comparisons of dual-pulse data with single-pulse data within each of the time-points of TMS. Significance threshold was therefore adjusted to $p = 0.008$ (6 comparisons) for the 1500 ms block, to $p = 0.007$ (7 comparisons) for the 1800 ms block and to $p = 0.006$ (8 comparisons) for the 2100 ms block. In addition to the planned analyses, we explored post-hoc the time-course of PMCd-dependent corticospinal inhibition during the SET-period by standardizing the duration of the period to the same value in all the 3 conditions. This was made simply by dividing the time points by the SET-period duration. For example, the 300 ms time point in the 1500 ms dataset became time point 0.2 (i.e. 300/1500 ms). Conversely, the same time point of 300 ms in the 2100 ms dataset became the time point 0.14 (i.e. 300/2100 ms). In this way, all 3 conditions of experiment 3 had the same standardized duration of the SET period, from 0 to 1. In order to quantify this data we used the curve fit tool box of the MATLAB software on the average data from experiments 2 and 3 to fit them first to a Gaussian function:

$$f(x) = a1 \times \exp\left(\frac{x - b1}{c1}\right)^2$$

Finally, an exponential component to account for the increase of the values in the right-hand tail of the distribution (i.e. towards the GO-signal) was added by using the following function:

$$f(x) = a1 \times \exp\left(\frac{x - b1}{c1}\right)^2 + a2 \times \exp(b2 \times x + c2)$$

~~With the parameters a2 = 0.007, b2 = 1.93 and c2 = − 0.39.~~ The the previous sentence on parameters a2, b2 and c2 has been deleted because it is a duplicate of the results data points and the fitted curve are represented in Fig. 8b. The data from Experiment 2 could not be analyzed alone in the same way because they contain too few data points.

Results

None of the subjects reported any significant discomfort from stimulation and no side-effects of TMS, neither immediate nor delayed, were observed in any of them. Across all 3 experiments, for the 55 mm coil the mean motor

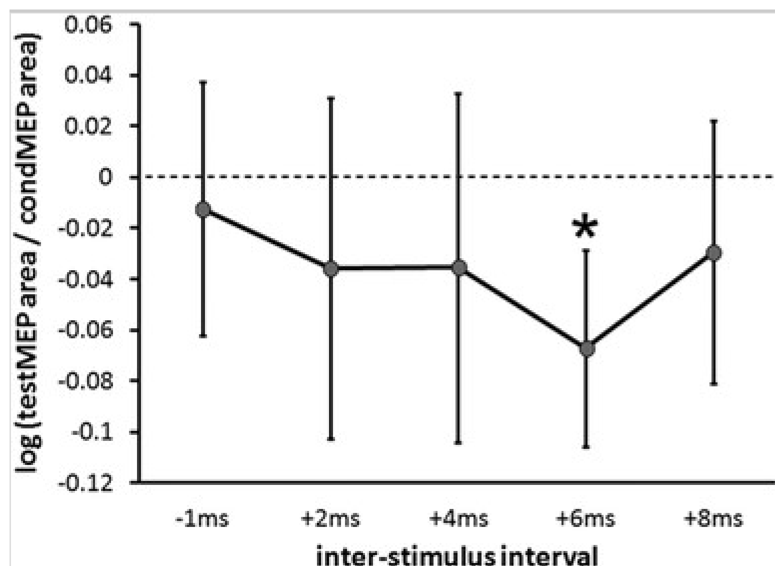
threshold for the OOr muscle was 61% (SD = 4.78) of the stimulator's output. Mean stimulation intensity was 70% (ranging 57–79%) of maximal stimulator output. For the 35 mm coil, the mean motor threshold for the OOr muscle was 65% (SD = 4.22) of the stimulator's output. Mean stimulation intensity was 77% (ranging 58–87%) of maximal stimulator output. Recordings from the 1DI muscle showed systematically the absence of MEPs (defined as waveforms time-locked to TMS with amplitude > 50 μV) in all subjects.

Experiment 1

All participants were able to keep the desired target activation of the OOr muscle. Frequent pauses in the experiment allowed for minimal fatiguing in an otherwise demanding task if performed continuously. Figure 5 illustrates the mean log-transformed MEP ratios. Statistical analysis by means of Bonferroni-corrected t-tests (corrected significance threshold: $p = 0.01$) showed significant deviation of the mean values of normalized MEP ratios from the zero value only for the 6 ms ISI ($p = 0.0033$), all others p values > 0.2.

Fig. 5

Results of Experiment 1. The average values ($n = 16$) of the logarithm of the normalized MEP areas subjects are shown for each of the ISIs. Error bars indicate 95% confidence intervals



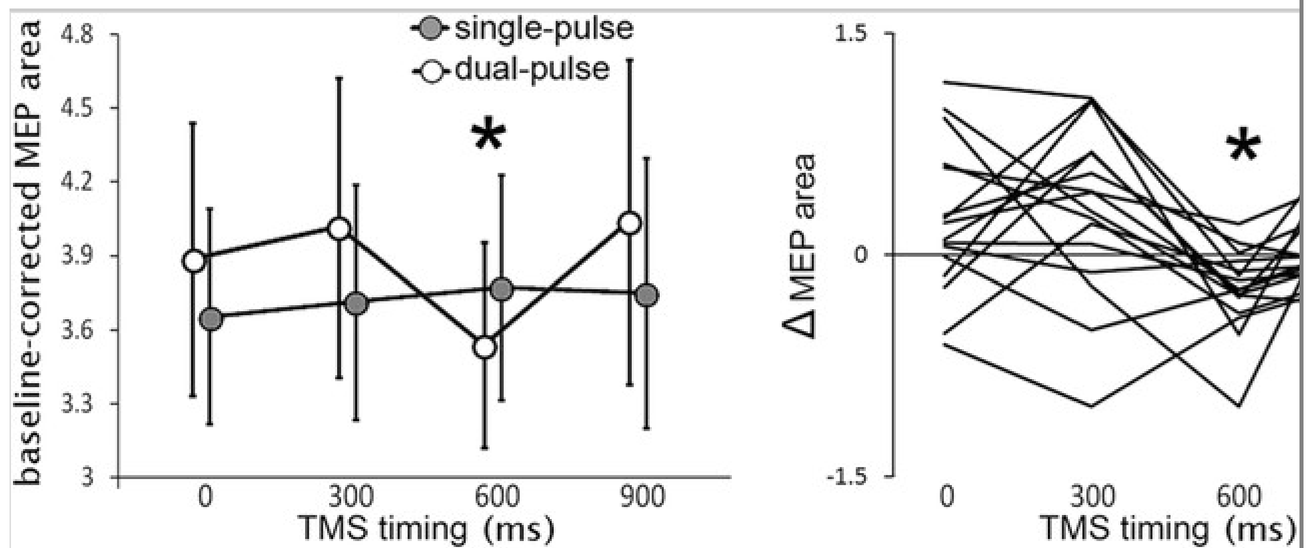
Experiment 2

The mean MEP areas are illustrated in Fig. 6. The two-way ANOVA showed a

significant interaction of TIME × TMS [$F(3, 45) = 5.60$, $p = 0.002$, $\eta^2 = 0.27$]. The data were then divided into two separate univariate ANOVAs, for each of the TMS modalities with TIME as the only factor. The results indicated that the single-pulse MEPs did not show any change in their distribution over time [$F(3, 45) = 0.22$, $p = 0.88$, $\eta^2 = 0.01$]. On the contrary, the partial analysis on paired-pulse indicated a significant main effect of TIME [$F(3, 45) = 4.12$, $p = 0.011$, $\eta^2 = 0.22$]. The 4 planned comparisons between the paired-pulse and the single-pulse data showed significant results only for the 600 ms interval, with paired pulse MEPs being significantly smaller than single-pulse MEPs ($p = 0.004$). The ANOVA on RTs showed a main effect of STIMULATION [$F(1,15) = 6.33$, $p = 0.024$, $\eta^2 = 0.31$] indicating that RTs were faster in dual-pulse trials than in single-pulse trials and a main effect of TIME [$F(3,45) = 19.4$, $p < 0.000001$]. This was entirely due to RTs in the 900 ms much slower than those in the remaining intervals (all p values < 0.000001) while no reciprocal differences were found between the other 3 intervals. The STIMULATION × TIME interaction was not significant [$F(3,45) = 0.45$, $p = 0.71$, $\eta^2 = 0.03$].

Fig. 6

Results of Experiment 2. Left panel: the average values ($n = 16$) of the MEP areas are shown for each stimulation time. MEPs from single-pulses (grey circles) and from dual-pulses (white circles) are plotted. Asterisks indicate significant differences between single-pulse MEPs and dual-pulse MEPs within the same time-interval of stimulation. Error bars indicate 95% confidence intervals. Right panel: in order to represent individual data, we plotted on the right side the individual *differences* between dual-pulse MEP areas and single-pulse MEP areas. By consequence, negative values indicate inhibition of the MEPs by conditioning TMS and positive values indicate facilitation



Experiment 3

The values of the MEP areas in the different experimental conditions are outlined in Fig. 7. The analysis was conducted separately for each of the 3 durations of the SET-periods (1500, 1800 and 2100 ms). A significant TIME × TMS interaction was found in all the 3 durations of the SET-period, i.e. in the 1500 ms duration [$F(5, 55) = 2.85$, $p = 0.023$, $\eta^2 = 0.21$], the 1800 ms duration [$F(6, 66) = 2.89$, $p = 0.014$, $\eta^2 = 0.22$] and the 2100 ms duration [$F(7, 77) = 2.33$, $p = 0.032$, $\eta^2 = 0.18$]. The pair-wise comparisons between single-pulse and paired-pulse data indicated that dual-pulse MEPs were significantly lower than single-pulse MEPs at the 600 ms timing ($p = 0.006$) in the 1500 ms block, at the 600 ($p = 0.003$) and 900 ms ($p = 0.001$) timing in the 1800 block. In the 2100 ms block we found uncorrected significant differences between dual-pulse and single-pulse MEPs in the 900 ($p = 0.02$) and 1200 ms ($p = 0.01$) timings, but the p-level did not survive multiple comparison correction. The results of the post-hoc standardization are shown in Fig. 8a, illustrating that corticobulbar inhibition tended to occupy the middle of the SET-period, irrespective of the duration. The time-course of PMCd–M1 effects appears therefore to be U-shaped, with a rightward skew, independently from the duration of the SET-period the results of the Gaussian function application yielded a satisfactory fit ($R^2 = 0.68$) with the parameters: $a1 = -0.08$, $b1 = 0.50$ and $c1 = -0.18$. However, to add an exponential component to account for the increase of the values in the right-hand tail of the distribution (i.e. towards the GO-signal) consistently increased the goodness of the fit, raising the value of R^2 to 0.88, by using the following function (parameters are $a2 = 0.007$, $b2 = 1.93$ and $c2 = -0.39$; see Fig. 8b):

Fig. 7

Results of Experiment 3. Left panels: the average values ($n = 12$) of the MEP areas are shown separately for each of the SET-period durations. MEPs from single-pulses (grey circles) and from dual-pulses (white circles) are plotted. Asterisks indicate significant differences between single-pulse MEPs and dual-pulse MEPs within the same time-interval of stimulation. Error bars indicate 95% confidence intervals. Right panels: similar to Fig. 4, in order to represent individual data on MEP areas, we represent on the right side the individual *differences* between dual-pulse MEP areas and single-pulse MEP areas. By consequence, negative values indicate inhibition of the MEPs by conditioning TMS and positive values indicate facilitation

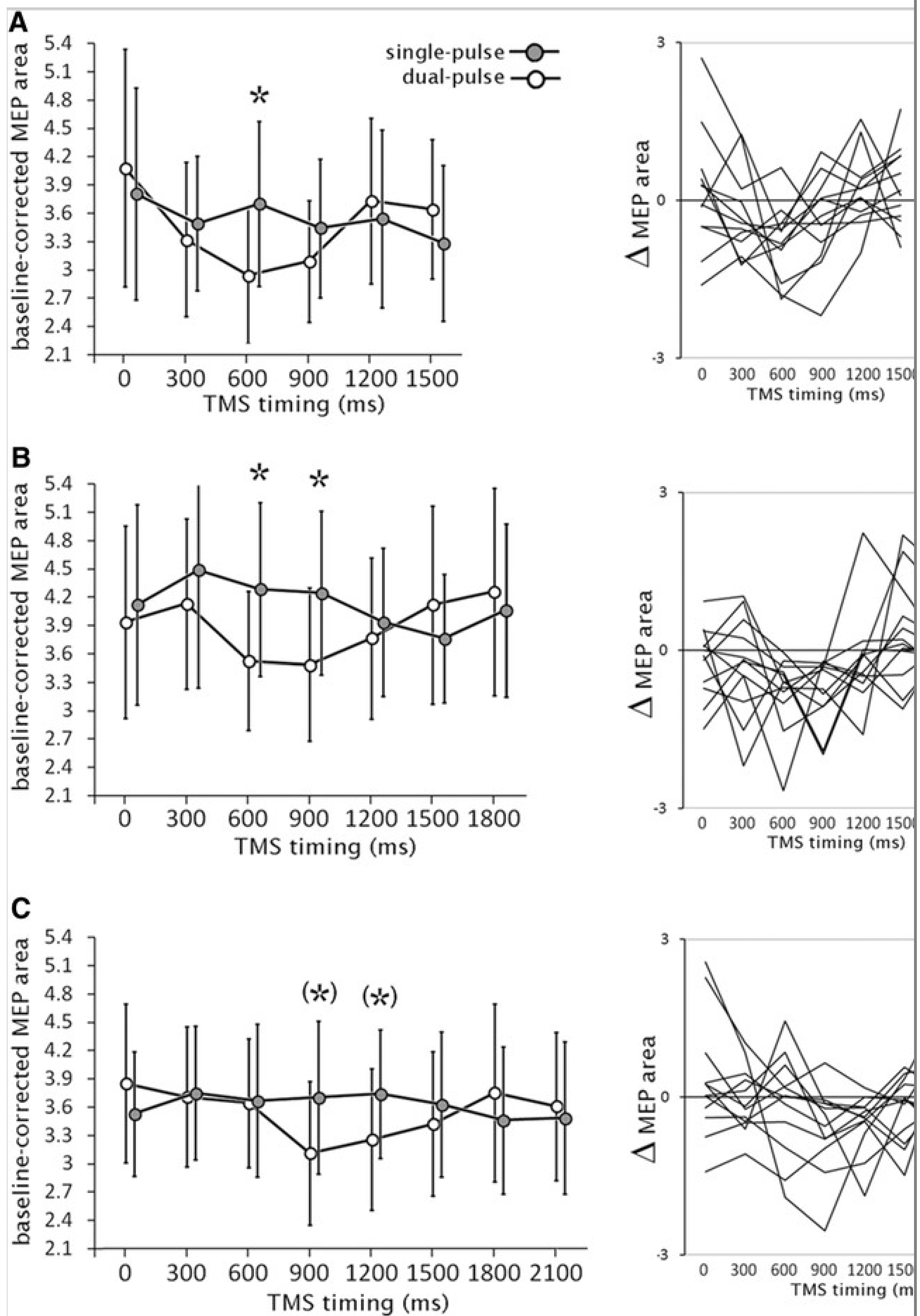
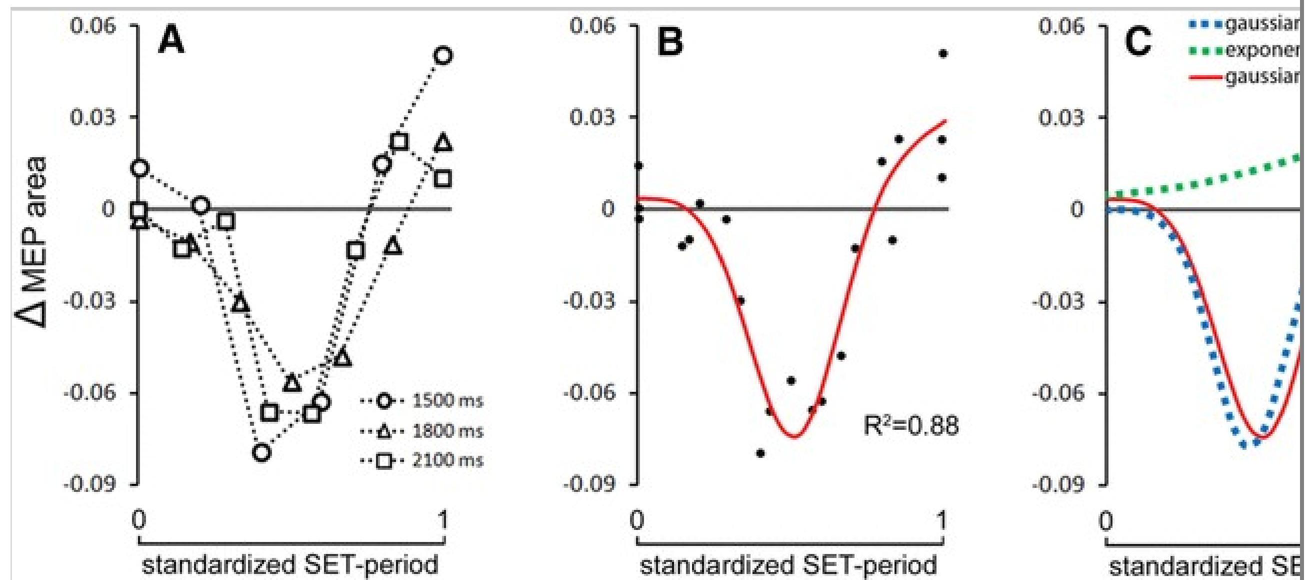


Fig. 8

Results of the analysis on data from Experiment 3, after standardization of the SET-period duration. **a** Average data (n = 12) from the 3 different SET durations. **b** Scatterplot of the average data, with the curve of the fitting function superimposed as a continuous red-line. **c** Representation of the two independent functions (Gaussian and exponential) that produce the fitting function



$$f(x) = a1 \times \exp\left(\frac{x - b1}{c1}\right)^2 + a2 \times \exp(b2 \times x + c2)$$

Discussion

In the present study, we aimed at investigating whether and how PMCd directly influences the ipsilateral M1 while a person is required not to move, waiting for a GO-signal, in the context of a simple delayed motor task. We used a variant of the canonical dual-coil paradigm, delivering test-TMS pulses over mouthM1 rather than on handM1. This allowed us to apply simultaneously TMS over both PMCd and the ipsilateral M1. Experiment 1 confirmed the feasibility of the approach, as has been preliminarily demonstrated in Parmigiani et al. (2015). We showed that condTMS applied over PMCd modified the excitability of testTMS to the orofacial M1 with an ISI of 6 ms. This finding implies that PMCd and the ipsilateral mouthM1 are functionally connected in humans because it is generally assumed that such a short-latency effect is a signature of a direct (or quasi-direct) cortico–cortical connectivity (see, for instance, Davare et al. 2008, 2009, 2010; Baumer et al.

2009; Cattaneo and Barchiesi 2011). This is not the only possible explanation of the results. Indeed, PMCd is known to send direct descending axons to the facial nucleus (Morecraft et al. 2001), our data cannot exclude the possibility that the interaction between the PMC and the mouthM1 occurs at the brainstem level rather than in a cortico–cortical pathway. However, the hypothesis of cortico–cortical interactions is nevertheless more likely because the effect at 6 ms ISI would imply that cortico-bulbar axons from the PMCd are much slower than those from the mouthM1. The cortico-bulbar conduction time to the facial nucleus is ~ 5 ms (Cattaneo and Pavesi 2013) and the supposed latency of the cortico-bulbar volley form the PMCd would therefore be ~ 11 ms, which is quite slow for pyramidal conduction.

Experiment 2 and Experiment 3 specifically investigated the functional properties of PMCd–M1 SET-related connectivity by using a 6 ms ISI. In both the experiments the task consisted in two distinct phases: an action-withholding phase (SET-period) and an action-releasing phase (after the GO-signal), in which participants had to lift a stick with their lips as fast as possible. The duration of the SET-period was fixed. This solution offers the unique possibility to sample equally the duration of the SET-period with TMS. On the downside, in this experimental setup the SET-period duration is entirely predictable by the participants. Consequently, there is the chance that the task can be solved in a predictive, top-down manner (i.e. independently from the actual presence of sensory cues) rather than with reactive, bottom-up processes (i.e. in response to the visual cues). In the present experiment we are testing a low-level process, i.e. direct inhibition of M1. It is likely that predictive and reactive processes do not have a dedicated projection to M1. The PMCd–M1 activity is probably shared between both types of action control.

Experiment 2 revealed an inhibitory activity of PMCd on M1 during the SET-period, specifically at 600 ms from the onset of the SET-period (see Fig. 6). Such phasic inhibitory activity was present also when varying the duration of the SET-period (1500, 1800, 2100 ms) in a similar blocked-design (Experiment 3; see Fig. 7). We then equalized the duration of the SET-period to a standard length of 1 and we modeled the pooled data from Experiment 3 to a biphasic inhibitory-facilitatory function, resulting in an excellent fit ($R^2 = 0.88$). This indicates that overall, the relative time course of the PMCd–M1 interactions is invariant with respect to the duration of the SET-period: inhibition builds up and peaks around the middle of the SET-period, but is

followed by facilitation as the (predictable) GO-signal approaches.

The finding of an inhibitory effect of PMCd on M1 is intriguing. As already mentioned in the Introduction, paired-pulse data are usually construed as revelatory of the information transmitted between the premotor and motor cortices (Koch et al. 2008; Davare et al. 2009; Vesia et al. 2013). Therefore, the inhibitory information flowing from PMCd to M1 could be interpreted as the neural correlate of the capability to withholding a preloaded action. This interpretation is in a good agreement with a previous work exploring the effect of offline TMS over the premotor regions on behavioral features of motor inhibition and on neurophysiological markers of corticospinal excitability. Kroeger et al. (2010) applied conditioning TMS pulses to the left PMCd 8 ms before a test pulse was given to the right M1 during a go/no-go task. Conditioning of the left PMCd led to interhemispheric inhibition at 300 ms after the presentation of the first stimulus instructing which movement will have to be performed but withheld until a the appearance of a second cue instructing to release the movement. Indeed, they found interhemispheric facilitation at 150 ms after the second cue appeared. They claimed that the excitability in left premotor–motor right motor pathways could be context-dependent, and that the left PMCd is engaged not only in action selection but also in withholding and releasing a preselected movement generated by the right motor cortex. Duque et al. (2012) showed that rTMS applied offline to the PMCd significantly diminished MEPs inhibition during an immobility phase preceding an imperative signal for movement. The authors construed these results by hypothesizing that PMCd would be responsible for a corticospinal inhibition while waiting for a GO-signal (“impulse control”, as recently proposed in Duque et al. 2017). The impulse control could be described as a characteristic feature of motor inhibition associated with action preparation.

Our findings support the hypothesis that PMCd–M1 connectivity carries inhibitory information whenever the impulse to release the response must be controlled and suppressed during the task. The findings also allow us to make a step further, by showing how inhibition and activation of a motor program could be intimately linked. Indeed, the finding of a reversal from inhibition to excitation provided evidence for this link, demonstrating that inhibitory and excitatory effects may coexist in a same cortical network such as PMCd–M1. The excitatory effect of PMCd on M1 showed up when the GO-signal was not appeared yet. This suggests that the excitatory information flowing from

PMCd to M1 could reflect the capability to release the withheld action at the right time. It is worth noting that our findings on a biphasic inhibitory-facilitatory function of PMCd are fully compatible with its canonical role in action preparation described in other works (O’Shea et al. 2007; Bestmann et al. 2008). For instance, O’Shea and colleagues (O’Shea et al. 2007) found facilitatory effects of the left PMCd on the contralateral handM1 when TMS was delivered just after the GO-signal presentation, when action releasing was required. In our study TMS was delivered during the SET-period before the GO-signal presentation, when action withholding was required. Besides this, the coexistence of an inhibitory and excitatory function in PMCd has been also previously reported by Koch et al. (2006), who found that PMCd–M1 activity varied with the varying of the task phase. They stimulated the left PMCd and the contralateral handM1 during a choice response task in which participants should squeeze one of the two hands as rapidly as possible after an arbitrary auditory cue was presented. Facilitatory influences from left PMCd to right M1 were found time-locked to the phase of the task which required action initiation, whereas inhibitory effects of PMCd over the contralateral M1 were found when the task required action suppression. Our findings do extend Koch et al.’s results, showing that the fine interplay between inhibition and facilitation in PMCd might be related not only to a choice between competing actions, but also to the performance of a single action, especially when this requires to withhold the action until the right time comes.

This does not rule out the possibility for our findings to be construed in an action selection framework. Indeed, although our task involved one type of action only and its performance was forced by the onset of the GO-signal, the finding of a biphasic inhibitory-excitatory function of PMCd can be also accounted for by the *action competition* theory developed by Cisek and colleagues (Cisek and Kalaska 2010; Thura and Cisek 2014, 2016). This theory postulates that action selection is the product of a competition between alternative actions, which takes place directly inside the premotor–motor networks. Action selection is therefore a commitment to action that occurs whenever a winner arises from the mutual competitive condition. When modeling our data (see Fig. 8b), we obtained the best fit ($R^2 = 0.88$) if we adopted two different functions, one to account for motor inhibition (the Gaussian function) and the other for response facilitation (the exponential function), which interacted reciprocally in a linear way, i.e. as independent but parallel processes (illustrated in Fig. 8c). This suggests that the commitment

on whether to move or not to move even in our simple delayed response task could therefore derive from competition between the two actions of withholding and releasing the impending movements.

Several methodological and theoretical concerns should be considered when interpreting our findings. First, one might be tempted to consider the inhibitory activity exerted by condTMS to be primarily due to an undesired spread of stimulation to the neighbor supplementary motor area (SMA) rather than to a selective PMCd stimulation. Indeed, SMA has been identified as a putative “negative motor area” (Burle et al. 2004; Nachev et al. 2008; Verbruggen and Logan 2008). However, we should resist this temptation. For two reasons, at least. First, the high focality of the stimulation provided by a 35 mm figure of-eight coil made a spread to the medial cortical surface unlikely, as already pinpointed by Parmigiani et al. (2015). The second reason why current spread to SMA is rather implausible is that the scalp localization of condTMS target in the present study was quite distant from the midline, as can be observed in Figs. 1 and 2.

Second, we adopted in our study a fixed and predictable duration of SET-periods. As well known, this does not allow for a clear separation between internally triggered movement and movement associated with the go signal. Experiment 3 suggest that the highly predictability of GO-signal induced the participants to make use of predictive strategies in order to succeed in the task. This could confine our findings to action inhibition and releasing processes involved in internally generated actions rather than in externally cued ones.

Third, the need for constant contraction of the target muscle is a possible confound. In most of the literature, TMS has been applied to the motor cortex while the target muscle was at rest. Some of the effects of TMS on corticospinal excitability are dependent on the resting condition and decrease or even disappear during active contraction, as is the case, for example, of intracortical inhibition (Zoghi et al. 2003) or of short-latency afferent inhibition (Ni et al. 2011). Data obtained during voluntary contraction are not immediately comparable to those obtained at rest. However, targeting the mouth-related M1 is the only viable possibility to test PMCd–M1 connectivity on the same hemisphere and obtaining MEPs from facial muscles requires voluntary contraction. We think therefore that the information obtained here is valuable, though it is difficult to compare it with the TMS literature in muscles at rest.

Finally, it should be clearly kept in mind that the physiological findings of inhibitory connectivity between PMCd and M1 cannot be considered per se as a conclusive argument for a distinctive role of PMCd in the behavioral function of motor inhibition. Such inference would be justified only in presence of behavioral effects of TMS over PMCd, and the current study was not designed to test the impact of TMs over PMCd on behavior. On the one side, even though our findings could account for the inhibitory behaviour, this mechanism is likely to be not the only one responsible for the final stopping behaviour observable in this task. On the other side, we managed to examine RTs in Experiment 2, where the number of trials per condition allowed such analysis. Our results indicated only a general speeding up of RTs in trials in which also PMCd was stimulated (i.e. dual-pulse trials). This result is ambiguous. On one hand some data in the literature had already shown that TMS over PMCd shortens reaction times (Chambers et al. 2007), on the other hand, this result could merely reflect the possibility that receiving two TMS pulses is more alerting than receiving only one. The lack of interaction between the stimulation modality and the timing of TMS argues against the specificity of such result. Therefore, further research in this direction is needed.

To sum up, at this stage the present study provides, for the first time, a direct investigation of functional connectivity between PMCd and the ipsilateral M1 during the SET-period of a delayed task. We found both an inhibitory effect of PMCd over M1, when the task required withholding a preloaded action, and a reversal from inhibition to facilitation when the right time to release the action was coming. This suggests that PMCd functions are richer than previously thought, supporting the notion that in this portion of the premotor cortex the connectivity with M1 underpins both an impulse control and facilitation function. The processes of motor inhibition and motor production seem to access independently and competitively the motor output, though both originating from a spatially overlapping region, i.e. the PMCd, coherently with the *action competition* account of motor behavior (Thura and Cisek 2016). As for the possible future directions of this research, the paradigm we developed could be employed to investigate also slightly different issues. For instance, premotor–motor long-latency interactions (e.g. Neubert et al. 2010; Fiori et al. 2016, 2017) are likely based on short-latency interactions as the one described here and could be investigated, both in healthy subjects as well as in neurological conditions affecting motor control (see for instance Li et al. 2007; Sattler et al. 2014). Furthermore, this paradigm could be associated to

repetitive TMS to investigate the plasticity of the PMCd–M1 circuit, by means of the cortico–cortical paired associative stimulation (ccPAS) (see Buch et al. 2011; Koch et al. 2013; Romei et al. 2016). Finally, several authors (Fadiga et al. 2002; Sato et al. 2010; Vicario et al. 2016) reported changes in cortico-bulbar excitability during cognitive and emotion processing that could be enriched with an exploration of the premotor–orofacialM1 interactions, taking advantage of the present TMS paradigm.

References

Aron AR (2007) The neural basis of inhibition in cognitive control. Neurosci 13:214–228

Aron AR, Durston S, Eagle DM, Logan GD, Stinear CM, Stuphorn V (2007) Converging evidence for a fronto-basal-ganglia network for inhibitory control of action and cognition. J Neurosci 27:11860–11864

Baumer T, Schippling S, Kroeger J, Zittel S, Koch G, Thomalla G, Rothwell JC, Siebner HR, Orth M, Munchau A (2009) Inhibitory and facilitatory connectivity from ventral premotor to primary motor cortex in healthy humans at rest—a bifocal TMS study. Clin Neurophysiol 120:1724–1731

Beck S, Houdayer E, Richardson SP, Hallett M (2009) The role of inhibition from the left dorsal premotor cortex in right-sided focal hand dystonia. Brain Stimul 2:208–214

Bestmann S, Swayne O, Blankenburg F, Ruff CC, Haggard P, Weiskopf N, Josephs O, Driver J, Rothwell JC, Ward NS (2008) Dorsal premotor cortex exerts state-dependent causal influences on activity in contralateral primary motor and dorsal premotor cortex. Cereb Cortex 18:1281–1291

Boy F, Husain M, Singh KD, Sumner P (2010) Supplementary motor area activations in unconscious inhibition of voluntary action. Exp Brain Res 206:441–448

Brinkworth RS, Türker KS, Savundra AW (2003) Response of human jaw muscles to axial stimulation of the incisor. J Physiol 547(1):233–245

Brodin P, Miles TS, Türker KS (1993) Simple reaction-time responses to

mechanical and electrical stimuli in human masseter muscle. Arch Oral Biol 38(3):221–226

Buch ER, Johnen VM, Nelissen N, O'Shea J, Rushworth MF (2011) Noninvasive associative plasticity induction in a corticocortical pathway of the human brain. J Neurosci 31(48):17669–17679

Burle B, Vidal F, Tandonnet C, Hasbroucq T (2004) Physiological evidence for response inhibition in choice reaction time tasks. Brain Cogn 56:153–164

Cattaneo L, Barchiesi G (2011) Transcranial magnetic mapping of the short-latency modulations of corticospinal activity from the ipsilateral hemisphere during rest. Front Neural Circuits 5:1–13

Cattaneo L, Pavesi G (2013) The facial motor system. Neurosci Biobehav Rev 38:135159

Cattaneo L, Macaluso GM, Pavesi G (2007) Inhibitory reflexes in human perioral facial muscles: a single-motor unit study. Clin Neurophysiol 118(4):794–801

Chambers CD, Garavan H, Bellgrove MA (2009) Insights into the neural basis of response inhibition from cognitive and clinical neuroscience. Neurosci Biobehav Rev 33:631–646

Cisek P, Kalaska JF (2010) Neural mechanisms for interacting with a world full of action choices. Annu Rev Neurosci 33:269–298

Davare M, Lemon R, Olivier E (2008) Selective modulation of interactions between ventral premotor cortex and primary motor cortex during precision grasping in humans. J Physiol 586:2735–2742

Davare M, Montague K, Olivier E, Rothwell JC, Lemon RN (2009) Ventral premotor to primary motor cortical interactions during object-driven grasp in humans. Cortex 45:1050–1057

Davare M, Rothwell JC, Lemon RN (2010) Causal connectivity between the human anterior intraparietal area and premotor cortex during grasp. Curr Biol 20:176–181

di Pellegrino G, Wise SP (1993) Visuospatial versus visuomotor activity in the premotor and prefrontal cortex of a primate. J Neurosci 13:1227–1243

Duque J, Labruna L, Verset S, Olivier E, Ivry RB (2012) Dissociating the role of prefrontal and premotor cortices in controlling inhibitory mechanisms during motor preparation. J Neurosci 32:806–816

Duque J, Greenhouse I, Labruna L, Ivry RB (2017) Physiological markers of motor inhibition during human behavior. Trends Neurosci 40:219–236

Fadiga L, Craighero L, Buccino G, Rizzolatti G (2002) Speech listening specifically modulates the excitability of tongue muscles: a TMS study. Eur J Neurosci 15(2):399–402

Fiori F, Chiappini E, Soriano M, Paracampo R, Romei V, Borgomaneri S, Avenanti A (2016) Long-latency modulation of motor cortex excitability by ipsilateral posterior inferior frontal gyrus and pre-supplementary motor area. Sci Rep 6:38396

Fiori F, Chiappini E, Candidi M, Romei V, Borgomaneri S, Avenanti A (2017) Long-latency interhemispheric interactions between motor-related areas and the primary motor cortex: a dual site TMS study. Sci Rep 7(1):14936

Ghosh S, Porter R (1988) Corticocortical synaptic influences on morphologically identified pyramidal neurons in the motor cortex of the monkey. J Physiol 400:617–629

Godschalk M, Lemon RN, Kuypers HGJM., Van Der Steen J (1985) The involvement of monkey premotor cortex neurones in preparation of visually cued arm movements. Behav Brain Res 18:143–157

Groppa S, Schlaak BH, Münchau A, Werner-Petroll N, Dünnweber J, Bäumer T, van Nuenen BFL, Siebner HR (2012) The human dorsal premotor cortex facilitates the excitability of ipsilateral primary motor cortex via a short latency cortico-cortical route. Hum Brain Mapp 33:419–430

Hardwick RM, Rottschy C, Miall RC, Eickhoff SB (2013) A quantitative meta-analysis and review of motor learning in the human brain.

Neuroimage 67:283–297. https://doi.org/10.1016/j.neuroimage.2012.11.020

Hardwick RM, Lesage E, Eickhoff CR, Clos M, Fox P, Eickhoff SB (2015) Multimodal connectivity of motor learning-related dorsal premotor cortex. Neuroimage 123:114–128

Hatanaka N, Nambu A, Yamashita A, Takada M, Tokuno H (2001) Somatotopic arrangement and corticocortical inputs of the hindlimb region of the primary motor cortex in the macaque monkey. Neurosci Res 40:9–22

Hoshi E, Tanji J, Gallivan JP, Mclean DA, Flanagan JR, Culham JC (2014) Contrasting neuronal activity in the dorsal and ventral premotor areas during preparation to reach. J Neurophysiol 87:1123–1128

Johansen-Berg H, Rushworth MFS, Bogdanovic MD, Kischka U, Wimalaratna S, Matthews PM (2002) The role of ipsilateral premotor cortex in hand movement after stroke. Proc Natl Acad Sci USA 99:14518–14523

Kaufman MT, Churchland MM, Santhanam G, Byron M, Afshar A, Ryu SI, Shenoy KV (2010) Roles of monkey premotor neuron classes in movement preparation and execution. J Neurophysiol 104(2):799–810

Kiefer M, Marzinzik F, Weisbrod M, Scherg M, Spitzer M (1998) The time course of brain activations during response inhibition: evidence from event-related potentials in a go/no go task. Neuroreport 9:765–770

Kirchner H, Thorpe SJ (2006) Ultra-rapid object detection with saccadic eye movements: visual processing speed revisited. Vis Res 46(11):1762–1776

Koch G, Franca M, Del Olmo MF, Cheeran B, Milton R, Alvarez Sauco M, Rothwell JC (2006) Time course of functional connectivity between dorsal premotor and contralateral motor cortex during movement selection. J Neurosci 26:7452–7459

Koch G, Fernandez Del Olmo M, Cheeran B, Schippling S, Caltagirone C, Driver J, Rothwell JC (2008) Functional interplay between posterior parietal and ipsilateral motor cortex revealed by twin-coil transcranial

magnetic stimulation during reach planning toward contralateral space. J Neurosci 28:5944–5953

Koch G, Ponzo V, Di Lorenzo F, Caltagirone C, Veniero D (2013) Hebbian and anti-Hebbian spike-timing-dependent plasticity of human cortico-cortical connections. J Neurosci 33(23):9725–9733

Kroeger J, Bäumer T, Jonas M, Rothwell JC, Siebner HR, Münchau A (2010) Charting the excitability of premotor to motor connections while withholding or initiating a selected movement. Eur J Neurosci 32(10):1771–1779

Kurata K, Wise SP (1988a) Premotor and supplementary motor cortex in. Exp Brain Res 72:237–248

Kurata K, Wise SP (1988b) Premotor cortex of rhesus monkeys: set-related activity during two conditional motor tasks. Exp Brain Res 69:327–343

Li JY, Espay AJ, Gunraj CA, Pal PK, Cunic DI, Lang AE, Chen R (2007) Interhemispheric and ipsilateral connections in Parkinson’s disease: relation to mirror movements. Mov Disord 22(6):813–821

Maule F, Barchiesi G, Brochier T, Cattaneo L (2015) Haptic working memory for grasping: the role of the parietal operculum. Cereb Cortex 25:528–537

Mirabella G, Pani P, Ferraina S (2011) Neural correlates of cognitive control of reaching movements in the dorsal premotor cortex of rhesus monkeys. J Neurophysiol 106(3):1454–1466

Moll L, Kuypers HG (1977). Premotor cortical ablations in monkeys: contralateral changes in visually guided reaching behavior. Science 198(4314):317–319

Morecraft RJ, Louie JL, Herrick JL, Stilwell-Morecraft KS (2001) 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 124(1):176–208

Muakkassa KF, Strick PL (1979) Frontal lobe inputs to primate motor

cortex: evidence for four somatotopically organized “premotor” areas. Brain Res 177:176–182

Nachev P, Kennard C, Husain M (2008) Functional role of the supplementary and pre-supplementary motor areas. Nat Rev Neurosci 9:856–869

Neubert FX, Mars RB, Buch ER, Olivier E, Rushworth MF (2010) Cortical and subcortical interactions during action reprogramming and their related white matter pathways. Proc Natl Acad Sci 107(30):13240–13245

Ni Z, Charab S, Gunraj C, Nelson AJ, Udupa K, Yeh I-J, Chen R (2011) Transcranial magnetic stimulation in different current directions activates separate cortical circuits. J Neurophysiol 105:749–756

O’Shea J, Sebastian C, Boorman ED, Johansen-Berg H, Rushworth MFS (2007) Functional specificity of human premotor-motor cortical interactions during action selection. Eur J Neurosci 26:2085–2095

Parmigiani S, Barchiesi G, Cattaneo L (2015) The dorsal premotor cortex exerts a powerful and specific inhibitory effect on the ipsilateral corticofacial system: a dual-coil transcranial magnetic stimulation study. Exp Brain Res 233:3253–3260

Pavesi G, Macaluso GM, Marchetti P, Cattaneo L, Tinchelli S, De Laat A, Mancia D (2000) Trigemino-facial reflex inhibitory responses in some lower facial muscles. Muscle Nerve 23(6):939–945

Pirio Richardson S, Beck S, Bliem B, Hallett M (2014) Abnormal dorsal premotor-motor inhibition in writer’s cramp. Mov Disord 29:797–803

Riehle A, Grammont F, MacKay WA (2006) Cancellation of a planned movement in monkey motor cortex. Neuroreport 17:281–285

Romei V, Chiappini E, Hibbard PB, Avenanti A (2016) Empowering reentrant projections from V5 to V1 boosts sensitivity to motion. Curr Biol 26(16):2155–2160

Rossi S, Hallett M (2009) Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical

practice and research. Clin Neurophysiol 120:2008–2039

Rothwell JC (2011) Using transcranial magnetic stimulation methods to probe connectivity between motor areas of the brain. Hum Mov Sci 30:906–915

Sato M, Buccino G, Gentilucci M, Cattaneo L (2010) On the tip of the tongue: modulation of the primary motor cortex during audiovisual speech perception. Speech Commun 52:533–541

Sattler V, Dickler M, Michaud M, Meunier S, Simonetta-Moreau M (2014) Does abnormal interhemispheric inhibition play a role in mirror dystonia? Mov Disord 29(6):787–796

Sawaguchi T, Yamane I, Kubota K (1996) Application of the GABA antagonist bicuculline to the premotor cortex reduces the ability to withhold reaching movements by well-trained monkeys in visually guided reaching task. J Neurophysiol 75(5):2150–2156

Schluter ND, Rushworth MFS, Passingham RE, Mills KR (1998) Temporary interference in human lateral premotor cortex suggests dominance for the selection of movements. A study using transcranial magnetic stimulation. Brain 121:785–799

Thura D, Cisek P (2014) Deliberation and commitment in the premotor and primary motor cortex during dynamic decision making. Neuron 81:1401–1416

Thura D, Cisek P (2016) Modulation of premotor and primary motor cortical activity during volitional adjustments of speed-accuracy trade-offs. J Neurosci 36:938–956

Tokuno H, Nambu A (2000) Organization of nonprimary motor cortical inputs on pyramidal and nonpyramidal tract neurons of primary motor cortex: an electrophysiological study in the macaque monkey. Cereb Cortex 10:58–68

Tokuno H, Tanji J (1993) Input organization of distal and proximal forelimb areas in the monkey primary motor cortex: a retrograde double labeling study. J Comp Neurol 333:199–209

Tukey JW (1977) Exploratory data analysis, vol 2. Addison-Wesley Publishing Company, Boston

Verbruggen F, Logan G (2008) Response inhibition in the stop-signal pardigm. Trends Cogn Sci 12:418–424

Vesia M, Bolton DA, Mochizuki G, Staines WR (2013) Human parietal and primary motor cortical interactions are selectively modulated during the transport and grip formation of goal-directed hand actions. Neuropsychologia 51:410–417

Vicario CM, Rafal RD, Borgomaneri S, Paracampo R, Kritikos A, Avenanti A (2016) Pictures of disgusting foods and disgusted facial expressions suppress the tongue motor cortex. Soc Cogn Affect Neurosci 12(2):352–362

Watkins K, Paus T (2004) Modulation of motor excitability during speech perception: the role of Broca's area. J Cogn Neurosci 16:978–987

Watkins KE, Strafella AP, Paus T (2003) Seeing and hearing speech excites the motor system involved in speech production. Neuropsychologia 41:989–994

Weinrich M, Wise SP, Mauritz KH (1984) A neurophysiological study of the premotor cortex in the rhesus monkey. Brain 107(Pt 2):385–414

Wise SP (1985) The primate premotor cortex: past, present, and preparatory. Annu Rev Neurosci 8:1–19

Wise SP, Mauritz KH (1985) Set-related neuronal activity in the premotor cortex of rhesus monkeys: effects of changes in motor set. Proc R Soc Lond B 223(1232), 331–354

World Medical Association (2009) Declaration of Helsinki. Ethical Principles for Medical Research Involving Human Subjects

Zoghi M, Pearce SL, Nordstrom M (2003) Differential modulation of intracortical inhibition in human motor cortex during selective activation of an intrinsic hand muscle. J Physiol 550:933–946