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Original Article

Connecting the dots: harnessing dual-site transcranial magnetic stimulation to quantify the causal influence of medial frontal areas on the motor cortex

Cécilia Neige[1,2,3,*], Pierre Vassiliadis[4,5], Abdelkrim Ali Zazou[4], Laurence Dricot[4], Florent Lebon[1], Thomas Brees[4], Gerard Derosiere[4,6]

[1]Université Bourgogne Franche-Comté, INSERM UMR1093-CAPS, UFR des Sciences du Sport, F-21078, Dijon, France,
[2]Université Claude Bernard Lyon 1, CNRS, INSERM, Centre de Recherche en Neurosciences de Lyon CRNL U1028 UMR5292, PsyR2 Team, F-69500, Bron, France,
[3]Centre Hospitalier le Vinatier, 95 Boulevard Pinel, 300 3969678 Bron Cedex, France,
[4]Institute of Neuroscience, Université Catholique de Louvain, Avenue E. Mounier 53 & 73, 1200, Brussels, Belgium,
[5]Defitech Chair for Clinical Neuroengineering, Neuro-X Institute (INX) and Brain Mind Institute (BMI), École Polytechnique Fédérale de Lausanne (EPFL), 1202, Geneva, Switzerland,
[6]Université Claude Bernard Lyon 1, CNRS, INSERM, Centre de Recherche en Neurosciences de Lyon CRNL U1028 UMR5292, Impact Team, F-69500, Bron, France

[*]Corresponding author: Cécilia Neige. Université Claude Bernard Lyon 1, CNRS, INSERM, CRNL U1028 UMR5292, Centre Hospitalier le Vinatier, PsyR2 Team. F69500, Bron, France. Email: cecilia.neige@inserm.fr

Dual-site transcranial magnetic stimulation has been widely employed to investigate the influence of cortical structures on the primary motor cortex. Here, we leveraged this technique to probe the causal influence of two key areas of the medial frontal cortex, namely the supplementary motor area and the medial orbitofrontal cortex, on primary motor cortex. We show that supplementary motor area stimulation facilitates primary motor cortex activity across short (6 and 8 ms) and long (12 ms) inter-stimulation intervals, putatively recruiting cortico-cortical and cortico-subcortico-cortical circuits, respectively. Crucially, magnetic resonance imaging revealed that this facilitatory effect depended on a key morphometric feature of supplementary motor area: individuals with larger supplementary motor area volumes exhibited more facilitation from supplementary motor area to primary motor cortex for both short and long inter-stimulation intervals. Notably, we also provide evidence that the facilitatory effect of supplementary motor area stimulation at short intervals is unlikely to arise from spinal interactions of volleys descending simultaneously from supplementary motor area and primary motor cortex. On the other hand, medial orbitofrontal cortex stimulation moderately suppressed primary motor cortex activity at both short and long intervals, irrespective of medial orbitofrontal cortex volume. These results suggest that dual-site transcranial magnetic stimulation is a fruitful approach to investigate the differential influence of supplementary motor area and medial orbitofrontal cortex on primary motor cortex activity, paving the way for the multimodal assessment of these fronto-motor circuits in health and disease.

Key words: corticospinal excitability; effective connectivity; medial orbitofrontal cortex; supplementary motor area; magnetic resonance imagery.

Introduction

The execution of most volitional actions relies on pyramidal cells located in the primary motor cortex (M1) that project down to the spine and connect with peripheral motoneurons. This so-called corticospinal pathway is under the constant influence of distributed areas of the cerebral cortex that rely on effective connectivity to either facilitate or suppress M1 activity and, ultimately, exert control over behavior. As such, two key areas of the medial frontal cortex– the supplementary motor area (SMA) and the medial orbitofrontal cortex (mOFC)—are known to play a central role in human behavior, being involved in processes as diverse as motor planning and learning (Makoshi et al. 2011; Carlsen et al. 2015; Neige et al. 2018; Vassiliadis et al. 2023), decision-making (Fellows 2007; Klein-Flügge et al. 2016; Derosiere et al. 2018; Loh and Rosenkranz 2022) and inhibitory control (Aron et al. 2007; Boy et al. 2010; Hu and Li 2012). SMA and mOFC project to M1 via both cortico-cortical and cortico-subcortico-cortical circuits, providing candidate pathways through which they might implement these processes. Crucially, however, tools to probe their effective influence on M1 remain scarce at present.

In humans, a specific type of transcranial magnetic stimulation (TMS) protocol—called dual-site paired-pulse TMS (ppTMS)—allows probing the effective connectivity between specific cortical areas and M1 (for recent reviews, see Derosiere and Duque 2020; Neige et al. 2021). As such, the excitability of the corticospinal pathway can be quantified by recording of motor-evoked potentials (MEPs), which can be elicited in muscles by applying single-pulse TMS over the contralateral M1. The amplitude of MEPs provides a global readout of corticospinal excitability, reflecting the simultaneous influence of multiple brain structures projecting to M1 (Bestmann and Duque 2016; Di Lazzaro et al. 2018). Dual-site ppTMS allows to isolate the influence of a targeted cortical area on M1. In such protocols, a first, conditioning stimulation is used to pre-activate the targeted area, while a second, test stimulation is applied over M1 with another coil to elicit a MEP and assess the nature of the influence (i.e. facilitatory or inhibitory) of the pre-activated area on corticospinal excitability. Potentiation of conditioned MEP amplitudes (i.e. relative to unconditioned MEPs) reflects a facilitatory influence of the pre-activated area, whereas a reduction

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of conditioned MEPs reflects an inhibitory effect. Interestingly, varying the inter-stimulation interval allows to probe different circuits, with short inter-stimulation intervals (e.g. between 4 and 8 ms) recruiting cortico-cortical circuits preferentially, and longer ones (e.g. >10 ms) recruiting more indirect circuits presumably funneling through subcortical structures (Neubert et al. 2010).

Over the past two decades, dual-site ppTMS has been widely used in humans, with studies probing the causal influence of several areas of the premotor cortex (Civardi et al. 2001; Koch et al. 2006; Davare et al. 2008; Bäumer et al. 2009; Groppa et al. 2011; Vesia et al. 2018), of the lateral prefrontal cortex (Neubert et al. 2010; Wang et al. 2020) and of the parietal cortex (Koch et al. 2009; Koch and Rothwell 2009; Lebon et al. 2012; Vesia et al. 2017; Allart et al. 2019) on ipsi- and contralateral M1 (Oliveri et al. 2003; Matsunaga et al. 2005; Byblow et al. 2007). However, several issues remain open, in particular regarding the use of this approach on some areas of the medial frontal cortex—including SMA and mOFC.

To date, the few ppTMS studies targeting SMA have mostly focused on short inter-stimulation intervals (6 to 8 ms, Arai et al. 2011, 2012; Green et al. 2018; Rurak et al. 2021). Given this, there is currently a lack of data on the nature of the influence (i.e. facilitatory vs. inhibitory) of SMA stimulation on M1 activity when probed with longer inter-stimulation intervals (i.e. 10 to 15 ms). In fact, the SMA projects to M1 through multiple cortico-subcortico-cortical circuits (Nachev et al. 2008), some of which exert a net facilitatory influence on motor activity (e.g. the direct basal ganglia pathway) and some of which play an inhibitory role (e.g. the indirect and hyperdirect pathways). Interestingly, ppTMS studies focusing on the preSMA—i.e. another key area of the medial frontal cortex—with intervals of 12 ms, reported a potentiation of conditioned MEP amplitudes, which strongly covaried with white matter density in preSMA-basal ganglia-M1 circuits (Mars et al. 2009; Neubert et al. 2010). Taken together, these two sets of findings suggest that, when applied over preSMA at such intervals, ppTMS recruits circuits that have a facilitatory influence on M1 and funnel through the basal ganglia. Still, the SMA and the preSMA are functionally and anatomically distinct (Nachev et al. 2008), with cortico-subcortico projections from the former area mainly directed to the posterior putamen and projections from the latter one directed to more rostral parts of the striatum (Lehéricy et al. 2004). A first goal of the present study is to shed light on to the influence of SMA stimulation on M1 at such intervals, by testing the idea that SMA-originating circuits bear a similar facilitatory influence on motor activity.

As such, ppTMS studies targeting SMA with short inter-stimulation intervals (6 to 8 ms) have reported a potentiation of MEP amplitudes, which has been assumed to reflect the operation of cortico-cortical, facilitatory circuits from SMA to M1 (Arai et al. 2011, 2012; Green et al. 2018; Rurak et al. 2021). Indeed, this assumption seems consistent with animal studies showing that SMA has direct glutamatergic projections to M1 (Muakkassa and Strick 1979; Luppino et al. 1993) and that electrical stimulation of SMA neurons elicits responses in M1 with short latencies, of about 4 ms (Aizawa and Tanji 1994; Tokuno and Nambu 2000). Importantly though, other observations challenge the validity of this assumption. Indeed, like M1, SMA has pyramidal cells that project to the spine (Dum and Strick 1996) and, in certain contexts, unique stimulation of SMA with single-pulse TMS can evoke MEPs (Spieser et al. 2013; Entakli et al. 2014), suggesting that these pyramidal cells can also recruit motoneurons. Thus, it is possible that the MEP potentiation reported in ppTMS studies using short intervals reflects the summation of volleys descending from SMA and M1 and converging at close times on motoneurons. In other words, it is currently unclear whether this potentiation can be taken as a pure measure of effective connectivity between SMA and M1 or not. Addressing this issue is fundamental for any investigation targeting motor areas (e.g. the dorsal or the ventral premotor cortex; Civardi et al. 2001; Koch et al. 2006; Davare et al. 2008; Bäumer et al. 2009; Groppa et al. 2011; Vesia et al. 2018, which, for the most part, present corticospinal projections (Dum and Strick 1991). This is the second goal of the current study. Specifically, we tested the effect of SMA conditioning on MEP amplitudes using a very short inter-stimulation interval of 1 ms. The rationale here is that a 1 ms interval would be too short for a MEP potentiation to result from the recruitment of cortico-cortical circuits (Aizawa and Tanji 1994; Tokuno and Nambu 2000); any potentiation occurring at this interval would instead provide evidence for a summation of neural inputs occurring at the spinal level.

As mentioned earlier, mOFC is another major area of the medial frontal cortex (Amodio and Frith 2006). mOFC is not directly connected to M1 (Carmichael and Price 1995), but rather sends cortico-cortical outputs to the lateral prefrontal cortex (Saleem et al. 2014), which in turn, projects extensively into motor and premotor regions. Consequently, mOFC exerts its influence over a variety of cognitive and motor functions, including action planning and execution (Padoa-Schioppa and Conen 2017). Strikingly though, ppTMS has never been used to probe the influence of this area on M1, potentially because of the presumed difficulty of reaching it with magnetic fields. Hence, it is currently unknown whether TMS could be exploited to probe effective connectivity between mOFC and M1 and, if so, what would be the nature of the influence of the recruited circuits on motor activity. In fact, while former investigations on caudal areas of the medial frontal cortex (i.e. SMA and preSMA) generally reported a facilitatory influence on M1, ppTMS studies on more rostral areas of the frontal lobe, such as the dorsolateral PFC, revealed the operation of inhibitory circuits (Wang et al. 2020, but see also Brown et al. (2019a) for an absence of influence of DLPFC stimulation on ipsilateral M1 activity). A third goal of the present study is to test the feasibility of using ppTMS to probe effective connectivity between mOFC and M1 and, in turn, to determine the influence of mOFC stimulation on M1 activity.

Finally, previous studies utilizing dual-site TMS have consistently observed variations in effective connectivity between cortical areas and M1 (Ferbert et al. 1992; Brown et al. 2019b; Wang et al. 2020; Rurak et al. 2021). However, the underlying neurophysiological mechanisms responsible for this inter-individual variability have remained elusive. In fact, single-pulse TMS studies have demonstrated how individual morphometric features of M1, such as cortical thickness, gray matter volume, and microstructural properties of cerebral white matter, can significantly influence cortical excitability (Klöppel et al. 2008; List et al. 2013). Therefore, in the current study, we exploited structural MRI to characterize the relationship between the morphometric features of SMA and mOFC, particularly their cortical volume, and the facilitatory/inhibitory effect of their stimulation on M1 activity.

Overall, the current study addresses four main goals. The first one is to provide insight into the influence of SMA stimulation on M1 activity with long inter-stimulation intervals, which are thought to recruit cortico-subcortico-cortical circuits. As a second objective, we aim to clarify whether the MEP potentiation reported in studies targeting SMA and M1 with short intervals can be taken as a pure measure of cortico-cortical connectivity between these areas or if it could in part reflect the summation of volleys

descending from those on motoneurons. Moreover, we seek to test the feasibility of exploiting ppTMS to probe effective connectivity between mOFC and M1 and, relatedly, to determine the influence of mOFC stimulation on motor activity. As a last goal, we also investigated whether these TMS-based measures of fronto-motor connectivity were associated to the individual volume of SMA and mOFC.

Material and methods

Ethical approval

The protocol was approved by the institutional review board of the Catholic University of Louvain (#2018/22MAI/219) and complied with the latest version of the Declaration of Helsinki, except for registration in a database.

Participants

Twenty healthy subjects participated in the current study (11 females; mean age 26.9 years ±5.4; right-handed as assessed by the Edinburgh Handedness Inventory; Oldfield 1971). Subjects were recruited from the Research Participant Pool at the Institute of Neuroscience of the Catholic University of Louvain (Brussels, Belgium). None of them had any neurological disorder, history of psychiatric illness or drug or alcohol abuse, or presented any contraindication to TMS (Rossi et al. 2009, 2021).

MRI data acquisition

Anatomic sequence was acquired at the Cliniques Universitaires Saint-Luc (UCLouvain, Belgium) using a 3 T head scanner (Signa™ Premier, General Electric Company, USA) equipped with a 48-channel coil.) A three-dimensional (3D) T1-weighted data set encompassing the whole brain was selected to provide detailed anatomy (1 mm^3) thanks to a MPRAGE sequence (inversion time = 900 msec, repetition time (TR) = 2238.92 msec, echo time (TE) = 2.96 msec, flip angle (FA) = 8°, field of view (FOV) = 256∗256 mm^2, matrix size = 256∗256, 170 slices, slice thickness = 1 mm, no gap). Patients, instructed to remain still, were positioned comfortably in the coil and fitted with soft earplugs.

Transcranial magnetic stimulation protocol

Coil locations

Dual-site ppTMS involves applying a test stimulation (TS) with one coil over M1 preceded, in a certain proportion of trials, by a conditioning stimulation (CS) delivered with another coil over an area of interest. Our aim here was to investigate intra-hemispheric influences of SMA and mOFC on M1. To do so, we applied both stimulations over the left, dominant hemisphere, with the TS administered over the left M1 and the CS targeting either the left SMA or the left mOFC in separate blocks of trials. TS and CS were delivered with two small figure-of-eight coils (Magstim D25-Alpha model; wing internal diameter: 35 mm) connected to two monophasic Magstim stimulators (200^2 and $Bistim^2$ stimulators; Magstim, Whitland, Dyfed, UK).

M1 coil

The M1 coil was placed tangentially to the scalp with the handle pointing backward and laterally at a 45° angle away from the midsagittal line, resulting in a postero-anterior current flow within the cortex (Rossini et al. 1994; Rossi et al. 2009; Derosiere et al. 2015, 2020). The equipment resources associated with our neuronavigation system did not allow us to neuronavigate two coils simultaneously. Therefore, to define the optimal location for M1 stimulation (i.e. the so-called "hotspot"), we relied on markers disposed on an electroencephalography (EEG) cap fitted on the participant's head (Zrenner et al. 2018; Derosiere et al. 2019). Of note, due to the anatomical proximity of left M1 and left SMA, in a number of subjects ($n = 12/20$), the position of the M1 coil had to be slightly adjusted when the SMA coil was settled over the scalp; this position was exploited for TS during SMA blocks. Hence, we defined two different positions for M1 stimulation: the real hotspot and an adjusted hotspot.

To find the real hotspot, we first applied the stimulation with the center of the M1 coil over the C3 location of the EEG cap (i.e. corresponding to the left M1 area; Derosiere et al. 2018; Alamia et al. 2019), in the absence of the second coil on the head. Stimulation intensity was increased until consistent MEP responses were obtained in the right first dorsal interosseous (FDI) muscle at this location. We then moved the coil by steps of ∼0.5 cm around this location in both the rostrocaudal and the mediolateral axes. Stimulation was applied with the previously defined intensity at each new location, and MEP amplitudes were visually screened. The real hotspot was defined as the location at which the largest and most consistent MEP amplitudes could be obtained (Rossini et al. 1994; Neige et al. 2018; Derosiere et al. 2019). The coil was then held at this location, and the edges of its shape were marked on tapes disposed on the EEG cap (Vassiliadis et al. 2020; Geers et al. 2021; Neige et al. 2022; Wilhelm et al. 2022). These marks allowed us to localize the real hotspot at any required time during the session. To determine the adjusted hotspot, we first positioned the SMA coil over the head and then reproduced the same procedure as described above, while trying to fit the two coils over the head. Once the largest MEP amplitudes obtained, the two coils were held at their respective locations, and the edges of the M1 coil were marked on the EEG cap. These marks allowed us to localize the adjusted hotspot during SMA blocks.

SMA and mOFC coils

To ensure that the coil exploited for the CS was precisely targeting SMA and mOFC in each subject, the T1-weighted images were used for neuronavigation. Precisely, we determined the SMA and mOFC locations on the individual images using MNI coordinates in a dedicated software (Visor 2.0 Advanced NeuroTechnologies, Enschede, The Netherlands). The locations were finally used during the experiment, in which we relied on head and coil trackers as well as a 3D tracking device to coregister the position of the SMA/mOFC coil with the individual MRI.

The MNI coordinates exploited to initially localize SMA and mOFC were $x = -8$, $y = -9$, $z = 77$ and $x = -7$, $y = 71$, $z = -4$, respectively (Codol et al. 2020). These two locations were then slightly adjusted for each subject using the Visor software, so that they corresponded to the point where the scalp-to-cortex distance was minimal. Following this procedure, the MNI coordinates for SMA and mOFC locations were $x = -7.9 \pm 0.3$, $y = -7.4 \pm 0.8$, $z = 82.9 \pm 1$, and $x = -9.4 \pm 0.7$, $y = 72.6 \pm 0.5$, $z = 8.2 \pm 1.7$, respectively (mean ± standard error (SE) of the group; see Fig. 1 and Table 1 for group-averaged and individual MNI coordinates, respectively).

For both SMA and mOFC stimulation, the center of the coil was placed over the corresponding target location (see Fig. 1). In SMA blocks, the coil was held tangential to the scalp with the handle pointing at a −100° angle away from the midsagittal line (i.e. in the counter-clockwise direction), resulting in a medio-lateral current flow within the cortex (Fig. 1). This coil position was chosen based on a previous experiment showing that it allows the most optimal recruitment of SMA neurons (Arai et al. 2012). In

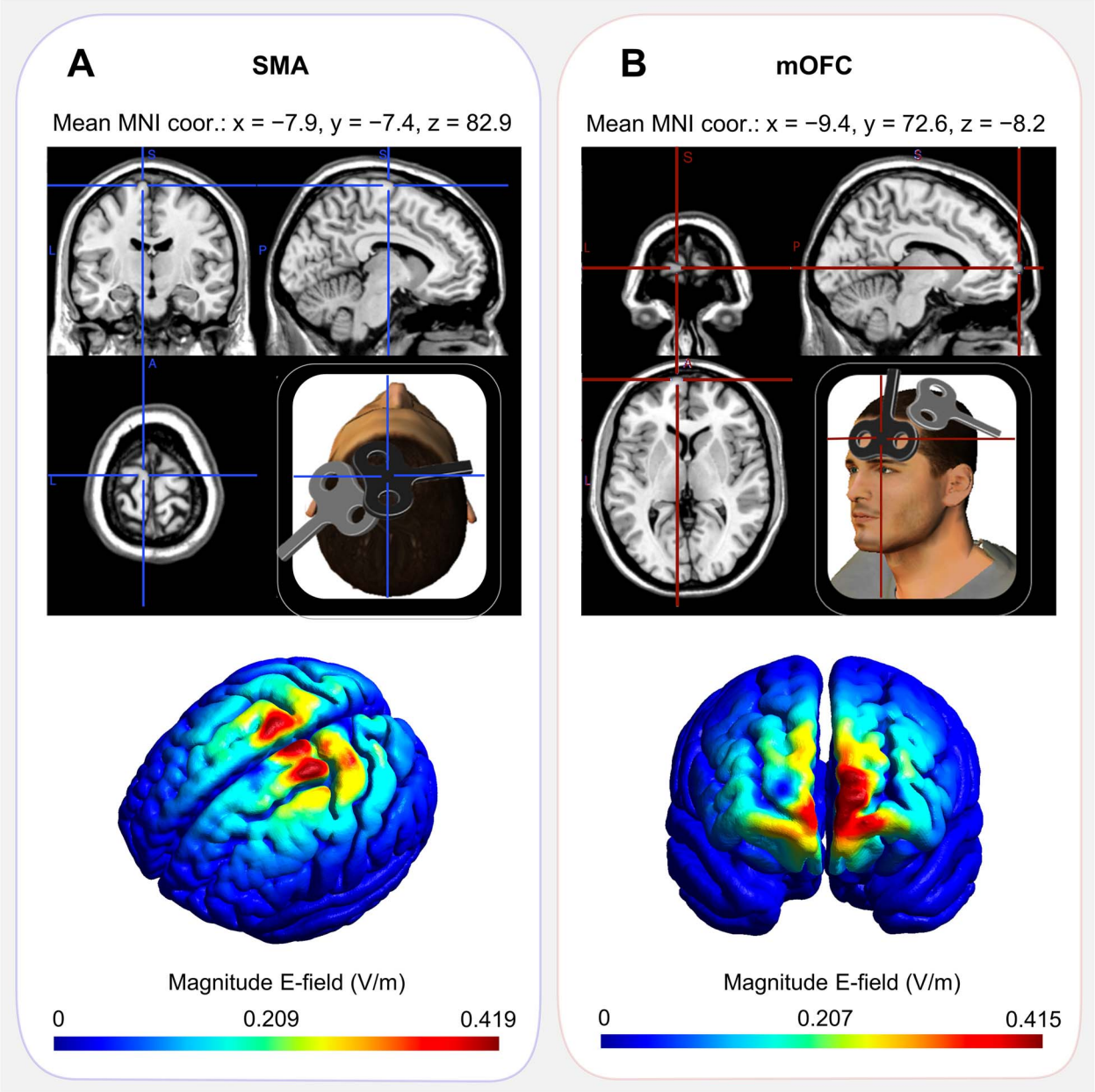

Fig. 1. Localization of the sites of stimulation and illustration of the coil positioning and induces E-fields modeling for SMA (A) and mOFC (B) targets. The group-averaged MNI coordinates are illustrated on a standard MRI template (coronal, sagittal and axial views) using MRIcron software (v1.0.20190902 http://www.mricro.com). Induced E-fields computed with the open-source package SimNIBS v4.0.1 (www.simnibs.org) and simulated on the cortical surface of a MNI 152 template. SMA and mOFC targets were defined using the group-averaged MNI coordinates. Simulations were performed using a model of the Magstim D25-alpha model figure-of-8 coil (Deng et al. 2013), a coil-to-scalp distance and a stimulation intensity set to the default values of 4 mm and dI/dt = 1 a/μs, respectively.

mOFC blocks, the coil was held tangential to the forefront with the handle directed upward and parallel to the midsagittal line (Codol et al. 2020), resulting in a downward current flow at the cortical level.

Importantly, neuronavigation allowed us to place the center of the coil over our predefined regions of interest. Nevertheless, a potential limitation of the present study is that the recruitment of the left SMA and of the left mOFC requires using suprathreshold intensities (i.e. of 120% rMT), which may lead to a partial activation of adjacent cortical areas. In fact, e-field modeling computed with SimNIBS v4.0.1 (www.simnibs.org) revealed that the CS also elicited partial activation of the contralateral (right) SMA as well as partial activation of the contralateral (right) mOFC and of the lower part of the ipsilateral (left) dorsomedial prefrontal cortex (see Fig. 1).

Stimulation intensities

Once the real and the adjusted hotspots were found (as mentioned earlier), we determined the resting motor threshold (rMT) for both locations. The rMT was defined as the lowest stimulation intensity (expressed in percentage of maximal stimulator output (%MSO)) required to evoke MEPs of 50 μV amplitude on 5 out of 10 consecutive trials in the relaxed FDI muscle (Rossini et al. 1994, 2015). The rMTs for the real and the adjusted hotspots were 39.9 ± 1.5% and 44.6 ± 1.9%MSO, respectively. These rMT values were exploited to determine the stimulation intensities to be

Table 1. Individual MNI coordinates for SMA and mOFC target locations. The MNI coordinates exploited to initially localize SMA and mOFC were x = −8, y = −9, z = 77 and x = − 7, y = 71, z = −4, respectively. These two locations were then slightly adjusted for each subject using the visor software, so that they corresponded to the point where the scalp-to-cortex distance was minimal.

Subject	SMA coordinates			mOFC coordinates		
	x	y	z	x	y	z
1	−6	−7.7	84.2	−7.1	72.5	21.7
2	−7.6	−9.4	76.2	−8.1	70.3	−1.7
3	−8	−2.5	80.6	−10.3	73.2	2.6
4	−8	−8.9	77.8	−17.7	70.8	7.5
5	−6.9	−8.8	88.6	−14.5	73.4	25.4
6	−7.9	−7.4	82.3	−9.4	72.6	8.2
7	−11.9	−8.1	81.7	−9	65.3	19
8	−7.8	−1.6	88	−13.3	73	9.7
9	−8.3	−9.9	86.1	−10	76	6.3
10	−7.5	−10	86.2	−10.7	73.7	4
11	−7.5	−6.6	79.7	−9.9	72.5	12.9
12	−7.6	−5.4	86.3	−8.4	69.7	3.4
13	−10.4	2.1	74.3	−8.7	72.8	−1.1
14	−8	−9.8	76.3	−5.8	72.9	2.6
15	−6.7	−10.6	84.9	−8	70.9	3.7
16	−8.3	−10.5	84.9	−7.3	73.5	11.1
17	−7.4	−6	79.9	−6.4	73.9	8.1
18	−7.6	−10.9	85.4	−8.5	76	9.6
19	−7.2	−7.1	85.7	−8.5	73.8	5.8
20	−7.8	−9.6	88.4	−5.9	74.8	5.8
Mean	**−7.9**	**−7.4**	**82.9**	**−9.4**	**72.6**	**8.2**
SE	**0.3**	**0.8**	**1**	**0.7**	**0.5**	**1.7**

used for the rest of the experiment. In SMA blocks, the M1 coil was positioned over the adjusted hotspot. Hence, we based on the rMT obtained at this location to define the stimulation intensity for M1; we stimulated M1 at 120% of this rMT (Derosiere et al. 2017a, 2017b). Importantly, slightly adjusting the M1 coil location should not affect neural recruitment within M1. As such, we stimulated with an adjusted rMT to compensate for the putative increase between the coil and the real hotspot and the potential decrease in electric field with distance. This adjusted rMT can evoke the same motor responses as the real rMT (i.e. MEPs of 50 μV amplitude on 5 out of 10 consecutive trials). In fact, the mean MEP amplitude elicited in single-pulse trials did not differ between the participants in whom we exploited the real and the adjusted hotspot (0.794 ± 0.47 mV vs. 0.716 ± 0.47 mV; $t_{18} = 0.375$, $P = 0.712$).

In mOFC blocks, the M1 coil could be easily positioned over the real hotspot and we thus stimulated at 120% of the rMT obtained for the real hotspot. Finally, CS intensity was set at 120% of the rMT obtained for the real hotspot, both in SMA and in mOFC blocks (Brown et al. 2019a).

Inter-stimulation intervals and blocks

As mentioned in the Introduction section, the goal of the present study was fourfold. First, we aimed to test the influence of SMA stimulation on M1 activity with long inter-stimulation intervals, presumed to recruit cortico-subcortico-cortical circuits. To this aim, we exploited intervals of 12 and 15 ms (Neubert et al. 2010). As a second objective, we sought to clarify whether the MEP potentiation reported in studies targeting SMA and M1 with short intervals (Arai et al. 2011, 2012; Green et al. 2018; Rurak et al. 2021) can be taken as a pure measure of cortico-cortical connectivity between these areas or if it could in part reflect the summation of volleys descending from those on motoneurons. To address this issue, we tested the effect of SMA conditioning on MEP amplitudes using a very short inter-stimulation interval of 1 ms. The rationale here was that a 1 ms interval would be too short for a MEP potentiation to result from the recruitment of cortico-cortical circuits (Aizawa and Tanji 1994; Tokuno and Nambu 2000); any potentiation occurring with this interval would instead provide evidence for a summation of neural inputs occurring at the spinal level. We also included other short inter-stimulation intervals of 4, 6, and 8 ms in the experiment to be able to compare the effect obtained on MEP amplitudes when using the 1 ms interval vs. when exploiting more classical intervals. Finally, we aimed to test the feasibility of exploiting ppTMS to probe effective connectivity between mOFC and M1 and, relatedly, to determine the influence of mOFC stimulation on motor activity. Given the current lack of data regarding the latter issue, we exploited all of the intervals mentioned above in mOFC blocks too. Note that, contrary to SMA, mOFC does not present corticospinal projections. Hence, the use of a 1 ms interval in mOFC blocks allowed us to verify that any effect of SMA stimulation on MEPs using this interval was specific to SMA. Altogether, the experiment involved six inter-stimulation intervals, both in SMA and in mOFC blocks: 1, 4, 6, 8, 12, and 15 ms.

The experiment was divided into 10 blocks of 42 trials (i.e. 5 SMA blocks and 5 mOFC blocks). Each block comprised trials with single-pulse (i.e. TS only) and paired-pulse TMS (i.e. CS + TS with the six intervals mentioned above), occurring in a randomized order. As such, within each block, a total of six trials was recorded for each of the seven conditions (i.e. single-pulse, and paired-pulse with 1, 4, 6, 8, 12, and 15 ms), leading to 30 trials per condition over the whole experiment. These high numbers are quite unusual for TMS studies (i.e. which typically involve 10 to 20 MEPs per condition; e.g. see Arai et al. 2012; Beaulieu et al. 2017; Neige et al. 2017; Derosiere et al. 2019). Having a large number of MEPs reduces within-subject variability and may help increase the reliability of the findings (Chang et al. 2016; Beaulieu et al. 2017). Finally, to prevent subjects from anticipating the stimulations, we varied

the inter-trial interval, which ranged between 3.6 and 4.4 s (i.e. rectangular distribution centered over 4 s; Rothwell et al. 1999).

Electromyographic recordings

Electromyography was used to record MEPs in the right FDI muscle. To do so, pairs of surface electrodes (Ag/AgCl, Medicotest, USA) were disposed on the FDI in a belly-tendon montage. A ground electrode was placed on the styloid process of the right ulna. EMG signals were recorded for 800 ms on each trial, starting and ending 400 ms before and after the TS, respectively. The signals were amplified with a gain of 1000, and band-pass and notch-filtered (10–500 Hz and 50 Hz, respectively) online using a dedicated amplifier (Digitimer D360; Digitimer Ltd, Welwyn Garden City, UK). Signals were digitized at a sampling rate of 2000 Hz (CED Power 1401; CED Ltd, Cambridge, UK) and collected using the Signal software (version 6.04; CED Ltd) for further offline analyses.

Data analyses

EMG data

EMG data were analyzed with custom Signal and R scripts (R Core Team, 2020). Of note, to prevent contamination of the MEP measurements from background muscular activity, participants were reminded to relax during the whole experiment based on the EMG signals, which were continuously screened by the experimenters. In addition, trials in which the root mean square of the EMG signal exceeded 2.5 SD above the mean before stimulation (i.e. −250 to −50 ms from the pulse) were discarded from the analyses. Besides, to attenuate any effect of MEP variability on our measures, MEPs with an amplitude exceeding 2.5 SD around the mean within a given condition were excluded too. Following this cleaning procedure, we had 84.9 ± 7.55% and 86.63 ± 5.53% trials left on average for SMA and mOFC blocks, respectively.

We then extracted the peak-to-peak MEP amplitude for each subject, each condition and each single trial. Trials were subsequently pooled together, by computing the median amplitude for each subject and each condition. As such, we chose to use the median rather than the mean to reduce the effect of outlying single-trial data within each subject, in the same line as former studies (Fitzgibbon et al. 2014; Nieminen et al. 2019). The nature of the influence of the SMA/the mOFC over M1 (i.e. facilitatory vs. inhibitory) was quantified by computing a ratio expressing MEPs elicited by the TS in paired-pulse trials relative to MEPs elicited in the TS in single-pulse trials (Lafleur et al. 2016; Derosiere et al. 2020; Koch 2020; Neige et al. 2021). Following this procedure, one MEP ratio was obtained for each subject and each inter-stimulation interval. MEP ratios above 1 were taken as a marker of a facilitatory influence of the conditioned area on M1, whereas ratios below 1 were considered as reflecting an inhibitory effect on M1.

MRI data

Individual MRI volumetric data were used to investigate whether the facilitatory/inhibitory effect of the SMA/mOFC over M1, demonstrated by dual-site ppTMS at specific, significant intervals, could be related to the cortical volume of these two areas.

The segmentation and parcellation of brain cortical and subcortical regions were performed using FreeSurfer (version 7.2.; http://surfer.nmr.harvard.edu). Following the completion of the pipeline, each segmentation was visually inspected and corrected if necessary. Minor errors in the cranial stripes were identified and rectified before re-running the data through the pipeline. The regions of interest (ROIs) were defined based on the Brainnetome atlas (https://atlas.brainnetome.org/) (Fan et al. 2016a), which provides a fine-grained, connectivity-based parcellation of the human brain into 246 regions. Specifically, we focused on the left SMA (left medial area 6 (A6m; ID #9) region from the Brainnetome Atlas) and the left mOFC (left medial area 14 (A14m; ID #41) and left medial area 11 (A11m; ID #47)) regions which were projected onto the Freesurfer's parcellation of each individual's brain. The occipital polar cortex (OPC; ID #204) was also selected as a control area. Then, the automated pipeline enabled the generation of cerebral volume measures (in mm^3). The volume obtained for each ROI was normalized to the total intracranial volume of the subject to account for individual brain size.

Statistical analyses

All statistical analyses were performed using the JASP software (JASP Team, version 0.14.1.0; https://jasp-stats.org/).

First, we aimed to identify whether the influence of the CS on MEP amplitudes varied as a function of the inter-stimulation interval (e.g. whether the effect for an interval of 1 vs. 4, 6 and 8 ms differed for SMA blocks; see section *Inter-stimulation intervals and blocks*, described earlier). Data were normally distributed, as evidenced by non-significant results of the Shapiro–Wilk tests. When running repeated-measures (rm) ANOVA, Mauchly's tests were systematically exploited to check for data sphericity and a Greenhouse-Geiser correction was applied if the sphericity assumption was violated. To address this point, MEP ratios obtained for SMA and mOFC blocks were analyzed using two separate one-way rm ANOVAs with INTERVAL (1, 4, 6, 8, 12, and 15 ms) as a within-subject factor. Pre-planned post-hoc analyses were performed on significant interactions after applying a Bonferroni correction for multiple comparisons. Effect sizes were estimated for the main effect of INTERVAL, by calculating partial eta squared (η^2_p). In accordance with conventional interpretation partial η^2_p, a value of 0.01 is interpreted as indicating a small effect size, a value of 0.06 a medium effect size and a value of 0.14 or more as a large effect size (Lakens, 2013). Second, we sought to determine whether the CS had a significant facilitatory or inhibitory effect on MEP amplitude. To this aim, MEP ratios were compared against a constant value of 1 (i.e. reflecting the amplitude obtained in TS only trials) using one-sample t-tests. We chose to compare MEP ratios against a constant value of 1 rather than comparing raw MEP amplitudes between different conditions, as raw MEP amplitudes vary widely between individuals, in an idiosyncratic way, and are thus rarely used in TMS studies. Hence, we exploited the same procedure as in other studies (Arai et al. 2012; Quoilin et al. 2019; Neige et al. 2020; Wang et al. 2020), by first normalizing MEP amplitudes within each individual as ratios to cancel out the within-subject variability and then comparing the ratios against a constant value of 1.

To complement the frequentist statistics, we conducted a Bayes factor (BF) analysis, allowing us to quantify statistically the level of evidence for the presence of an effect on MEP ratios. These analyses were performed using the JASP default parameters (i.e. Cauchy prior width of 0.707; van Doorn et al. 2021). BFs (expressed as BF_{10}) provided us with a ratio of the likelihood probability of the alternative hypothesis (i.e. H_1: the probability that data exhibit the effect; Morey and Rouder 2011) over the null hypothesis (i.e. H_0: the probability that data do not exhibit an effect of the tested factor). A BF_{10} of 1 reflect an equal probability that H_1 and H_0 are correct, whereas a value higher than 1 would reflect a higher probability that H_1 is correct. In accordance with conventional interpretation of BF values (Jeffreys 1961), a BF_{10} value ranging between 1 and 3 is interpreted as indicating anecdotal evidence in favor of H_1, a value between 3 and 10 as indicating substantial

evidence for H_1, a value between 10 and 30 a strong evidence for H_1, a value between 30 and 100 a very strong evidence for H_1, and a value above 100 a decisive evidence for H_1. Conversely, a BF_{10} value between 0.1–0.33 and 0.33–1 indicates substantial and anecdotal evidence for the null hypothesis, respectively.

Lastly, we aimed to determine whether the individual cortical volumes of the SMA and the mOFC could account for their observed facilitatory/inhibitory influence on MEP amplitudes. To assess these non-normally distributed data, Spearman's correlations (nonparametric) were performed between the median MEP ratios for significant facilitatory/inhibitory intervals and the respective normalized cortical volume values of the SMA and the mOFC (see Supplementary material for more details). Additionally, to evaluate the specificity of these findings, Spearman's correlations were also performed between the same MEP ratios and the normalized cortical volume of a control occipital polar area, as well as the normalized total gray matter volume.

Results

SMA stimulation induced facilitatory and inhibitory effects on MEP amplitudes depending on the inter-stimulation interval

Figures 2A and B illustrate the MEP ratios obtained as a function of the inter-stimulation interval for SMA blocks. Interestingly, the rmANOVA revealed a main effect of the factor INTERVAL on MEP ratios (GG-corrected $F_{(2.41,45.87)} = 10.75$, $P < 0.0001$). The n^2_p for this effect was 0.361, denoting a large effect size. Further, the BF_{10} was 560,807, indicative of a "decisive" evidence in favor of H_1 (i.e. H_1: presence of an effect of INTERVAL) over H_0 (i.e. H_0: lack of effect of INTERVAL). Post-hoc analyses indeed showed that MEP ratios strongly varied as a function of the inter-stimulation interval: ratios for 6 ms ($P < 0.001$; $BF_{10} = 114.17$), 8 ms ($P < 0.001$; $BF_{10} = 87.44$), 12 ms ($P < 0.001$; $BF_{10} = 35.29$) and 15 ms ($P < 0.001$; $BF_{10} = 119.87$) were significantly higher than at 1 ms. Moreover, ratios for intervals of 8 ms ($P < 0.001$; $BF_{10} = 12.67$) and 12 ms ($P = 0.006$; $BF_{10} = 8.46$) were significantly higher than for an interval of 4 ms. Finally, the ratio at 12 ms was not significantly different from ratios obtained at 6 ms ($P > 0.999$; $BF_{10} = 0.603$) and 8 ms ($P > 0.999$; $BF_{10} = 0.261$) intervals, nor was the ratio at 15 ms when compared to 6 ms and 8 ms intervals ($P > 0.999$, $BF_{10} = 0.246$ and $P > 0.999$, $BF_{10} = 1.235$, respectively).

As mentioned above, to directly test whether the CS had a significant facilitatory or inhibitory effect on MEP amplitudes, MEP ratios were compared to a constant value of 1 (i.e. representing the mean amplitude of test MEPs) using one-sample t-tests. Interestingly, this analysis confirmed the presence of a significant facilitatory influence of SMA stimulation on MEP amplitudes (i.e. MEP ratios > 1) for classical, short intervals of 6 ms ($t_{19} = 2.161$, $P = 0.044$; $BF_{10} = 1.55$) and 8 ms ($t_{19} = 2.766$, $P = 0.012$; $BF_{10} = 4.32$), in accordance with the literature. Most importantly, a similar facilitatory effect was found for 12 ms ($t_{19} = 2.435$, $P = 0.025$; $BF_{10} = 2.42$). Of note, these effects did not reach statistical significance anymore if corrected for multiple testing by a conservative Bonferroni correction (i.e. P-value threshold = 0.05/6 = 0.008). However, as highlighted above, BF analyses revealed a moderate to strong evidence for the presence of a facilitatory effect at these three inter-stimulation intervals (average $BF_{10} = 2.76 \pm 0.81$), indicating that the data were 2.76 times more likely to show facilitation than no difference from the constant value of 1. As such, 75%, 70%, and again 75% of subjects had a MEP ratio >1 at 6, 8, and 12 ms, respectively, indicating that SMA stimulation potentiated MEP amplitudes for most of the subjects at these intervals (see Fig. 2C). Finally, another interesting finding revealed by the t-tests was the presence of a significant inhibitory influence of SMA stimulation on MEP amplitudes for the 1 ms interval ($t_{19} = 4.034$, $P < 0.001$; $BF_{10} = 49.39$). In fact, 85% of subjects presented a MEP ratio below 1 when using the 1 ms interval, indicating that SMA stimulation had an inhibitory effect for a very large proportion of subjects. Other t-tests failed to achieve the level of statistical significance (p-values were of 0.081 and 0.070 for 4 and 15 ms).

Medial orbitofrontal cortex stimulation induced a moderate, interval-dependent inhibitory effect on motor evoked potential amplitudes

Figures 3A and B illustrate the MEP ratios obtained as a function of the inter-stimulation interval for mOFC blocks. The rmANOVA performed on MEP ratio did not indicate a significant main effect of INTERVAL ($F_{(1,19)} = 1.148$, $P = 0.340$, $n^2_p = 0.057$; $BF_{10} = 0.14$).

Despite this lack of a main effect of INTERVAL, we tested whether the CS had a significant facilitatory or inhibitory effect on MEP amplitudes at specific intervals (i.e. by comparing ratios to a constant value of 1 with t-tests). Interestingly, this analysis revealed a significant inhibitory influence of mOFC stimulation on MEP amplitudes (i.e. MEP ratios < 1) for short intervals of 6 ms ($t_{19} = -2.425$, $P = 0.025$; $BF_{10} = 2.38$) and 8 ms ($t_{19} = -2.201$, $P = 0.040$; $BF_{10} = 1.65$) as well as for a longer interval of 15 ms ($t_{19} = -2.370$; $P = 0.029$, $BF_{10} = 2.17$). Although the BFs for these effects provided anecdotal evidence in favor of H_1, a total of 75%, 70%, and 60% of subjects presented a MEP ratio greater than 1 at intervals of 6, 8 and 15 ms, respectively. This indicates that mOFC stimulation decreased MEP amplitudes for a large proportion of subjects at these intervals (Fig. 3C). Interestingly, other t-tests did not reach the level of statistical significance (all p-values ranged from 0.077 to 0.595), including for the ratio obtained with the 1 ms interval ($t_{19} = -0.541$, $P = 0.595$; $BF_{10} = 0.265$). The latter observation suggests that the inhibitory effect observed with this interval was specific to SMA conditioning.

The facilitatory influence of supplementary motor area stimulation on the primary motor cortex depends on the volume of the supplementary motor area

Our aim here was to investigate whether the impact of SMA and mOFC stimulation on MEP amplitudes at significant short and long ISIs, as illustrated in Fig. 2A and 3A, could be attributed to individual variations in the volume of these areas. In an effort to reduce the number of correlations performed and mitigate the multiple comparison problem, we combined the MEP ratios obtained at short ISIs of 6 and 8 ms, treating them as an overall proxy of cortico-cortical circuit recruitment. Hence, we present two correlations below for both SMA and mOFC stimulation. One correlation predominantly reflects the recruitment of cortico-cortical circuits (resulting from the pooling of short ISIs of 6 and 8 ms), while the other emphasizes the recruitment of cortico-subcortical–cortical circuits (ISIs of 12 and 15 ms, for SMA and mOFC, respectively). However, for a more comprehensive examination of the data, we also present separate correlations for ISIs of 6 and 8 ms, as well as for an ISI of 1 ms, in the Supplementary Materials section.

Spearman's rank-order analyses revealed significant positive correlations between SMA cortical volume and the facilitatory effect of SMA stimulation over M1 activity at both short (median MEP ratios at 6 and 8 ms averaged together; Spearman's Rho = 0.521, $P = 0.024$) and long intervals (median MEP ratio at 12 ms; Spearman's Rho = 0.732, $P < 0.001$; Bonferroni-corrected p-value threshold = 0.05/2 = 0.025; Fig. 4A). Importantly, these correlations between MEP ratios and cortical volume were specific

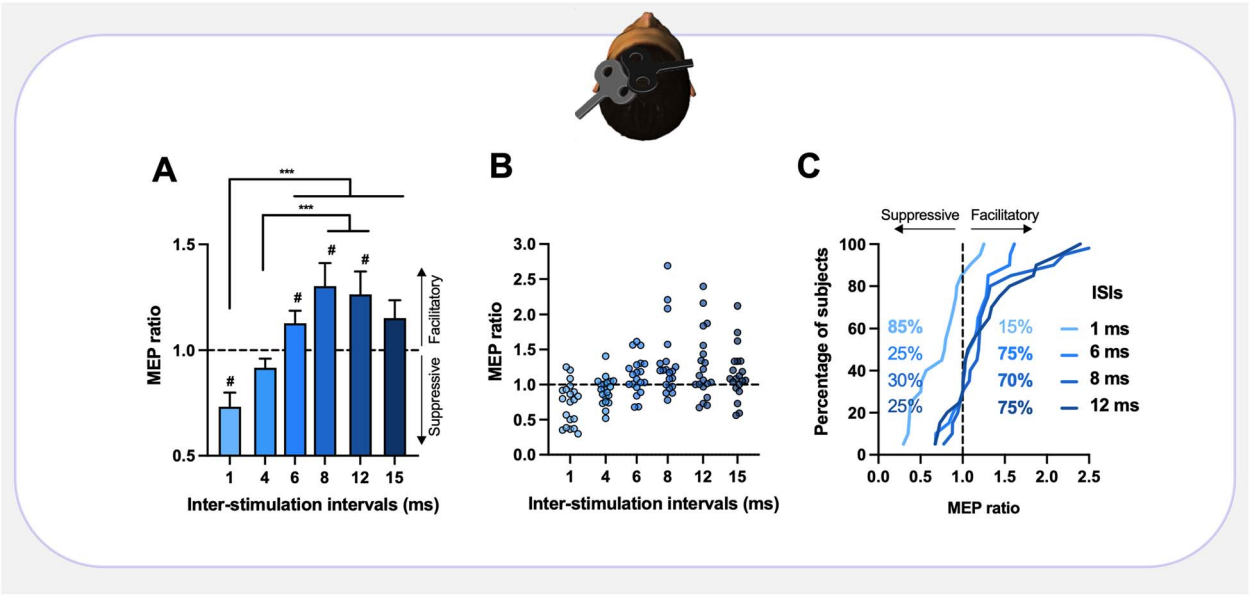

Fig. 2. SMA stimulation induced a mix of facilitatory and inhibitory effects on MEP amplitudes depending on the inter-stimulation interval. A) Group-averaged MEP ratios. Ratios above 1 indicate a facilitatory influence of SMA on M1, whereas ratios below 1 reflect an inhibitory effect on M1. *** indicates significant differences between inter-stimulation intervals at $P < 0.001$). # indicates a significant difference of the ratio with respect to 1. Error bars represent 1 SEM. B) Individual MEP ratios for each inter-stimulation intervals. C) Cumulative percentage of subjects for inter-stimulation intervals at which MEP ratios were significantly different than 1. 75%, 70%, and 75% of subjects presented a ratio above 1 at 6, 8, and 12 ms, respectively, while 85% of subjects presented a ratio below 1 when using the 1 ms interval.

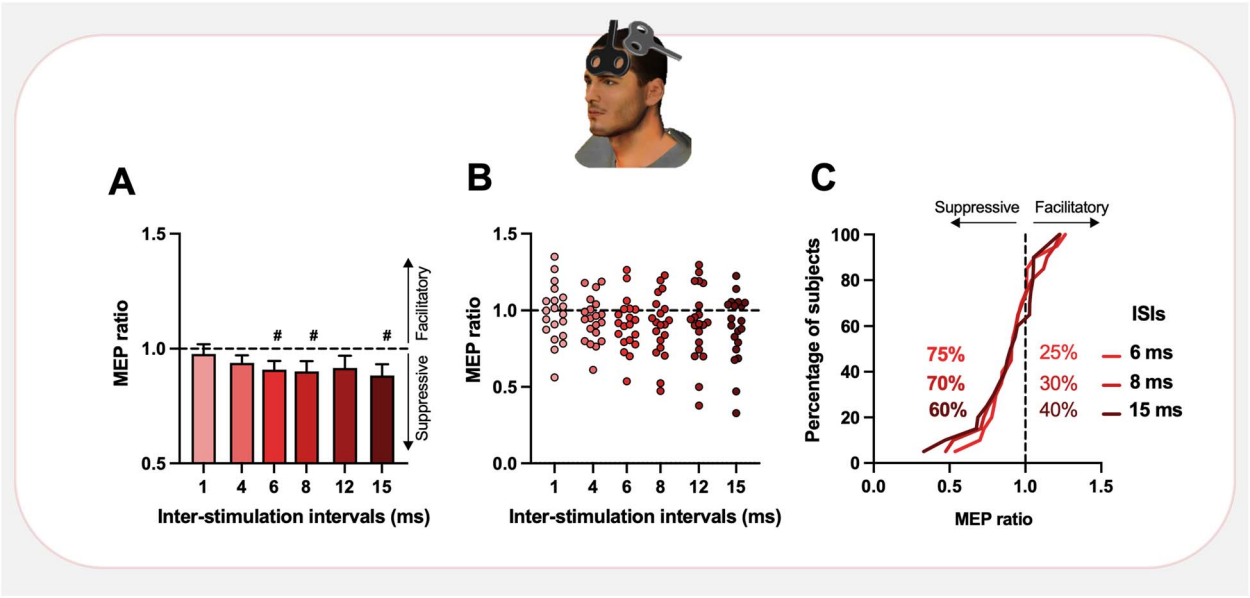

Fig. 3. mOFC stimulation induced a moderate inhibitory effect on MEP amplitudes at some inter-stimulation intervals. A) Group-averaged MEP ratios. Ratios below 1 reflect an inhibitory effect on M1. # indicates a significant difference of the ratio with respect to 1. Error bars represent 1 SEM. B) Individual MEP ratios for each inter-stimulation intervals. C) Cumulative percentage of subjects for inter-stimulation intervals at which MEP ratios were significantly different than 1. 75%, 70%, and 60% of subjects presented a ratio below 1 at 6, 8, and 15 ms, respectively.

to the SMA area, as no significant correlations were observed between MEP ratios and our control area's volume—i.e. the occipital polar cortex (short intervals: Spearman's Rho = 0.358; $P = 0.133$; long interval: Spearman's Rho = 0.112; $P = 0.646$), or total gray matter volume (short intervals: Spearman's Rho = 0.218; $P = 0.369$; long interval: Spearman's Rho = 0.086; $P = 0.726$). Hence, the facilitatory effect of SMA stimulation on M1 activity seems to depend specifically on SMA cortical volume, independently of the interval considered.

Spearman's rank-order analyses showed that mOFC cortical volume was not significantly correlated with the inhibitory effect of mOFC stimulation over M1 activity, neither at short (Spearman's Rho = −0.374; $P = 0.116$) nor at long intervals (Spearman's Rho = = −0.102; $P = 0.678$; Fig. 4A). Hence, our data reveal that, contrary to the effect of SMA stimulation, the net impact of mOFC stimulation on M1 activity does not significantly depend on mOFC volume.

Discussion

Over the past two decades, dual-site ppTMS has been widely used in humans, with studies probing the causal influence of multiple

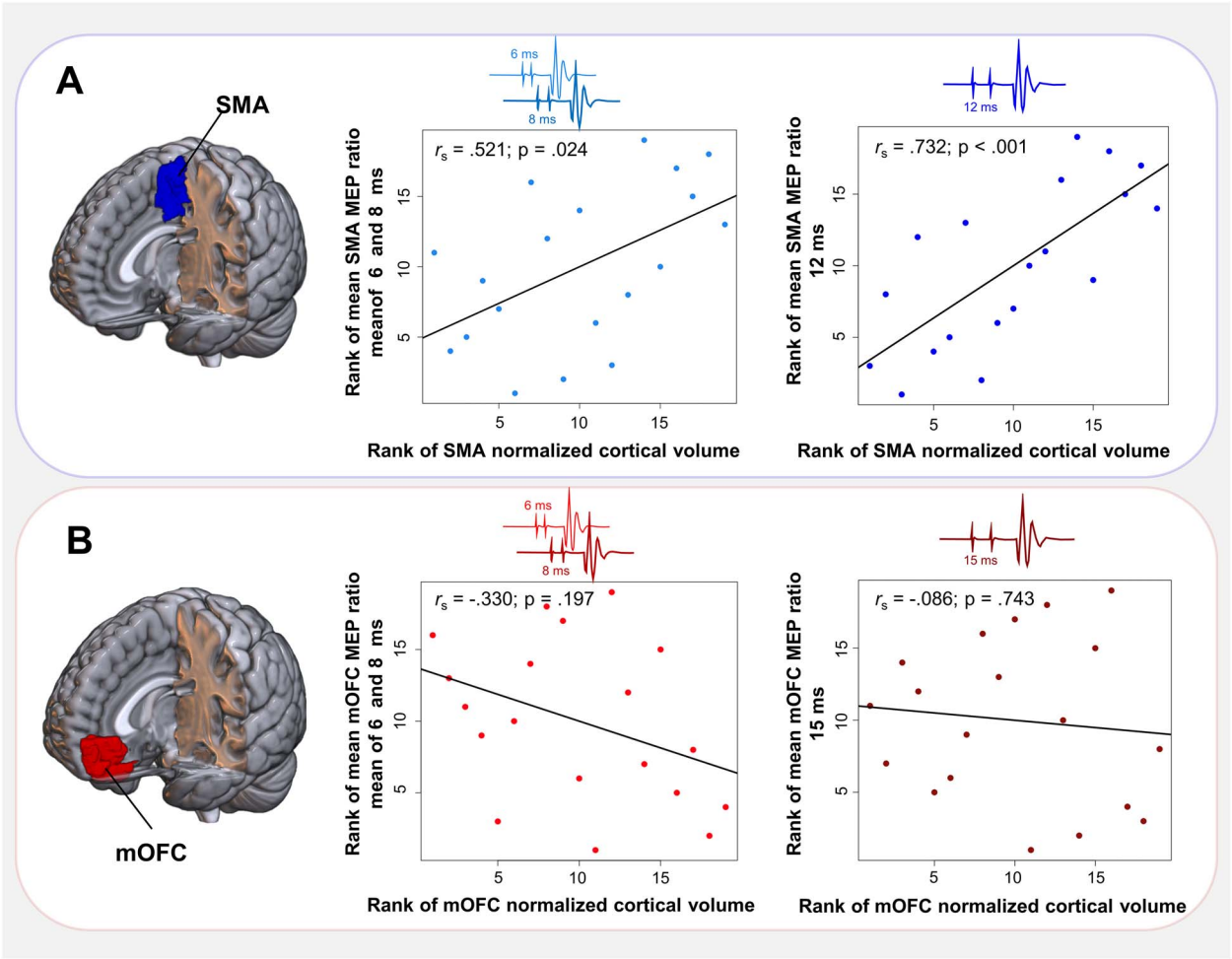

Fig. 4. The cortical volume of the SMA (but not the mOFC) contributes to the facilitatory influence on MEP amplitude observed in the dual-site ppTMS data. A) 3D representation of the SMA, area 9 of the Brainnetome atlas. Significant Spearman's correlation between the MEP ratios of the SMA for significant short facilitatory intervals (median MEP ratios at 6 and 8 ms averaged together) and long facilitatory interval (median MEP ratio at 12 ms) and the SMA normalized cortical volume. B) 3D representation of the mOFC, encompassing areas 41 and 47 of the Brainnetome atlas. Non-significant Spearman's correlation between MEP ratios of the mOFC for significant short inhibitory intervals (mean MEP ratios at 6 and 8 ms) and long inhibitory interval (MEP ratio at 15 ms) and normalized cortical volume of the mOFC. The 3D images were generated using MRIcroGL software (v1.0.20220720). r_s: Spearman's rho.

frontal and parietal areas on M1. However, several important questions currently remain unanswered, notably regarding the application of this approach to key areas of the medial frontal cortex—including SMA and mOFC. The present study directly addressed three of these issues. First, we aimed to provide insight into the influence of SMA stimulation on M1 activity with long inter-stimulation intervals (12 to 15 ms), which are thought to recruit cortico-subcortico-cortical circuits (Neubert et al. 2010). Our data reveal that SMA stimulation significantly potentiates MEP amplitudes with a 12 ms interval, indicating the recruitment of circuits that bear a facilitatory influence on M1. Second, we sought to clarify whether the MEP potentiation reported in studies targeting SMA and M1 with short intervals (6 to 8 ms) can be taken as a pure measure of cortico-cortical connectivity between these areas or whether it might in part reflect the summation of descending volleys on motoneurons at the spinal level. Here, we were able to replicate the MEP potentiation previously observed for such intervals. More importantly, our data show that this facilitation does not occur when using a very short interval of 1 ms, assumed to recruit spinal circuits. Rather, we found an inhibitory influence of SMA stimulation on MEP amplitudes at this interval. Finally, we tested the feasibility of exploiting ppTMS to probe effective connectivity between mOFC and M1 and, relatedly, we determined the influence of mOFC stimulation on motor activity. We found that mOFC stimulation induced a moderate inhibitory effect on MEP amplitudes with both short and long inter-stimulation intervals. Interestingly, mOFC stimulation did not alter MEP amplitudes with the 1 ms interval, suggesting that the inhibitory effect observed with this interval was specific to SMA conditioning.

As mentioned above, SMA stimulation induced a significant potentiation of MEP amplitudes with the 12 ms interval. This finding is not trivial because SMA projects to M1 through multiple cortico-subcortico-cortical circuits (Nachev et al. 2008; Accolla et al. 2016; Oswal et al. 2021), some of which exert a net facilitatory influence on motor activity (e.g. the direct pathway of the basal ganglia) and others of which play an inhibitory role (e.g. the indirect and hyperdirect pathways). One possibility is that SMA stimulation preferentially recruits the direct pathway of the basal ganglia. In this pathway, areas of the frontal cortex (including SMA) rely on their projections to the striatum to inhibit the internal segment of the globus pallidus, which in turn suppresses

neural activity in the subthalamic nucleus (Alexander and Crutcher 1990; Aron et al. 2007; Calabresi et al. 2014; Niranjan et al. 2018). Since the latter structure exerts an inhibitory influence on the motor system (Frank 2006; Aron et al. 2016; Quartarone et al. 2020), the recruitment of this whole circuit ultimately leads to a disinhibition of M1, putatively explaining the MEP potentiation observed at 12 ms interval. Interestingly, the MEP potentiation did not reach statistical significance when a 15 ms interval was used, suggesting that the facilitatory effect uncovered here is interval-dependent, with an optimal temporal window of about 12 ms. The latter observation is relevant for future ppTMS studies aimed at investigating these circuits.

An alternative interpretation would be that the observed MEP potentiation at an ISI of 12 ms results from the disinhibition of the left M1 by the contralateral right SMA, via transcallosal interactions. We employed neuronavigation to target the SMA on the same side as the stimulated M1 with the CS, situated in the right hemisphere. Nevertheless, our e-field modeling analysis revealed that the CS also elicited partial activation in the contralateral (right) SMA, as depicted in Fig. 1. Moreover, prior studies utilizing dual-site ppTMS have documented the presence of interhemispheric interactions between the left and right M1 at ISIs of 12 ms (Ni et al. 2009), as well as long-latency interactions between SMA and M1 at an ISI of 40 ms (Fiori et al. 2016, 2017). However, in those investigations, the use of such intervals typically resulted in a reduction in MEP amplitude rather than MEP potentiation, which indicates the involvement of interhemispheric inhibition, suggesting that the latter interpretation is less plausible than that of the recruitment of a disinhibition from the direct pathway mentioned above. Still, further research is warranted to elucidate the precise neural circuits recruited with long intervals.

Of note, we were able to replicate the MEP potentiation previously observed for intervals of 6 and 8 ms (Arai et al. 2011, 2012; Green et al. 2018; Rurak et al. 2021). More importantly, our data show that this facilitatory effect does not arise when using a very short interval of 1 ms, which is thought to recruit spinal circuits. The hypothesis of a modulation occurring at the spinal level rather than at the cortico-cortical or corticospinal levels during this very short inter-simulation interval was based on studies employing electrical stimulation in monkeys, which suggest that the cortico-cortical circuits connecting SMA and M1 exhibit latencies longer than 1 ms (Aizawa and Tanji 1994; Tokuno and Nambu 2000). Therefore, it is reasonable to assume that the MEP potentiation reported in ppTMS studies using intervals of 6 to 8 ms does not result from the summation of excitatory volleys descending from SMA to motoneurons. In fact, we found an inhibitory influence of SMA stimulation on MEP amplitudes when using the 1 ms interval, which was present in up to 85% of the subjects. Although this effect was quite unexpected, a closer look at the literature reveals that a similar reduction in MEP amplitudes could also be observed when the primary somatosensory cortex ipsilateral to the target M1 was stimulating with a 1 ms interval (Brown et al. 2019b). Interestingly, the primary somatosensory cortex also has pyramidal cells that project to the spine. Still, this does not explain the reduction of MEP amplitudes. One possible explanation is that corticospinal cells do not exclusively synapse with motoneurons through direct, excitatory connections, which in fact represent only a minority of cortico-motoneuronal connections (Lemon 2008). Rather, a large proportion of cells connect to motoneurons through complex inhibitory circuits. The SMA, for instance, innervates a specific set of spinal interneurons (Cheney et al. 2004). One possibility is that SMA stimulation led to the recruitment of such inhibitory interneurons, which reduced motoneurons excitability and ultimately reduced the amplitude of MEPs elicited by M1 stimulation. As highlighted above, our data show that the stimulation of mOFC did not reduce MEP amplitudes when we exploited the 1 ms interval. Interestingly, mOFC does not contain corticospinal cells, suggesting that the presence of such cells is a prerequisite for this inhibitory effect to emerge at 1 ms. Similarly, we showed in two previous studies that conditioning the contralateral M1—which has pyramidal cells that mostly project to the other side of the spine—with a 1 ms interval does not alter MEP amplitudes neither (Grandjean et al. 2018; Vassiliadis et al. 2018). Taken together, these findings indicate that the inhibitory effect observed with a 1 ms interval occurs specifically when stimulating areas that present pyramidal cells *and* project to the same side of the spine as the targeted M1. Further experiments are needed to disentangle the respective contributions of the putative cortico-cortical and spinal circuits to the facilitatory/inhibitory influence of SMA stimulation on M1 activity.

Another central aim of the current study was to test the feasibility of using dual-site ppTMS to probe effective connectivity between mOFC and M1, and to determine the influence of mOFC stimulation on motor activity. In fact, while former investigations on caudal areas of the medial frontal cortex (i.e. SMA and preSMA) generally reported a facilitatory influence on M1, ppTMS studies on more rostral areas of the frontal lobe, such as the dorsolateral PFC, revealed the operation of inhibitory circuits (Wang et al. 2020). The latter inhibitory effect (Wang et al. 2020), however, seemed to be more variable between individuals or even groups of individuals as other studies using very similar stimulation parameters did not replicate it in healthy humans (Brown et al. 2019a). In a similar line, we found that mOFC stimulation moderately reduced MEP amplitudes at specific short and long inter-stimulation intervals, with a high inter-individual variability that likely plays a role in the observed moderate effect. Hence, while the dorsolateral PFC and mOFC differ in their connections with premotor and motor regions and have been shown to exert an influence on motor functions (Padoa-Schioppa and Conen 2017; Neige et al. 2021; Xia et al. 2022), both appear to bear an inhibitory influence on M1 with a high inter-individual variability. In fact, mOFC is not directly connected to M1 (Carmichael and Price 1995), but rather sends cortico-cortical outputs to the lateral prefrontal cortex (Saleem et al. 2014), and the inhibitory effect of mOFC on M1 emerging in our data may rely on the recruitment of the dorsolateral PFC as an intermediate structure. Altogether, our finding suggests the existence of two of frontal lobe systems with opposite influences on the motor output pathway at rest, with a more caudal system—i.e. including SMA and preSMA—bearing a facilitatory influence preferentially, and a more rostral one—i.e. including mOFC and the DLPFC—exerting an inhibitory influence, though with more inter-individual variability. The opposing influence of these systems on M1 may allow them to implement distinct functional roles in motor behavior.

Previous studies utilizing dual-site TMS have consistently observed variations in effective connectivity between cortical areas and M1, as measured by intra-sample variations in MEP ratios (Ferbert et al. 1992; Rurak et al. 2021). However, the underlying neurophysiological mechanisms responsible for this inter-individual variability have remained elusive. Our structural MRI analyses offer a compelling explanation for this crucial issue, as they reveal that individuals with larger SMA volumes demonstrate a stronger facilitatory influence of SMA stimulation on M1 activity. Existing evidence also supports a link between M1 cortical volume and inter-individual differences in M1 excitability,

measured through intra-sample variations in MEP amplitudes (Rosso et al. 2017; Dayan et al. 2018). These findings collectively suggest that greater cortical tissue volume may be associated with an increased number of cells projecting to other neural structures. Consequently, when employing a dual-site ppTMS approach to stimulate the SMA, a broader population of cortico-cortical cells projecting to M1 may be recruited. In the same line, stimulating M1 with a single-pulse TMS approach may activate a wider population of corticospinal neurons projecting to motoneurons. Therefore, our results suggest the presence of a common source of inter-individual variability in connectivity across different circuits of the nervous system.

However, cortical volume alone does not account for all aspects of this inter-individual variability. Notably, we did not find any association between mOFC cortical volume and its inhibitory influence on M1 activity. In fact, it is likely that the inhibitory influence of mOFC stimulation on M1 occurs indirectly through polysynaptic connections (Derosiere and Duque 2020), thereby reducing the contribution of mOFC cortical volume to inter-individual differences in effective connectivity. Future studies should explore the potential contribution of other anatomical features to inter-individual variability in effective connectivity (Neubert et al. 2010). Moreover, a replication of significant findings is required to confidently draw conclusions about the effects of mOFC stimulation on M1 activity.

While the current findings offer promising prospects for future ppTMS studies, it is essential to address some methodological considerations. The inhibitory effects of mOFC stimulation on MEP amplitudes (i.e. with 6, 8, and 15 ms intervals) exhibited substantial inter-individual variability, resulting in marginal group-effects level. A potential factor contributing to this inter-individual variability is the presumed difficulty in reaching the mOFC with the magnetic field (Dancy et al. 2023). One way to enhance the recruitment of mOFC neurons would be to adjust the intensity of the conditioning stimulation according to the individual scalp-to-cortex distance (Stokes 2005; Stokes et al. 2007, 2013) as previously done in repetitive TMS studies (Hanlon et al. 2017; Kearney-Ramos et al. 2018). Such an adjustment could result as 10 to 30% increase in intensity given the greater scalp-to-cortex distance for most prefrontal regions relative to M1 (Kähkönen et al. 2004). Nevertheless, this issue warrants further studies, as the application of a high intensity stimulation to this orbitofrontal location may be particularly uncomfortable for the subjects. A second, critical aspect to consider is the coil placement. This is especially true when stimulating SMA due to its spatial proximity with M1. Here, we used a neuronavigation system to target the MNI coordinates of the SMA based on individual MRI images. In the majority of the subjects ($n = 12/20$), we had to slightly adjust the M1 stimulation site (i.e. the hotspot) and its corresponding rMT. To the best of our knowledge, this type of adjustment has never been reported before, despite the existence of several ppTMS studies on areas lying close to M1. For the sake of transparency, we believe that future studies should systematically report any adjustment of coil positions. In addition, the precision of targeting SMA and mOFC structures can be improved in the future by taking into account peak activations identified by functional MRI during tasks directly related to these two regions.

As mentioned above, our data shows that some of the facilitatory and inhibitory effects are ISI-dependent. Of note, other key TMS parameters can affect the directionality (i.e. facilitation versus inhibition) and the strength of the effects obtained in the present study, such as the current intensity and the cortical current direction induced by the CS. Indeed, the observed inhibitory and facilitatory effects within cortico-cortical and cortico-subcortical networks are contingent upon these TMS parameters (Bäumer et al. 2009; Fiori et al. 2016, 2017). Furthermore, these effects may exhibit variability when evaluated during the execution of cognitive tasks (Neige et al. 2021), in older adult populations (Green et al. 2018), or among individuals with neurological or psychiatric disorders (Koch et al. 2008). To acquire a more comprehensive understanding of how the interactions between SMA-M1 and mOFC-M1 evolve in response to variations in these TMS parameters, it is crucial to expand our investigation to encompass additional CS intensities, as well as other current directions. For example, rotating the coil to direct the cortical current flow upward and laterally could increase the magnitude of the induced E-field over the left mOFC at the expense of stimulation focality (see our a posteriori e-field modeling analyses in Supplementary Fig. S3), highlighting the presence of a strength/focality tradeoff. Future studies interested in stimulating mOFC with TMS should consider this strength/focality tradeoff to decide about the cortical current direction to be used.

Overall, the current study shows that SMA and mOFC conditioning exerts interval- and region-specific facilitatory and inhibitory influences on motor activity. Our findings pave the way for both fundamental and clinical investigations aimed at understanding the causal role of these areas in the modulation of motor activity, as may occur in motor planning, decision-making, and inhibitory control, in which SMA and mOFC play a central role.

Supplementary material

Supplementary material is available at *Cerebral Cortex* online.

Author contributions

CN and GD designed the study; CN, AAZ, TB and GD performed data collection; CN, PV, AAZ, LD, FL, TB and GD analyzed and interpreted the data; CN, PV and GD drafted the paper; AAZ, LD, FL and TB revised it critically for important intellectual content. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work and ensured that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Data availability

All datasets will be freely available on the Open Science Framework repository upon publication at https://osf.io/up45j/.

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