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RESEARCH ARTICLE

Effective connectivity between human supplementary motor area and primary motor cortex: a paired-coil TMS study

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Abstract The supplementary motor area (SMA) is important for preparation and execution of voluntary movements and densely anatomically connected with the hand area of primary motor cortex (M1). However, little is known about the effective connectivity between SMA and ipsilateral M1 (SMA → M1). Here, we used paired-coil transcranial magnetic stimulation (pcTMS) to study the SMA → M1 effective connectivity in healthy human subjects. In Experiment 1, we tested the effects of different induced current directions in the SMA and M1, and different intensities of conditioning SMA stimulation. Coil placement over the SMA-proper was verified by MRI-navigation. We found a SMA → M1 facilitatory effect on motor evoked potential (MEP) amplitude that occurred very specifically only with an induced conditioning current directed from the midline towards the targeted SMA, an induced test current in M1 directed antero-medially and sufficient intensity of conditioning SMA stimulation. In Experiment 2, we selected these effective parameters to explore the effects of SMA → M1 on the active MEP amplitude, cortical silent period (CSP) duration, and using a triple-pulse protocol, on short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF). None of these measures was affected by conditioning SMA stimulation. Our findings demonstrate that pcTMS identifies predominantly facilitatory connections from SMA-proper to the hand area of the ipsilateral M1. The successful activation of this connection depends on effective SMA-proper stimulation, is state dependent and likely mediated via excitatory interneurons in M1.

Keywords Supplementary motor area · Primary motor cortex · Effective connectivity · Paired-coil stimulation · Transcranial magnetic stimulation · Human

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Introduction

The supplementary motor area (SMA-proper, here shortly referred to as SMA) is important for preparation and execution of voluntary movements and seems to play an important role in linking cognition to action (Goldberg 1985; Tanji 1996; Nachev et al. 2008). In monkeys and humans, the SMA is densely and reciprocally connected with the hand area of the primary motor cortex (M1) (Muakassa and Strick 1979; Luppino et al. 1993; Johansen-Berg et al. 2004). In contrast, the anteriorly adjacent pre-SMA does not have direct connections with M1 (Geyer et al. 2000; Liu et al. 2002; Johansen-Berg et al. 2004). Beyond this anatomical connectivity, relatively little is known about the functional and effective connectivity

along this SMA → M1 pathway. Electrical microstimulation of the SMA leads to short-latency action potential firing in pyramidal tract neurons of the monkey M1 (Aizawa and Tanji 1994; Tokuno and Nambu 2000). Epicortical electrical stimulation of the SMA in patients with intractable epilepsy results in short-latency evoked potentials over the M1 region (Matsumoto et al. 2007). Two paired-coil transcranial magnetic stimulation (pcTMS) studies demonstrated inhibitory and facilitatory effects but the conditioning stimulating coil was likely located over the pre-SMA rather than the SMA-proper (Civardi et al. 2001; Byblow et al. 2007). High-frequency repetitive TMS of the SMA resulted in facilitation of corticospinal excitability, as measured by motor evoked potential (MEP) amplitude, outlasting the period of stimulation (Matsunaga et al. 2005; Hamada et al. 2009; Raux et al. 2010). Finally, recent functional MRI studies indicated state-dependent effective SMA → M1 connectivity that was dynamically enhanced during hand movements (Grefkes et al. 2008).

However, the physiological characteristics of the SMA → M1 facilitation have not been explored in detail. Here, we used the pcTMS technique that employs two stimulating coils through which the conditioning and test pulses are applied [for review, (Ni et al. 2011)]. The test coil is typically located over the hand area of M1, and the effects of conditioning stimulation elsewhere are measured by modulation of the MEP amplitude. Pathways that have been explored by pcTMS in previous studies comprise the interhemispheric connection between the two M1 (Ferbert et al. 1992), the cerebello-dentato-thalamo-M1 projection (Ugawa et al. 1995), and projections from parietal cortex (Koch et al. 2007), ventral premotor cortex (Davare et al. 2008) and ipsi- (Groppa et al. 2012) and contralateral dorsal premotor cortex (Koch et al. 2006) to M1. To the best of our knowledge, no pcTMS experiment so far has tested the connection between SMA and M1. The present experiments were designed to explore optimal conditions with respect to the induced current directions in SMA and M1, intensity of conditioning SMA stimulation and state (resting vs. tonically activated target muscle) for unravelling SMA → M1 effective connectivity in healthy subjects. In Experiment 1, we used modulation of MEP amplitude as index for significant input from SMA to M1, and in Experiment 2, other measures of M1 excitability (cortical silent period duration, short-interval intracortical inhibition and intracortical facilitation) were tested.

Materials and methods

Subjects

Twelve subjects (five female) aged 21–38 years (mean ± SEM, 27.8 ± 1.9 years) participated in the study (10 subjects in Experiment 1; 8 subjects in Experiment 2). None of the subjects had a history of neurological disease or was on central nervous system active drugs at the time of the experiments. Ten subjects were right-handed and two were left-handed according to the Edinburgh Handedness Inventory (Oldfield 1971). Written informed consent was obtained prior to participation. The experiments conformed to the Declaration of Helsinki and were approved by the ethics committee of the medical faculty, Goethe-University of Frankfurt am Main, Germany.

Recording and stimulation procedures

Subjects were seated comfortably in a reclining chair. Both forearms were placed in a prone position on an arm rest, and the head was always supported by a pillow to maintain a fully relaxed position. MEPs were recorded from first dorsal interosseous (FDI) muscle of the dominant hand by surface electromyogram (EMG) using Ag–AgCl cup electrodes in a belly tendon montage. With the exception of cortical silent period (CSP) measurements, experiments were performed in the resting FDI. The EMG raw signal was amplified and band-pass filtered (20 Hz–2 kHz; Counterpoint Mk2 electromyograph; Dantec, Skovlunde, Denmark), digitized at an A/D rate of 5 kHz (CED Micro 1401; Cambridge Electronic Design, Cambridge, UK) and stored on a laboratory computer for offline analysis using customized data collection and conditional averaging software (Spike 2 for Windows, version 3.05, CED). Complete voluntary muscle relaxation was monitored audio-visually by high-gain EMG (50 μV/division). Trials contaminated with voluntary activity were discarded from the analysis.

M1 stimulation

Focal TMS was applied over the hand area of dominant primary motor cortex (M1) through a 'branding iron' figure-of-eight stimulating coil (diameter of each wing, 70 mm; handle orthogonal to coil plane) connected to a Magstim 200 magnetic stimulator with a monophasic current waveform (The Magstim Company, Carmarthenshire, Wales, UK). The coil was held tangential to the scalp and rotated away from the mid-line by 45°, so that the induced current in the motor cortex was directed from posterior–lateral to anterior–medial (M1–AM) in order to activate the corticospinal system preferentially transsynaptically via horizontal cortico-cortical connections (Sakai et al. 1997; Di Lazzaro et al. 2004). The optimal coil position for activation of the FDI representation was determined as the site where TMS at a slightly suprathreshold stimulus intensity consistently produced the largest MEPs. This optimal coil position was marked with a pen on the scalp in

order to ensure consistent placement of the coil throughout the experiment. In Experiment 1, we examined also the opposite current direction (i.e. directed from anterior–medial to posterior–lateral, M1–PL) by rotating the coil by 180°. While M1–AM elicits preferentially I1-waves, M1–PL elicits preferentially I3-waves (Di Lazzaro et al. 2004). For both current directions, the stimulus intensity was adjusted to elicit MEPs of on average 1 mV in peak-to-peak amplitude (S_{1mV}). The mean MEP latencies were 22.16 ± 0.09 ms for M1–AM and 24.08 ± 0.10 ms for M1–PL.

SMA stimulation

The supplementary motor area (SMA) was stimulated using a small 'branding iron' double coil (25 mm diameter of the wing) ('SMA coil', The Magstim Company). The SMA coil was centred on the midline 4 cm anterior to the vertex (C_z according to the 10–20 International EEG System), targeting the SMA-proper (Matsunaga et al. 2005; Arai et al. 2011; Lu et al. 2012). In five subjects, we verified the target position of the SMA coil on individual brain anatomy using a frameless TMS navigation system (Localite TMS Navigator, Localite GmbH, Sankt Augustin, Germany). The mean (±SEM) location of the centre of the SMA coil was −0.22 ± 0.32 cm relative (i.e. posterior) to the vertical line from the anterior commissure perpendicular to the anterior–posterior commissure line in the sagittal plane (VCA line, white line in Fig. 1a), which is a standardized anatomical separator for the SMA-proper and the anteriorly adjacent pre-SMA (Picard and Strick 1996; Vorobiev et al. 1998).

Experiment 1: SMA → M1 effects on MEP amplitude

Paired-coil TMS (pcTMS) was employed in a conditioning-test stimulus protocol. The conditioning stimulus (CS_{SMA}) and test stimulus (TS) were applied over the SMA-proper and the hand area of M1, respectively. Measurements always consisted of 15 trials each of TS alone and CS_{SMA} + TS in pseudo-randomized order. The intertrial interval varied randomly between 4 and 6 s to limit anticipation of the next trial. The effect of the CS_{SMA} on the test MEP was expressed by the ratio of the mean conditioned MEP (elicited by CS_{SMA} + TS) over the mean test MEP (elicited by TS alone). Four different directions of the induced current in the SMA were examined: posterior (SMA-P), lateral (SMA-L), postero-lateral (SMA-PL) and anterior (SMA-A) (Fig. 1b). We selected these current directions because previous studies demonstrated SMA → M1 effects with SMA-P (Civardi et al. 2001; Byblow et al. 2007; Arai et al. 2011) and SMA-L (Matsunaga et al. 2005; Hamada et al. 2009; Raux et al. 2010; Lu et al. 2012), but the influence of SMA current direction on M1 excitability has never been assessed systematically. For all four current directions, the SMA was stimulated with a fixed CS_{SMA} intensity of either 140 or 90 % of the active motor threshold (AMT) because previous experiments had indicated a dependency of magnitude and direction of SMA → M1 effects on stimulation intensity (Civardi et al. 2001; Arai et al. 2011). AMT was determined by using the small SMA coil with the induced current in AM-direction over the hot spot of the FDI representation of the dominant M1 hand area during slight isometric FDI contraction (~10 % of maximum voluntary contraction, verified by online audio-visual feedback of the EMG signal). AMT was measured to the nearest 1 % of maximum stimulator output and was defined as the lowest stimulus intensity that produced a MEP of ≥100 μV from the average of five consecutive single trials (Ziemann et al. 1996b).

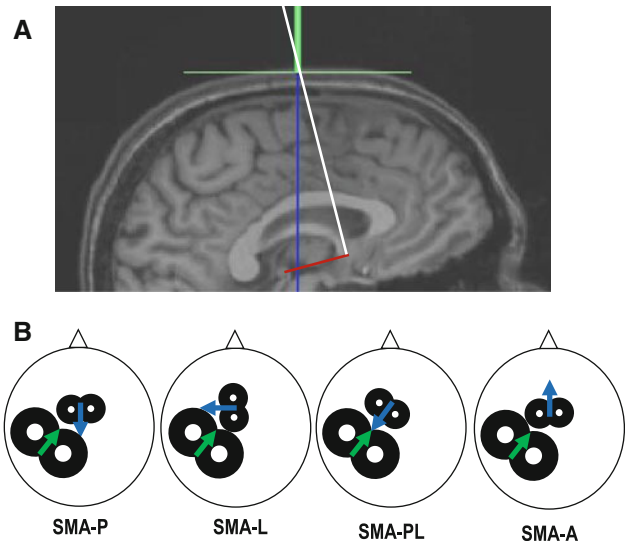

Fig. 1 Experimental set-up. **a** MRI-navigated TMS of SMA-proper (data from one representative subject). The *vertical line* from the anterior commissure, perpendicular to the *anterior–posterior commissure line* (*red line*) in the sagittal plane (*VAC line*, *white*) was used to divide SMA-proper and anteriorly adjacent pre-SMA anatomically. *Green*, schematic representation of the stimulating coil. *Blue*, orthogonal projection from the intersection of the TMS coil wings targeting SMA-proper. **b** Schematic display of the paired-coil protocol with a small (25 mm) figure-of-eight coil over SMA-proper and a larger (70 mm) coil over the hand area of the *left* M1. Four different current directions induced in the SMA (indicated by *blue arrows*) were tested in Experiment 1: *posterior* (SMA-P), *lateral* (SMA-L), *postero-lateral* (SMA-PL and *anterior* (SMA-A). *Green arrows* indicate the direction of the induced current in M1 (*antero-medial*). In addition, the opposite current direction (*postero-lateral*) was also tested in Experiment 1 (not shown) (colour figure online)

The effects of the four SMA current directions on M1 excitability (conditioned MEP/test MEP) were tested in the following six conditions: (1) M1–MA, CS_{SMA} 140 % AMT, ISI 6 ms; (2) M1–PL, CS_{SMA} 140 % AMT, ISI 3 ms; (3) M1–PL, CS_{SMA} 140 % AMT, ISI 6 ms; (4)

M1–MA, CS_{SMA} 90 % AMT, ISI 6 ms; (5) M1–PL, CS_{SMA} 90 % AMT, ISI 3 ms; (6) M1–PL, CS_{SMA} 90 % AMT, ISI 6 ms. The ISI of 6 ms was chosen because it was proven effective in previous studies (Civardi et al. 2001; Byblow et al. 2007). The adjustment to an ISI of 3 ms in some of the M1–PL experiments was done to accommodate for the preferential activation of I3-waves with the M1–PL current direction, which excite the corticomotoneuronal cells ~3 ms later than I1-waves (Day et al. 1989; Di Lazzaro et al. 2001).

Experiment 2: SMA → M1 effects on active MEP amplitude, CSP duration, SICI and ICF

The protocol of Experiment 2 was based on the findings in Experiment 1 (see "Results"), which demonstrated a very selective facilitatory SMA → M1 effect on MEP amplitude with M1–MA, TS intensity S_{1mV} (determined in the resting FDI), SMA-L, CS_{SMA} intensity 140 % AMT and ISI 6 ms. Therefore, the testing of SMA → M1 effects on various measures of M1 inhibition and facilitation was performed by using these stimulation condition.

Active MEP amplitude and CSP duration

Subjects maintained a slight isometric contraction of dominant FDI (~10 % of maximum voluntary contraction). CSP duration was measured in the single-trial rectified conditional averaged recordings from MEP onset to the return of consistent voluntary EMG activity exceeding 50 % of the 100 ms pre-stimulus EMG (Garvey et al. 2001). MEP amplitudes were measured peak-to-peak. Fifteen TS alone and 15 CS_{SMA} + TS trials were obtained in pseudo-randomized order.

Short-interval intracortical inhibition (SICI) and intracortical facilitation (ICF)

SICI and ICF were measured using conventional methods (Kujirai et al. 1993; Ziemann et al. 1996b; Peurala et al. 2008). The 'M1 coil' was connected to a BiStim module (The Magstim Company) to enable paired M1 stimulation. The intensity of the conditioning stimulus over M1 (CS_{M1}) was set to 90 % AMT of the FDI (tested as above, but with the larger 'M1 coil' connected to the BiStim). In two separate blocks of trials, CS_{M1} preceded the TS by 2 ms (SICI) or 12 ms (ICF). Within each block, the following four conditions were tested (15 trials per condition in pseudo-randomized order): TS alone, CS_{M1} + TS, CS_{SMA} + TS, CS_{SMA} + CS_{M1} + TS. SMA → M1 effects on SICI and ICF were expressed by comparing the MEP amplitude ratio of CS_{M1} + TS/TS (unconditioned SICI or ICF) with CS_{SMA} + CS_{M1} + TS/CS_{SMA} + TS (conditioned SICI or ICF). This calculation takes into account a possible facilitatory effect of CS_{SMA} on the MEP amplitude elicited by TS and therefore addresses specifically the effect of CS_{M1} in the absence versus presence of CS_{SMA} [for review of triple-stimulation protocols, (Ni et al. 2011)].

Statistical analysis

For Experiment 1, six separate one-way repeated-measures analyses of variance ($ANOVA_{RM}$) of the MEP ratio (CS_{SMA} + TS/TS) were performed with the within-subject effect of CS_{SMA} current direction (four levels, SMA-P, SMA-L, SMA-PL and SMA-A) for the six experimental conditions listed above (i.e. variations of current direction in M1, CS_{SMA} intensity and ISI). In case of a significant main effect, post hoc paired *t* tests were performed using the Bonferroni method for adjustment for multiple comparisons to determine differences between CS_{SMA} current directions. For Experiment 2, we applied paired *t* tests separately for the various measures of M1 excitability (active MEP amplitude, CSP duration, SICI, ICF) to compare the unconditioned with the SMA-conditioned recordings. All data are expressed as means ± 1 SEM. For all tests, statistical significance was assumed if $P < 0.05$. Statistical analyses were conducted using StatView for Windows 5.0.1. software (SAS Institute Inc., Cary, NC, USA).

Results

None of the subjects reported any adverse event. AMT, SI_{1mV} for M1–AM and SI_{1mV} for M1–PL were 35.0 ± 12.7 %, 49.1 ± 12.6 % and 64.8 ± 15.1 % of maximum stimulator output, respectively. AMT for the small 'SMA coil' was 50.1 ± 15.3 % of maximum stimulator output.

Experiment 1

Unconditioned mean test MEP amplitudes in the 6 experimental conditions ($a_{1\text{-}3}$ and $b_{1\text{-}3}$ in Fig. 2) 4× CS_{SMA} current directions ranged between 0.92 and 1.46 mV and were not different between conditions ($P > 0.05$), excluding a significant influence of test MEP amplitude on the SMA → M1 effects.

$ANOVA_{RM}$ demonstrated a significant effect of CS_{SMA} current direction for M1–MA, CS_{SMA} intensity 140 % AMT and ISI = 6 ms ($F_{3,27} = 6.84$, $P = 0.0014$). Post hoc paired *t* tests showed that SMA-L resulted in a larger MEP ratio than SMA-PL and SMA-A ($P < 0.001$; hash in Fig. $2a_1$). In addition, a paired *t* test revealed that the conditioned MEPs with SMA-L were significantly larger than the unconditioned ones ($P = 0.0007$; asterisk in

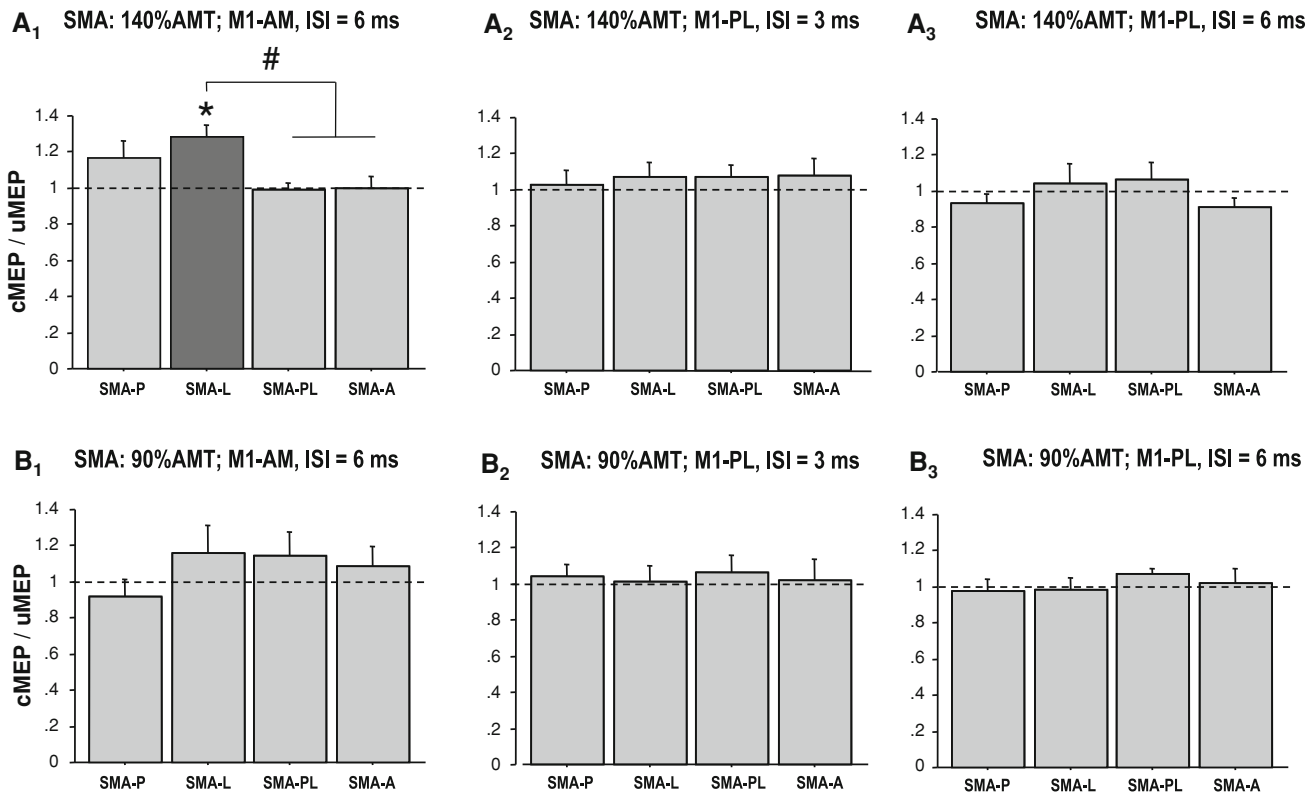

Fig. 2 Results of Experiment 1. The ratios of the mean ($n = 10$ subjects) conditioned MEP amplitude (cMEP) elicited by SMA + M1 stimulation over the mean unconditioned MEP amplitude (uMEP) elicited by M1 stimulation alone are shown as a function of current direction in the SMA (SMA-P, SMA-L, SMA-PL, SMA-A). The intensity of the SMA stimulus was 140 % AMT ($\mathbf{a}_{1–3}$) or 90 % AMT ($\mathbf{b}_{1–3}$). Current direction in M1 was AM ($\mathbf{a}_1$, $\mathbf{b}_1$) or PL ($\mathbf{a}_{2–3}$, $\mathbf{b}_{2–3}$) and the ISI between SMA and M1 stimulation was 6 ms ($\mathbf{a}_1$, $\mathbf{a}_3$, $\mathbf{b}_1$, $\mathbf{b}_3$) or 3 ms ($\mathbf{a}_2$, $\mathbf{b}_2$). The hatched *horizontal lines* indicate ratios of 1.0 (i.e. no effect of conditioning SMA stimulation). *Asterisk* indicates a significant MEP facilitation (paired *t* test, conditioned vs. unconditioned MEPs, $P = 0.0007$) and the *hash symbol* indicates significant differences of the MEP ratios between SMA current directions (paired *t* tests, $P < 0.001$). *Error bars* are 1 S.E.M

Fig. 2a_1), while no such effect occurred with any of the other SMA current directions (all $P > 0.05$). No significant SMA → M1 effects on MEP amplitude were observed in any of the other experimental conditions (Fig. 2$a_{2–3}$, $b_{1–3}$).

Experiment 2

When using the optimal facilitatory experimental conditions from Experiment 1 (M1–AM, SMA-L, CS_{SMA} intensity 140 % AMT, ISI 6 ms), SMA → M1 had no effects on active MEP amplitude or CSP duration (Table 1). For SICI, the test MEP elicited by TS was 1.05 ± 0.14 mV and the test MEP elicited by CS_{SMA} + TS was 1.31 ± 0.21 mV ($P = 0.04$), replicating the facilitatory SMA → M1 effect of Experiment 1. However, SMA-conditioning stimulation had no additional effect on SICI (Table 1). For ICF, the test MEP elicited by TS was 1.03 ± 0.17 mV and the test MEP elicited by CS_{SMA} + TS was 1.22 ± 0.21 mV ($P = 0.01$), again replicating the facilitatory SMA → M1 effect of Experiment 1. However, SMA-conditioning stimulation had no extra effect on ICF (Table 1).

Table 1 SMA → M1 effects on M1 excitability

Measure	Unconditioned by CS_{SMA}	Conditioned by CS_{SMA}	*P*
Active MEP (mV)	2.03 ± 0.27	2.06 ± 0.26	0.66
CSP duration (ms)	116.7 ± 19.0	115.4 ± 19.0	0.52
SICI (%)	55.1 ± 8.6	45.0 ± 3.6	0.18
ICF (%)	130.0 ± 7.2	129.1 ± 9.5	0.87

CS_{SMA}-conditioning stimulus applied to the SMA with lateral current direction, intensity of 140 % AMT and 6 ms prior to the test stimulus over M1, *MEP* motor evoked potential, *CSP* cortical silent period, *SICI* short-interval intracortical inhibition, *ICF* intracortical facilitation. All data are means ($n = 8$) ±SEM

Discussion

This study addressed systematically effective connectivity from human SMA to ipsilateral M1 using a paired-coil TMS protocol at an interstimulus interval of 6 ms. The major finding was facilitation of MEP amplitude that depended specifically on current direction in the SMA

(only significant when the induced current was directed towards the stimulated SMA), stimulus intensity (only significant with 140 % AMT but not 90 % AMT) and state (only significant when the target hand muscle was in the resting state but when voluntarily activated). Furthermore, this MEP facilitation was present only with antero-medially directed current in M1 (preferentially eliciting I1-waves) but not with postero-laterally directed current (preferentially eliciting I3-waves). Finally, measures of motor cortical inhibition (CSP duration and SICI) and facilitation (ICF) in M1 remained unaffected by conditioning stimulation of the SMA.

Effective ISI

Our findings are in largely agreement with extracellular recordings of single pyramidal tract neurons and/or movement related neurons in monkey M1, a majority of which showed short-latency excitatory responses within 3–4 ms (range 1–9 ms) to intracortical microstimulation of the SMA (Aizawa and Tanji 1994; Tokuno and Nambu 2000). Pharmacological experiments showed that these excitatory responses of M1 neurons are mediated by glutamatergic NMDA and non-NMDA receptors (Shima and Tanji 1998). The effective ISI in the present experiments of 6 ms is compatible with the slightly shorter action potential response latencies of pyramidal tract neurons in the monkey because of the longer SMA → M1 conduction distance in humans. Admittedly, we have not tested other ISIs because our previous study suggested that 6 ms but not other intervals are effective for facilitatory SMA → M1 transmission (Arai et al. 2011).

Effective CS_{SMA} intensity

We demonstrated that the facilitatory SMA → M1 effect on MEP amplitude was expressed only at sufficient CS_{SMA} intensity (i.e. 140 % AMT but not 90 % AMT). Identical results were obtained in a recent study from our group where we demonstrated that associative long-term potentiation-like plasticity in the SMA–M1 network induced by repetitive pairing of SMA + M1 stimulation required sufficient SMA stimulation intensity (Arai et al. 2011). One might argue that the observed facilitatory SMA → M1 effect at the higher CS_{SMA} intensity has been caused by current spread from SMA to M1 rather than by transsynaptic activation of M1 neurons along SMA-to-M1 anatomical connections. However, this is unlikely because current spread (i.e. direct M1 stimulation by CS_{SMA}) would not explain why the SMA → M1 facilitation with SMA-L was not seen with SMA-PL when possible current spread towards M1 should be most effective (cf. Fig. 1b). In addition, one previous ICF study demonstrated that the clear ICF observed with AM orientation of the induced currents of CS_{M1} and TS turned into inhibition if CS_{M1} and TS induced currents were turned by 180° into PL direction (Di Lazzaro et al. 2006). However, an inhibitory SMA → M1 effect was not observed with M1–PL (cf. Fig. $2a_{2-3}$), making a direct stimulation of M1 by spread from CS_{SMA} further unlikely.

We cannot entirely exclude spread from CS_{SMA} to the pre-SMA, which is located directly anteriorly adjacent to the SMA-proper (Nachev et al. 2008). Recent paired-coil TMS studies demonstrated effective connectivity between pre-SMA and M1 via cortico–cortical and cortical–subcortical routes (Mars et al. 2009; Neubert et al. 2010), but these effects were specifically dependent on motor tasks requiring action reprogramming. For this reason, it is unlikely that current spread to the pre-SMA has contributed to the MEP facilitation in the present results, which was obtained specifically at rest but not during voluntary activation. In addition, in contrast to the SMA-proper, the pre-SMA has no direct anatomical connections with ipsilateral or contralateral M1 in monkeys (Luppino et al. 1993) or in humans (Bestmann et al. 2003; Johansen-Berg et al. 2004; Kim et al. 2010), making it further unlikely that activation of the pre-SMA had significantly contributed to the observed SMA → M1 facilitation.

Finally, another possibility is that current spread has occurred to the dorsal premotor cortex (PMd) on the lateral convexity, which is densely connected to M1 (Dum and Strick 2005). One recent pcTMS study demonstrated effective PMd → M1 facilitation at ISIs of 2.4–2.8 ms and 4.0 ms but not longer ISIs (tested up to 5.2 ms) (Groppa et al. 2012). This is at variance with the effective ISI of 6 ms in this and our previous study (Arai et al. 2011) and therefore renders a contribution by current spread to the PMd also unlikely. However, we cannot entirely rule out the possibility that the proposed activation of neurons in SMA-proper was relayed to M1 via other premotor areas, such as the PMd, rather than reaching M1 directly because all of these areas are mutually densely connected in a cortico-cortical motor network (Luppino et al. 1993; Dum and Strick 2005; Kim et al. 2010).

Effective CS_{SMA} current direction

This is the first study that systematically investigated the role of induced current direction in the SMA. Several previous studies showed that high-frequency repetitive TMS stimulation of the SMA at lateral current orientation induced MEP facilitation outlasting the period of SMA stimulation (Matsunaga et al. 2005; Hamada et al. 2009; Raux et al. 2010), but other current orientations were not tested. The most parsimonious explanation for the selective efficacy of SMA-L compared to all other tested CS_{SMA}

current directions in the present study (cf. Fig. 2a$_1$) is that the strength of the induced electrical field in grey matter is maximized by current directions that are perpendicular to local gyrus orientation (Thielscher et al. 2011). These data were obtained by TMS of M1 and were explained by an increase in the induced electrical field strength at the boundary of cerebrospinal fluid and grey matter, where the current passes into the less conductive grey matter (Miranda et al. 2003; Thielscher et al. 2011). This principle of a comparable dependency of the TMS effect strength on the local current orientation should hold in general for all brain areas [cf. for visual cortex: (Kammer et al. 2007)]. Therefore, for targeting the SMA of one hemisphere, which is the most dorso-medial part of Brodmann area 6 extending into the wall of the interhemispheric fissure, SMA-L (i.e. current directed from the midline towards the target SMA) should be the most efficient current direction for SMA activation. In addition to conductivity boundaries, the orientation of the cortico-cortical fibres connecting SMA with M1 may influence optimal current direction because fibre bends away from the orientation of the induced electrical field represent low-threshold points of excitation (Amassian et al. 1992). The exact orientation of these fibres in humans has not been elucidated yet. Future diffusion tensor imaging studies may help to model the effects of optimized current direction with respect to orientation of the SMA → M1 pathway (De Lucia et al. 2007).

Effective TS current direction

SMA → M1 facilitation was only observed when the current direction in M1 was directed antero-medially (M1–AM) but not when directed postero-laterally (M1–PL). The possible problem of missing the optimal ISI due to predominant activation of I3-waves (rather than I1-waves) with M1–PL was addressed by testing an ISI of 3 ms in addition to the ISI of 6 ms. However, this also did not result in SMA → M1 facilitation (cf. Fig. 2a$_2$). This strongly suggests that the facilitatory input from SMA to M1 acts predominantly on first-order excitatory interneurons that are responsible for the generation of the I1-wave. To verify the proposed predominant action of the facilitatory input from SMA to M1 onto the I1-wave generating interneurons would, however, require invasive epidural recordings of the multiple descending corticospinal volleys and their modulation by the SMA input from the cervical spinal cord. Previous spinal epidural recording studies showed that a selective modulation of the I1-wave occurred by other interventions, for example, after continuous theta-burst stimulation of M1 (Di Lazzaro et al. 2005).

State dependency of the SMA → M1 facilitation

State dependency of effective connectivity tested by pcTMS protocols is a well-established phenomenon and likely signifies the task-specific involvement of cortico-cortical pathways in the human brain. For example, facilitatory effects from the left dorsal premotor cortex to the right M1 observed in the resting state are largely switched off during a choice reaction time task, except for one short period during response preparation when the left hand has to be moved (Koch et al. 2006). On the other hand, a net inhibitory effect from the ventral premotor cortex to the ipsilateral M1 is abolished during a power grip of the contralateral hand and even turns into muscle-specific facilitatory effective connectivity during a precision grip (Davare et al. 2008; Davare et al. 2009). The abolition of the facilitatory SMA → M1 effect in the present study during maintenance of a slight isometric contraction is compatible with the notion that the SMA, in contrast to M1, does not play a relevant role in this simple motor task (Tanji 1996). In contrast, effective connectivity between SMA and ipsilateral M1 is dynamically enhanced during hand movements, as recently indicated by a functional MRI study (Grefkes et al. 2008).

Lack of effect on CSP duration, SICI and ICF

The CSP duration and SICI are currently thought to reflect GABAB and GABAA receptor-mediated postsynaptic inhibition of corticospinal neurons, respectively (Ziemann et al. 1996a; Siebner et al. 1998; Di Lazzaro et al. 2000; Paulus et al. 2008). The lack of effect of SMA-conditioning stimulation on these measures strongly suggests that the MEP facilitation (Fig. 2a$_1$) has not been caused by disinhibition but represents a true excitatory input from SMA to M1. This is in line with the glutamatergic dense anatomical connectivity between SMA and M1 (Muakassa and Strick 1979; Luppino et al. 1993) and abolition of action potentials evoked by SMA microstimulation in corticospinal M1 neurons by pharmacological blockade of glutamatergic receptors (Shima and Tanji 1998). The lack of ICF modulation by SMA-conditioning stimulation is not surprising because many examples for dissociation of interventional effects [e.g. pharmacological testing, cf. (Paulus et al. 2008)] on MEP amplitude versus ICF have been described. For example, slight voluntary contraction of the target muscle leads to MEP facilitation but ICF depression (Ridding et al. 1995). The physiological mechanisms underlying ICF are as of yet still largely unclear (Di Lazzaro et al. 2006) and limit further conclusions on the meaning of the absence of its modulation by SMA input.

Neurobiological relevance

The present results support the notion on the usefulness of the pcTMS technique to measure effective connectivity between motor areas of human cortex. The identified facilitatory SMA → M1 effect is in excellent agreement with many previous invasive studies in monkeys and recent functional MRI and repetitive TMS studies in humans that provided evidence for such an excitatory interaction. Future pcTMS studies could address in more detail the state dependency of this pathway and its potentially pathological regulation in neurological disorders such as Parkinson's disease, dystonia, or after cerebral stroke.

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