Facilitatory conditioning of the supplementary motor area in humans enhances the corticophrenic responsiveness to transcranial magnetic stimulation

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1Department of Experimental Medicine, Transcranial Magnetic Stimulation Laboratory, McGill University Health Centre, Montréal, Quebec, Canada; and 2Assistance Publique-Hôpitaux de Paris, Département d’Anesthésie RéanIMATION, Groupe Hospitalier Pitié-Salpêtrière, 3Université Paris 6, ER10UPMC, Laboratoire de Physiopathologie Respiratoire, and 4Assistance Publique-Hôpitaux de Paris, Service de Pneumologie et RéanIMATION, Groupe Hospitalier Pitié-Salpêtrière, Paris, France

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Raux M, Xie H, Similowski T, Koski L. Facilitatory conditioning of the supplementary motor area in humans enhances the corticophrenic responsiveness to transcranial magnetic stimulation. J Appl Physiol 108: 39–46, 2010. First published November 5, 2009; doi:10.1152/japplphysiol.91454.2008.—Inspiratory loading in awake humans is associated with electroencephalographic signs of supplementary motor area (SMA) activation. To provide evidence for a functional connection between SMA and the diaphragm representation in the primary motor cortex (M1DIA), we tested the hypothesis that modulating SMA activity using repetitive transcranial magnetic stimulation (rTMS) would alter M1DIA excitability. Amplitude and latency of diaphragm motor evoked potentials (MEPFDI), evoked through single pulse M1DIA stimulation, before and up to 16 min after SMA stimulation, were taken as indicators of M1DIA excitability. MEPs from the first dorsal interosseous muscle (FDI, MEPFDI) served as a control. Four SMA conditioning sessions were performed in random order at 1-wk intervals. Two aimed at increasing SMA excitatory connectivity to the cingulate gyrus (30). In addition, multiple lines of evidence indicate the importance of higher cortical motor inputs to the respiratory muscles, delineating a refined voluntary control of breathing in humans.

Converging evidence points to the supplementary motor area (SMA) as well as primary motor cortex (M1) as the source of these cortical inputs. Neuroimaging studies frequently report coactivation of the SMA with the M1 during various respiratory tasks and particularly during human volitional inspiratory maneuvers (e.g., 20). There is also electroencephalographic evidence for SMA activation during volitional respiratory tasks (15). A premotor potential, known to originate within the premotor and motor cortical areas (SMA, premotor cortex and M1), precedes voluntary self-paced sniffing maneuvers (15). This premotor potential has characteristics similar to that of premotor potentials recorded during voluntary repetitive hand movements (27). A premotor potential is also present during inspiration against a mechanical load (threshold valve or resistive pipe) in awake subjects (23), highlighting the preinspiratory activation of SMA during loaded breathing (5). The exact ventilatory role of the SMA is not yet known. Understanding it first requires one to elucidate the functional connectivity of the SMA with corticophrenic pathways. This can be achieved by TMS studies, as follows.

Using transcranial magnetic stimulation (TMS), Sharshar et al. (25) provided functional evidence for SMA spinal projections on phrenic motoneurons in humans. The SMA projects fibers both to M1 (17) and to the intermediate and ventral zones of the spinal cord (4) in macaques. However, how the SMA representation of the diaphragm projects on its M1 representation (M1DIA) is unknown. The primary objective of the present study was thus to provide functional evidence for these connections in healthy humans. Matsunaga et al. (18) probed SMA cortical projections on the first dorsal interosseous (FDI) representation within M1 (M1FDI). They reported an increase in the excitability of FDI corticospinal excitability at the cortical level (henceforth referred to as M1FDI excitability) following excitatory repetitive TMS (rTMS) (6) over the SMA (18). They claimed these remote effects were “due to rTMS after-effects on the level of on-going activity” in connection between SMA and M1FDI (18). We hypothesized that similar excitatory conditioning would have similar effects on diaphragmatic corticospinal excitability at the cortical level (henceforth referred to as M1DIA excitability). To this aim, we applied high-frequency rTMS over SMA and studied the impact of this excitatory conditioning on the characteristics of diaphragm motor evoked potentials (MEPFDI), taken as indicators of M1DIA excitability.
Our second objective was to assess the level of baseline tonic activity within the SMA-to-M1DIA connections. If SMA exerts a tonic facilitatory influence on M1DIA excitability, then suppressing activity in the SMA should in turn reduce excitability of M1DIA. We tested this prediction using a low-frequency rTMS protocol to stimulate SMA (6). A similar protocol has previously been shown to have inhibitory effects on M1FDI excitability when applied over lateral premotor cortex (8). The effects of this inhibitory conditioning on M1DIA excitability were then assessed through MEPDIA recording.

We also included measurements of the FDI as a control condition to provide evidence for the effectiveness of the conditioning protocol. This was possible because rTMS cannot selectively stimulate somatotopic subregions of the SMA.

MATERIALS AND METHODS

Subjects

Eighteen healthy subjects (age 18–56 yr, 10 women, 16 right-handed) gave written informed consent to participate in the study, which was approved by the Research Ethics Board of the McGill University Health Centre. A screening questionnaire was used to exclude subjects with a neurological, psychiatric, or serious medical history. Exclusion criteria also included contraindications to TMS: presence of a pacemaker, aneurysm clips, heart/vascular clips, prosthetic valves, any type of metal prosthesis, pregnancy, personal or familiar history of seizures, or use of antipsychotic or antidepressant medications lowering the seizure threshold.

Four subjects subsequently withdrew their consent: two withdrew before the first session with no given reason and two during the first session because of discomfort during single-pulse stimulation (n = 1) or postsession headaches (n = 1). Two subjects were invited to withdraw from the study because their resting motor thresholds exceeded 90% of the maximum stimulator output, and hence had an increased risk of post-rTMS headaches (31). The study could not be completed in one additional subject who fainted during the hot spot localization (see below). In the end, 11 subjects completed the study.

Measurements

Abdominal circumference. Abdominal circumference was monitored using a mechanical strain gauge (Pneumotrace II, UFI, Morro Bay, CA) attached to an elastic belt placed at the level of the umbilicus.

Electromyographic responses. Surface recordings of the dominant-side hemidiaphragm electromyogram were obtained using a pair of Ag/AgCl adhesive electrodes. The active electrode was placed in the last intercostal space between the midclavicular line and the edge of the sternum. The reference electrode was placed 2 cm away, on the above rib. This montage is known to reduce the risk of signal contamination by extradiaphragmatic muscle activity in response to transcranial magnetic stimulation (2). Raw diaphragmatic electromyographic signals were preamplified (Electronique du Mazet, Mazet Saint-Voy, France) close to the electrodes with a gain of 300, filtered to a bandwidth of 10–1,000 Hz, digitized at 20 kHz, and then stored (Biopac MP150, Biopac System, Goleta, CA) for subsequent analysis.

The electrical activity of the dominant FDI was recorded using a pair of Ag/AgCl adhesive electrodes, amplified with a gain of 1,000, bandpass filtered (100 Hz-1 kHz), digitized at 20 kHz, and stored (Biopac MP150, Biopac System) for subsequent analysis. For the purpose of the SMA hot spot localization, the electromyographic activity of the dominant abductor hallucis was also recorded using a pair of Ag/AgCl adhesive electrodes placed over the landmarks of the muscle, with the same settings as the FDI (see below and 18).

The offline analysis of electromyographic signals was performed using a modular Matlab time-based data analysis tool (dataWizard, version 0.8.20a, A.D. Wu, UCLA). For the purpose of maintaining a constant level of muscle contraction during the assessment of active motor thresholds, the electromyographic signal was rectified, integrated, and displayed on-line to the subject.

TMS

Corticospinal excitability. Corticospinal excitability was assessed using single-pulse TMS (spTMS) over the primary motor cortex. SMA conditioning was performed using rTMS. FDI spTMS and SMA rTMS were performed using a 70-mm air-cooled figure-of-eight coil (Air-film-coil, Magstim, Sheffield, UK) held tangentially to the skull and connected to a repetitive biphasic stimulator (RapidStim, Magstim). For FDI stimulation, the coil handle was pointing backward and laterally with a 45° angle from the midline. For SMA stimulation, the coil handle was pointing ipsilaterally to the dominant hemisphere (18). spTMS with the 70-mm coil failed to produce diaphragm responses in most subjects. Diaphragm responses were then studied using a 90-mm cone coil connected to a Magstim 200 monophasic stimulator (Magstim), the stimulating current flowing anterior-posteriorly. The cone coil focuses stimulation on motor areas that are otherwise difficult to access and delivers higher stimulation intensities (32).

Hot spot localization. The diaphragm and FDI stimulation hot spots were localized during a dedicated session preceding the conditioning sessions. The hot spots were identified as the primary motor cortex sites from which spTMS elicited the largest MEPs (23). The SMA hot spot was identified according to the technique described by Matsunaga et al. (18) as located 1 cm anterior to the last site from which MEPs could be evoked in the active contralateral abductor hallucis. Of note, in this study spTMS over the SMA failed to elicit either MEPFDI or MEPDIA. Hot spots were stored using a neuronavigation device (BrainSight, Rogue Research, Montreal, Canada) to ensure consistent placement of the coil across sessions (Fig. 1).

Seven of the subjects subsequently participated in a separate functional MRI study (fMRI). We took this opportunity to confirm that the SMA hot spot coordinates corresponded to the SMA in these subjects, using the anatomic MRI acquisition performed during the fMRI study. We plotted
the SMA hot spots stimulated during the study on the individual native-space MRIs. In all cases, the stimulated area proved to actually fall within the area known to delimit the posterior part of the SMA (22), between the two vertical commissure lines (Fig. 2).

**Motor thresholds.** Motor thresholds were defined as the lowest stimulation intensity (expressed in percentage of the maximum stimulator output) that elicited a MEP of at least 50 μV (resting) or 200 μV (active) in the corresponding muscle in three of six trials.

**Corticospinal excitability.** Corticospinal excitability was assessed for the diaphragm and for the FDI by performing spTMS over the corresponding hot spots, with a stimulation intensity of 120% of the corresponding resting motor thresholds. Twenty MEPs (interpulse interval ≥ 5 s) were obtained for each muscle [diaphragm (MEP_{DIA}) and FDI (MEP_{FDI})]. Amplitudes were measured from peak to trough, and latencies were defined as the time elapsed between TMS pulse and the first departure of the EMG signal of >3 SDs from baseline. spTMS was delivered over the diaphragmatic hot spot at the end of expiration, as identified from the abdominal circumference signal. The timing of the pulse was selected to eliminate the possibility of contamination of the MEP by background diaphragm activity or by changes in the relative position of the recording electrode to the diaphragm.

**SMA conditioning.** Four rTMS protocols were applied over the SMA hot spot in random order, during four sessions separated by at least 1 wk. Two high-frequency rTMS protocols aimed at enhancing the activity of the SMA. With a stimulation intensity set at 110% of the FDI active motor threshold, they consisted of 1) 10-s 5-Hz trains repeated 10 times separated by 50-s intertrains (henceforth referred to as “5Hz”), and 2) 5-s 10-Hz trains repeated 10 times separated by 55-s intertrains (“10Hz”). Two low-frequency protocols aimed at inhibiting the activity of the SMA. They consisted of a single 1-Hz 20-min train (1,200 stimulations), intended to produce aftereffects of at least 15 min (6). In one protocol, modified from Gerschlager et al. (8), the intensity of stimulation intensity was set at 110% of the FDI active motor threshold (“aMT”). In the other protocol, it was set at 110% of the FDI resting motor threshold to test for the effect of increasing intensity (“rMT”). Few studies have investigated the safety of rTMS applied outside the primary motor cortex (31). We therefore took precautions to detect increases in cortical excitability that would have heralded an augmented risk of seizures (31) by continuously monitoring the FDI electromyogram during rTMS. Had the stimulation aimed at SMA been sufficiently strong and appropriately oriented to enhance excitability of neighboring M1, we might have observed the appearance of MEPs in the FDI. This would have been indicative of incipient seizures (31) and would have prompted the interruption of the study.

**Experimental Protocols**

Each of the four rTMS sessions started with motor threshold assessment (resting and active motor thresholds for the FDI, resting motor threshold for the diaphragm) to adjust the rTMS and spTMS...
intensities. Twenty MEPs dia and 20 MEPs FD were then recorded (BEFORE condition) at 120% of the corresponding resting motor threshold. This was followed by one of the four SMA-conditioning rTMS paradigms. During the post-rTMS period, 20 MEPs FD and 20 MEPs dia were collected (1) between the 1st and the 6th min (AFTER1); 2) between the 6th and the 10th minute (AFTER2); and (J) between the 11th and the 16th minute (AFTER3) (Fig. 3).

Sample Size Calculation

The sample size calculation was based on a hypothesized change of 40% (18) in the amplitude of diaphragmatic MEPs, using the mean and SD obtained in the Demoule et al. study (2). We assumed a 0.05 risk of type I error and a power of 0.99 to account for the power loss due to the use of the Friedman’s test (28). Finally, sample size calculation included three post-rTMS measures with an estimated correlation of 0.7 across measures (14) since no data regarding correlation were available for the diaphragmatic MEPs. Seven subjects were required to meet these objectives (Stata, Statacorp, College Station, TX).

Data Analysis

Quality criteria. MEPs were retained for analysis when no background electromyographic activity was observed during the 100 ms preceding the spTMS. For the diaphragm, electromyographic responses to stimulation were taken into account if and only if the stimulus pulse also produced a concomitant increase in abdominal circumference, the diaphragm being the only muscle capable of producing this mechanical effect.

Statistics. Statistical analyses were performed using Prism 4.0c software (GraphPad Software, La Jolla, CA). The results are presented as medians and interquartiles. Motor thresholds are presented as percentages of the maximum stimulator output. Amplitudes and latencies were compared using the nonparametric Friedman’s test for repeated measures (Q) followed by a Dunn’s test for post hoc comparisons. Motor thresholds were compared using the Wilcoxon rank test. Differences were considered significant when the probability of a type I error was ≤0.05.

RESULTS

Eleven subjects completed all the sessions. In the first subject, only the FDI data from the aMT session were recorded according to the protocol. In another subject, no diaphragmatic response could be evoked in any of the sessions. In three subjects, technical issues (signal contamination) precluded analysis of the diaphragmatic MEPs (one during aMT, two during 10Hz). As a result, the number of subjects varies across the analysis performed, but it is always at least equal to the calculated sample size.

Safety

The subject who fainted experienced typical prodromes of vasovagal syncope (paleness, sweating, nausea, feeling unwell) and recovered after spTMS was stopped and the legs were lifted up. There were no clinical elements suggestive of seizure and no postictal syndrome. The event was qualified as a vasovagal syncope and reported as such to the research ethics board.

The FDI monitoring during rTMS never evidenced MEPs suggestive of a spread of the stimulation to the primary motor cortex. As a result, none of the sessions had to be interrupted for fear of incipient seizure.

Motor Thresholds

The mean FDI resting motor threshold was within the expected range (13) and was always significantly higher than the mean active threshold [55% (51–61%) vs. 45% (39–51%), P = 0.001].

As expected (29), the diaphragm resting motor threshold was higher than the FDI one, as illustrated by the fact that the flat 70 mm figure-of-eight coil elicited a diaphragm response in only three subjects. Resorting to the use of a 90-mm cone coil allowed us to obtain a diaphragm response in all but one case, with a resting diaphragm motor threshold of 53% (45–57%), which is in line with published values (26). Of note, the difference between the two stimulation techniques prevents any direct comparison of the thresholds for the FDI and the diaphragm.

The FDI and diaphragmatic resting motor thresholds did not fluctuate significantly across sessions (respectively Q3 = 0.45, P = 0.93; and Q3 = 5.8, P = 0.12). In contrast, the FDI active motor threshold decreased across sessions (Q3 = 13.31, P = 0.004), becoming significantly lower during the fourth and the fifth sessions than during the second one [respectively: 44% (37–49%) and 45% (36–47%) vs. 47% (43–55%), P < 0.05]. Despite this, the highest stimulation intensities used during the study (110% of the resting motor threshold) were always lower than 150% of the active motor threshold. This is reassuring regarding a putative spread of the magnetic field over the primary motor cortex (18) during SMA conditioning.

Diaphragm Corticospinal Excitability

Figure 4 summarizes the effects of rTMS on the amplitude of the MEPs dia. After 5Hz conditioning, the MEPs dia increased significantly (Q3 = 8.33, P = 0.04) (Fig. 5A). The MEP amplitude amounted to 121.4% of the BEFORE value (interquartile range: 93.6–140.7%) during AFTER3. The other protocols had no significant effects on MEPs dia (Q3 = 3.51, P = 0.32; Q3 = 4.35, P = 0.23; and Q3 = 0.6, P = 0.90 for 10Hz, aMT, and rMT, respectively).

The latencies of the diaphragm MEPs were unaffected by rTMS (Table 1).

First Dorsal Interosseous

Figure 6 summarizes the effects of rTMS on the amplitudes of the MEPs FD. After both 5Hz and 10Hz conditioning, the
MEPFDI increased significantly ($Q_3 = 10.2, P = 0.02$; and $Q_3 = 8.76, P = 0.03$, respectively) (Fig. 5B). The amplitude amounted to 135.4% (128.5–218.3%) and 124.8% (114.8–209.2%), respectively, for 5Hz and 10Hz during AFTER3. The low-frequency protocols had no significant effects on MEPFDI ($Q_3 = 2.46, P = 0.48$; and $Q_3 = 5.4, P = 0.14$, for aMT and rMT).

As for the diaphragm, the latencies of the FDI MEPs were unaffected by rTMS (Table 2).

**DISCUSSION**

This study showed that applying a high-frequency rTMS paradigm over the SMA in normal humans increases the excitability of the diaphragmatic corticospinal pathway. This finding points to the existence of modifiable functional connections between the SMA and M1DIA. Conversely, a low-frequency rTMS paradigm modified from a protocol that is effective at reducing corticospinal excitability when applied over the lateral premotor cortex (8) was not associated with depressed electrophysiological responses of the diaphragm or of the FDI when applied over the SMA. We discuss the interpretation of this finding in more detail below.

**Methodological Considerations**

Evidence supporting the appropriateness of the location selected for stimulation. We defined the SMA hot spots in our subjects according to a previously described and effective approach (18). In the seven subjects where MRI data later became available, the SMA hot spots actually lay in the expected anatomic area. Moreover, their Talairach coordinates resulting from the registration of individual native-space MRIs into Talairach space remained within the range that defines SMA, according to functional imaging studies (19). We are thus confident that we did deliver rTMS over the SMA. Of note, we used fewer conditioning pulses during our high-frequency 5-Hz protocol than Matsunaga et al. (18), because we wanted the 5Hz and the 10Hz protocols to produce an identical and safe (31) number of pulses. The effect that we observed in the FDI was however of similar magnitude to that observed previously by others (see Fig. 2 in Ref. 18).

Conditioning stimulation. The high-frequency conditioning rTMS protocols were conducted with a stimulation intensity set to 110% of the active motor threshold of the FDI, which was determined at the beginning of each experimental session. This represents a compromise between rTMS effectiveness, safety,
Lower intensities carry the risk of reduced aftereffects (8, 18), whereas higher ones carry the risk of spreading the stimulus over the primary motor cortex (6, 18). The absence of recordable MEPs during SMA conditioning suggests that M1 was not directly stimulated by the rTMS, although we cannot completely rule out the possibility of a subthreshold change in excitability that was not sufficiently potent to produce observable effects. Moreover, we did not observe changes in the amplitude of the MEPs when we used low-frequency rTMS at 110% of the resting motor threshold of the FDI (protocol rMT), namely the highest conditioning intensity that we used. This suggests that the magnetic field generated by rTMS at 110% of the active motor threshold, which is lower than that generated by rTMS at 110% of the resting motor threshold, is unlikely to have spread beyond the SMA (7, 8).

SMA conditioning intensities were calibrated for each subject based on the motor thresholds of the FDI, rather than on the thresholds for the diaphragm. This choice was made because diaphragm motor thresholds are much higher than the motor thresholds of hand muscles (29). We wished to ensure that the stimulation protocols remained within the parameters known to be safe for rTMS, which are based on skeletomotor muscle thresholds (31). It ensues that the conditioning intensity was notably closer to the FDI motor threshold than to the diaphragm motor threshold. This may explain why the facilitatory effects of 5Hz rTMS appeared weaker for the diaphragm than for the FDI (Figs. 4 and 6). In our subjects, 10-Hz rTMS did not facilitate the diaphragm response, in contrast to 5-Hz rTMS and in contrast with what was observed for the FDI. The reason for this is not obvious. Beyond the possibility of an unfavorable statistical signal-to-noise ratio, this could be due to the 10Hz paradigm being ill adapted to produce optimal facilitation. It has indeed been reported that too short (16) and too long (10) trains can fail to produce facilitation or even produce inhibition (10). This alone, or in combination with the conditioning intensity vs. threshold issue mentioned above, could have explain our observations. What remains is that, even if our SMA conditioning paradigm was underpowered for the diaphragm, it was nonetheless sufficiently potent to elicit a significant facilitation of the diaphragm response to corticospinal inputs. On this point, reducing the number of pulses compared with Matsunaga’s study (from 750 to 500), which we did to avoid changing too many stimulation parameters at the same time, did not render the stimulation ineffective. It is clear that, having now established that it is possible to facilitate the response of the diaphragm to TMS by rTMS conditioning of the SMA, we need to determine what exactly the best facilitatory paradigm would be.

Table 1. Diaphragmatic motor evoked potential latencies (ms) before and between 1 and 6 min, 6 and 11 min, and 11 and 16 min following the end of rTMS over the supplementary motor area

<table>
<thead>
<tr>
<th>Protocol</th>
<th>BEFORE</th>
<th>AFTER1</th>
<th>AFTER2</th>
<th>AFTER3</th>
<th>Q</th>
<th>P</th>
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<tbody>
<tr>
<td>5Hz</td>
<td>15.6 (15.4–16.3)</td>
<td>16.2 (15.2–16.6)</td>
<td>15.9 (15.5–16.6)</td>
<td>15.8 (15.5–16.5)</td>
<td>1.4</td>
<td>0.71</td>
</tr>
<tr>
<td>10Hz</td>
<td>16.6 (15.4–17.0)</td>
<td>16.2 (15.0–16.7)</td>
<td>16.1 (15.4–16.7)</td>
<td>16.2 (15.5–16.8)</td>
<td>0.04</td>
<td>0.99</td>
</tr>
<tr>
<td>aMT</td>
<td>15.3 (14.9–16.0)</td>
<td>15.3 (15.0–15.9)</td>
<td>15.3 (15.1–15.8)</td>
<td>15.4 (15.2–15.9)</td>
<td>1.33</td>
<td>0.72</td>
</tr>
<tr>
<td>rMT</td>
<td>15.9 (15.2–16.4)</td>
<td>15.9 (15.2–16.7)</td>
<td>15.9 (15.3–17.4)</td>
<td>16.0 (15.2–17.9)</td>
<td>5.09</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Values are medians (interquartiles). BEFORE, before repetitive transcranial magnetic stimulation (rTMS); AFTER1, between 1 and 6 min following the end of rTMS over the supplemental motor area; AFTER2, between 6 and 11 min following the end of rTMS over the supplemental motor area; AFTER3, between 11 and 16 min following the end of rTMS over the supplemental motor area. For description of protocols 5Hz, 10Hz, aMT, and rMT, see text.

Fig. 6. Amplitudes of the first dorsal interosseous motor evoked potentials (BEFORE) value at 1 to 6 (AFTER1), 6 to 11 (AFTER2), and 11 to 16 min (AFTER3) following the end of rTMS over the supplementary motor area for each of the 4 rTMS protocol. Boxes and whiskers depict median, interquartile, and range. Figures indicate the number of subjects who completed each session. See MATERIALS AND METHODS for detail regarding the stimulation parameters.
Neurophysiological Considerations

SMA-to-M1 connections. The 5-Hz rTMS over the SMA increased corticospinal excitability. This increase may be related to changes at either the cortical or spinal level. A number of arguments suggest that spinal facilitation is an unlikely explanation for the observed results. First, we observed changes in MEP_{DIA} amplitudes but not in latencies. This points to a facilitation of cortical rather than spinal origin (11, 12). Theoretically, either spatial or temporal facilitation at the level of the spine would decrease latencies (3). To our knowledge, spinal facilitation without a concurrent decrease in MEP latency has never been reported. Second, our rTMS conditioning intensity was only half of the stimulation intensity that was required to elicit a motor response through activation of the corticophrenic pathway originating within the SMA in the study by Sharshar et al. (25). Third, previous work by Matsunaga et al. (18) demonstrated that SMA stimulation with a higher number of pulses did not affect the H-reflex recorded from the flexor carpi radialis. This strongly suggests that changes in MEP amplitude after rTMS are not related to direct effects of corticospinal projections originating in the SMA. Taken together, these arguments strongly suggest that the effects of SMA conditioning observed in the present study were not the result of changes occurring at the spinal level. On the contrary, we attribute the observed changes to a modification by rTMS of the input received by M1DIA from the SMA. This is in line with the proposition of Matsunaga et al. (18), who suggested that rTMS changes the activity of SMA neurons, which subsequently modifies the influence exerted by SMA on M1 through intracortical connections.

Building on the evidence for anatomic connections between SMA and M1, our results provide the first evidence of functional connections between SMA and M1DIA, whose activity was enhanced by the 5Hz stimulation. Whether the remote effects of rTMS over SMA on M1 excitability are related to changes in excitatory or inhibitory processes remains unknown and to be assessed. Of note, the lack of cortical activity related to ventilation during spontaneous breathing (5, 15, 23) makes it unlikely that changes in respiratory cortical excitability occurred during data collection for this study.

Like others before us (18), we observed that the 5Hz protocol was followed by an enhanced response of the FDI to transcranial magnetic stimulation. This was also the case with the 10Hz protocol, which is a new finding since the only previous study of 10-Hz SMA conditioning was conducted in patients with Parkinson’s disease, who were evaluated for the effects on motor function and not on neurophysiology (1).

Resting state activity of the SMA-M1 connections. Low-frequency conditioning over the SMA had no inhibitory effect on corticospinal excitability. This could suggest that SMA does not exert a permanent, or “tonic,” facilitatory influence on M1DIA, during expiration (Fig. 4). If the SMA exerted a permanent facilitatory effect on M1DIA, a reduction in the size of the MEP_{DIA} would have been expected after a low-frequency stimulation paradigm. However, we cannot rule out the possibility of insufficiently potent SMA conditioning, particularly as we did not control for the effectiveness of the rMT or the aMT stimulation paradigms over different areas. Indeed, our low-frequency aMT protocol differed from the one of Gerschlager et al. (8) with respect to the number of pulses and the intensity of stimulation (8). The number of pulses that we used was lower than in this study, but it remained above the threshold value for effectiveness that was reported (namely 900) and thus should not be the limiting factor. The stimulation intensity that we used was not established relative to diaphragm threshold but to the FDI for safety purposes and differed from the intensity used by Gerschlager and co-workers (110% instead of 90% of active motor threshold). This might have reduced the effectiveness of the low-frequency rTMS protocols for both muscles. Although our evidence on this point is weak, it constitutes some of the only evidence on this issue and highlights the need for further work to delineate the nature of the interaction between SMA and M1.

Perspectives

In our view, this study demonstrates functional connections between SMA and M1DIA, in addition to the SMA–spinal pathway described for the diaphragm by Sharshar et al. (25). The functional significance of the SMA-to-M1DIA corticocortical connections remains to be elucidated. Experimental approaches to this question could include investigation into the effects of SMA conditioning on inspiratory load compensation, a circumstance known to involve both the SMA and M1DIA (23). A facilitatory SMA conditioning with rTMS should enhance the load compensation capacities.

ACKNOWLEDGMENTS

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GRANTS

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Table 2. First dorsal interosseous motor evoked potential latencies (ms) before and between 1 and 6 min, 6 and 11 min, and 11 and 16 min following the end of rTMS over the supplementary motor area

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<td>22.4 (20.8–23.8)</td>
<td>22.2 (20.7–23.5)</td>
<td>0.96</td>
<td>0.81</td>
</tr>
<tr>
<td>10Hz</td>
<td>22.2 (21.2–23.4)</td>
<td>22.6 (21.5–23.4)</td>
<td>22.6 (21.6–23.5)</td>
<td>22.7 (21.1–23.9)</td>
<td>2.52</td>
<td>0.47</td>
</tr>
<tr>
<td>aMT</td>
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<td>22.5 (21.2–23.3)</td>
<td>22.3 (21.5–23)</td>
<td>22.1 (21.4–23)</td>
<td>2.67</td>
<td>0.44</td>
</tr>
<tr>
<td>rMT</td>
<td>22.5 (21.1–23.6)</td>
<td>22.6 (21.1–23.4)</td>
<td>22.4 (21.4–24.1)</td>
<td>22.6 (21.5–23.9)</td>
<td>3</td>
<td>0.39</td>
</tr>
</tbody>
</table>

Values are medians (interquartiles). See Table 1 for definitions.
l’Université de Paris”, France. M. Raux was a Lavoisier scholar of the French “Ministère des Affaires Etrangères.”

DISCLOSURES

No conflicts of interest are declared by the authors.

REFERENCES


