Effect of voluntary facilitation on the diaphragmatic response to transcranial magnetic stimulation

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Sharshar, Tarek, Ewen Ross, Nicholas S. Hopkinson, Mark Dayer, Annabel Nickol, Frédéric Lofaso, John Moxham, Thomas Similowski, and Michael I. Polkey. Effect of voluntary facilitation on the diaphragmatic response to transcranial magnetic stimulation. J Appl Physiol 95: 26–34, 2003. First published March 14, 2003; 10.1152/japplphysiol.00918.2002.—We assessed recruitment curves of the surface diaphragm motor-evoked potential (MEP) after transcranial magnetic stimulation during relaxation and at three different levels of facilitation (20, 40, and 60% of maximal inspiratory esophageal pressure) in 10 healthy subjects (six young and four elderly). MEP amplitude recruitment curves varied between individuals during relaxation and at each level of facilitation. Amplitude recruitment curves during relaxation were reproducible in individual subjects. Inspiratory maneuvers caused a decrease in motor threshold and latency and an increase in MEP amplitude, positively correlated to the intensity of facilitation. These changes were similar in young and elderly subjects. The best fit for MEP amplitude recruitment curves for each condition was obtained with a Boltzmann model. The performance of repeated submaximal inspiratory maneuvers did not affect the amplitude recruitment curves of the relaxed diaphragm. We conclude that the recruitment curve of the diaphragm with transcranial magnetic stimulation is repeatable and changes consistently with facilitation and will, therefore, be a robust experimental tool for the investigation of supraspinal pathways to the diaphragm.

RELAXATION; DIAPHRAGM; MOTOR CORTEX; MOTOR-EVOKED POTENTIAL; CORTICAL NEURONS AND THE FUNCTION OF THE PYRAMIDAL TRACT (3, 6, 11, 13, 17, 27).

Cortical excitability can be investigated by assessing motor cortical stimulus-response curves to TMS (21, 28). Motor cortical threshold and stimulus-response curves to TMS have been assessed in various skeletal muscles, including the diaphragm (24, 25, 27). However, because the motor cortical representation of the diaphragm is small (leading to high-motor thresholds), the span of diaphragm recruitment curves has been limited by the power of the stimulator-coil combinations used (25). In addition, although voluntary facilitation of the diaphragm has previously been found to increase the response to TMS (4, 9, 34), changes in recruitment curves with different levels of facilitation have not been assessed.

The hypothesis underlying the present study was that different levels of voluntary activation of the diaphragm might change both the motor threshold for eliciting a response to TMS and the slope of the stimulus-response curve. Because motor-evoked potential (MEP) amplitude for other muscle groups is known to be affected by drugs (1, 12, 23, 33) and fatiguing (15) and nonfatiguing exercise (20), we reasoned that a detailed knowledge of the MEP stimulus-response curve of the diaphragm would be a necessary prelude to studies of the effect of respiratory disease on cortical excitability.

METHODS

Subjects

Ten healthy subjects (8 men and 2 women, 29–69 yr of age, height: 1.60–1.95 m, weight: 63–95 kg) were studied; all 10 participated in study II, whereas six younger subjects participated in study I. The ethics committee of the Royal Brompton and Harefield National Health Service Trust and National Heart and Lung Institute approved the protocol. All subjects gave written, informed consent.

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Measurements

Positioning of electromyogram electrodes. Subjects were seated comfortably in a high-backed chair, which supported the head and neck. The optimum surface electrode position for the diaphragm was determined by using transcutaneous electrical stimulation of the phrenic nerve at the posterior border of sternocleidomastoid muscle, at the level of the thyroid cartilage. Compound motor action potentials were obtained by using Ag-AgCl electrodes placed on the skin in the right sixth to eighth intercostal spaces and amplified with band-pass filters at 10 Hz to 10 kHz via a five-channel electromyogram (EMG) recorder (Medelec, Synergy, Oxford Instruments, Oxford, UK).

Pressure recordings. Esophageal (Pes) and gastric pressures (Pga) were measured by using a balloon catheter 110 cm in length (Ackrad Laboratories, Cranford, NJ). The catheters were connected to differential pressure transducers (range ±300 cm H2O; Validyne, Northridge, CA). Signals were digitized via a 12-bit NB-M10–16 analog-digital converter (National Instruments, Austin, TX) and acquired into a personal computer running LabVIEW software (National Instruments). Data sampling was at 100 Hz. Transdiaphragmatic pressure (Pdi) was derived electronically by subtracting Pes from Pga. Pes, Pga, and Pdi were displayed continuously on a computer screen visible to the subject and the operator.

TMS

TMS was performed by using a magnetic stimulator (Magstim 200 mono-pulse, The Magstim, Whitland, UK) and a double-cone coil (coil model no. P/N 9902-00, The Magstim). The coil was oriented in the anterior-posterior direction. To assess the best position for TMS, the scalp was marked with indelible ink on the vertex and on the midline (nasal-inion) every centimeter up to 3 cm forward and 3 cm backward from the vertex. At each midline point, TMS was performed five times at 100% stimulator output at relaxed end expiration. Then the scalp was marked every centimeter from the best midline position to 3 cm to the left. At each lateral point, TMS was performed five times at 100% stimulator output at relaxed end expiration.

Assessment of the Motor Threshold and Diaphragmatic Recruitment Curves to TMS

Resting motor threshold was defined as the lowest stimulation intensity (expressed in percentage of maximal stimulator output) that evoked a diaphragm MEP peak-to-peak amplitude of 50 μV in one-half of seven or more stimulations. To assess motor threshold, stimulator output was increased from 40% by 5% increments, until threshold was reached, and seven successive stimuli were delivered at each level of stimulation intensity.

To assess MEP recruitment curves, stimulator output was increased by 10% of threshold up to 100% of stimulator output. Five successive stimuli were delivered at each level of stimulation intensity. The order in which stimuli from threshold to 100% were delivered was varied in a random order to avoid any possible confounding effect of a simple incremental protocol. The interstimulus interval was ~30 s.

Study Protocols

Assessment of reproducibility of recruitment curves for the relaxed diaphragm (study I). The recruitment curves for the relaxed diaphragm were assessed on three different occasions over a 4-wk period. Pes, Pga, and Pdi were monitored during the last study in each subject. TMS was performed with the subject at relaxed end expiration. During the last session, it was possible to confirm that the subject was at functional residual capacity (FRC) during stimulation by using Pes.

Assessment of the effect of facilitation on motor threshold and recruitment curves (study II). We first assessed each subject’s maximal inspiratory pressure by measuring Pes during a maximal inspiratory effort, sustained for 2 s, against a closed airway from residual volume. The maneuver was repeated between 6 and 12 times, until no further increase in Pes was seen, and the greatest value was taken as the maximal inspiratory pressure (Pes\text{max}).

MEP recruitment curves in response to TMS were determined at different levels of facilitation, by asking the subject to repeat inspiratory maneuvers from FRC, aiming to achieve a target Pes. The investigator triggered TMS manually once the Pes had reached the desired plateau pressure, as judged visually from on-line monitoring. Three levels of Pes were targeted (20, 40, and 60% of Pes\text{max}) in a random order. Recruitment curves of the relaxed diaphragm were also assessed before and within 5 min of the end of the facilitation sessions.

Data and Statistical Analysis

For each subject, mean peak-to-peak amplitude of the first deflection and latency of the MEP at each stimulation intensity were calculated and expressed in microvolts and milliseconds, respectively. Motor threshold was expressed as a percentage of stimulator output. However, because individual MEP amplitudes varied considerably, as is the case in other muscles (29), subsequent data were normalized by dividing each MEP amplitude by the highest MEP amplitude and each latency by the shortest latency obtained during any of the resting or facilitated studies.

To exclude facilitated MEP in test 3, data were excluded if the Pes value at end expiration was more than ±2.5 cm H2O from the baseline; excluded traces were 2% of the total.

To assess the effect of facilitation, data were excluded if Pes did not match the ascribed levels of inspiratory Pes (20, 40, and 60% Pes\text{max}) with a ±5-cm H2O tolerance. To assess the intersubject reproducibility of the diaphragm MEP recruitment curves without facilitation, the values of MEP amplitude and MEP latency were normalized by dividing each MEP amplitude by the highest MEP amplitude and each latency by the shortest latency obtained during the three tests performed at rest. The derived data for all subjects were then pooled and averaged for MEP amplitude and latency.

During inspiratory maneuvers, we assessed the amplitude of Pes, Pga, and Pdi as well as Pes-to-Pes\text{max} (Pes/Pes\text{max} %), Pdi-to-maximal inspiratory Pdi (Pdi/maximal inspiratory Pdi %), Pes-to-Pga (Pes/Pga %), and Pes-to-Pdi (Pes/Pdi %) ratios. During the same maneuvers, we also assessed the twitch Pes, Pga, and Pdi elicited by TMS at 100% stimulator output.

Recruitment curves of tests 1, 2, and 3 were compared by using a two-factor ANOVA (stimulation intensity as within-group factor and subject or occasion as the between-group factor). Relaxed baseline, relaxed end, 20% facilitated, 40% facilitated, and 60% facilitated recruitment curves were compared by a two-factor ANOVA (stimulation intensity as within-group factor and subject or facilitation as the between-group factor). Where significant differences were noted, the effect of varying facilitation was compared by using post hoc analysis with Tukey’s multiple-comparison test. Relaxed
The best scalp position for the cone coil was over the vertex in seven subjects, vertex + 1 cm forward in two subjects, and vertex + 1 cm forward and 1 cm lateral in one subject.

RESULTS

The best scalp position for the cone coil was over the vertex in seven subjects, vertex + 1 cm forward in two subjects, and vertex + 1 cm forward and 1 cm lateral in one subject.
during the inspiratory maneuvers. In all cases, twitch Pes and twitch Pdi were negative and positive, respectively. Figure 2 shows the EMG and pressure responses to TMS in one young individual during relaxation and facilitation at 40% of the Pesmax. It is interesting to note that both the Pes and Pga are positive when the diaphragm was relaxed, suggesting the movement of antagonistic respiratory muscle groups that could have been unselectively activated by TMS. By contrast, Pes was negative, and a much greater Pdi was generated during inspiration, suggesting a greater relative activation of the diaphragm during TMS in this state.

Additionally, there was a significant relationship between the Pdi/maximal inspiratory Pdi and normalized amplitude of MEP elicited at 100% of TMS (Fig. 3).

Table 3 shows the individual values of motor threshold, minimal MEP latency, and maximal MEP amplitude obtained during each condition of study II. Figure 4 shows the pooled data stimulus-response curves for

Table 2. Pes, Pdi, Pes/maximal inspiratory Pes, Pdi/maximal inspiratory Pdi, Pes/Pdi, twitch Pes, and twitch Pdi during the assessment of facilitation effect on recruitment curves

<table>
<thead>
<tr>
<th></th>
<th>Relaxed Baseline</th>
<th>Facilitation 20%</th>
<th>Facilitation 40%</th>
<th>Facilitation 60%</th>
<th>Relaxed End</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pes, cmH2O</td>
<td>23.3 ± 3.1</td>
<td>45.3 ± 5.6</td>
<td>64.4 ± 8.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pdi, cmH2O</td>
<td>31.6 ± 15.8</td>
<td>54.1 ± 16.4</td>
<td>76.3 ± 26.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pes/MIPes, %</td>
<td>21.7 ± 2.4</td>
<td>37.7 ± 10.2</td>
<td>58.6 ± 11.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pdi/MIPdi, %</td>
<td>23.3 ± 7.9</td>
<td>39.8 ± 9.3</td>
<td>58.5 ± 14.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pes/Pdi, %</td>
<td>84.6 ± 24.9</td>
<td>92.9 ± 34.7</td>
<td>95.3 ± 35.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Twitch Pes, cmH2O</td>
<td>1.9 ± 0.9</td>
<td>−6.5 ± 2.9</td>
<td>−5.9 ± 3.3</td>
<td>−5.2 ± 2.6</td>
<td>2.2 ± 1.8</td>
</tr>
<tr>
<td>Twitch Pdi, cmH2O</td>
<td>2.1 ± 0.6</td>
<td>16.8 ± 8.1</td>
<td>14.0 ± 12.7</td>
<td>10.7 ± 6.2</td>
<td>3.0 ± 3.6</td>
</tr>
<tr>
<td>Twitch Pes/twitch Pdi, %</td>
<td>91.2 ± 46.7</td>
<td>−39.6 ± 13.3</td>
<td>−54.5 ± 33.9</td>
<td>−52.8 ± 16.5</td>
<td>86.5 ± 21.7</td>
</tr>
</tbody>
</table>

Values are means ± SD of each variable derived from the 10 subjects. The mean maximal inspiratory esophageal (Pes) and transdiaphragmatic pressures (Pdi) were 105 ± 16 and 135 ± 27 cmH2O, respectively. MIPes, maximal inspiratory Pes; MIPdi, maximal inspiratory Pdi; Pes/MIPes, Pdi/MIPdi, Pes/Pdi, and twitch Pes/twitch Pdi: ratios of Pes to MIPes, Pdi to MIPdi, Pes to Pdi, and twitch Pes to twitch Pdi, respectively.
MEP amplitude during relaxation and the three levels of facilitation studied. With increased facilitation, there was a significant decrease in motor threshold ($P < 0.001$) and minimum MEP latency ($P < 0.0008$), as well as a significant increase in maximal MEP amplitude ($P < 0.0001$). The two-factor ANOVA confirmed that facilitation has a statistically significant effect on the recruitment curve of the MEP amplitude ($P < 0.0001$) and latency ($P < 0.0001$). The MEP amplitude recruitment curve of the baseline-relaxed diaphragm did not differ from that of the 20% facilitated diaphragm and end-relaxed diaphragm. The MEP amplitude and latency recruitment curves of the 40 and 60% facilitated diaphragm were not statistically different but differed significantly from those of the baseline-relaxed diaphragm (Tukey’s multiple-comparison test, $P < 0.001$ and $P < 0.001$, respectively), 20% facilitated diaphragm ($P < 0.05$ and $P < 0.001$, respectively), and end-relaxed diaphragm ($P < 0.001$ and $P < 0.001$, respectively). Similar results were found for MEP latency recruitment curves. In addition, two-factor ANOVA showed that there was significant intersubject variability in recruitment curves of MEP amplitude ($P < 0.0001$) and latency ($P = 0.005$). This effect did not appear to be influenced by age (two-factor ANOVA, $P = 0.40$).

The goodness of fit ($r^2$) and comparison of fit ($F$-test) showed that, among the four models tested, the Boltzmann model was the best to fit MEP amplitude recruitment curves for each condition, as shown in Table 4 and Fig. 5. With increasing facilitation, there was a significant decrease in $V_{50}$ ($P < 0.0001$), which ranged from 93.1 (baseline) to 70.8 (facilitation 60%), and in slope ($P < 0.0001$), which ranged from 10.8 (baseline) to 8.7 (facilitation 60%).

**DISCUSSION**

We assessed the recruitment curves of the diaphragm MEP at relaxed end expiration (FRC) and the effect of increasing levels of inspiratory effort on diaphragm MEP recruitment curves. We demonstrated that the stimulus-response curve of the diaphragm motor area is reproducible and is influenced, like other skeletal muscles, by facilitatory efforts.

Our results raise several methodological issues. First, diaphragm surface MEP recording might have been contaminated by MEP of the adjacent intercostal muscles, particularly as these muscles can be costimulated by TMS. We did not use a needle to record the diaphragm MEP for two reasons. First, because of the risk of pneumothorax during repeated vigorous inspiratory effort required by the protocol, and second

**Table 3. Threshold and maximal amplitude and latency of relaxed and facilitated diaphragm motor-evoked potential in each subject during the assessment of facilitation effect on recruitment curves**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Baseline</th>
<th>20%</th>
<th>40%</th>
<th>60%</th>
<th>Relaxed End</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Threshold, %</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subject 1</td>
<td>65</td>
<td>55</td>
<td>50</td>
<td>45</td>
<td>60</td>
</tr>
<tr>
<td>Subject 2</td>
<td>65</td>
<td>55</td>
<td>50</td>
<td>50</td>
<td>70</td>
</tr>
<tr>
<td>Subject 3</td>
<td>70</td>
<td>60</td>
<td>55</td>
<td>55</td>
<td>60</td>
</tr>
<tr>
<td>Subject 4</td>
<td>65</td>
<td>55</td>
<td>50</td>
<td>50</td>
<td>60</td>
</tr>
<tr>
<td>Subject 5</td>
<td>80</td>
<td>65</td>
<td>65</td>
<td>60</td>
<td>80</td>
</tr>
<tr>
<td>Subject 6</td>
<td>80</td>
<td>75</td>
<td>70</td>
<td>70</td>
<td>75</td>
</tr>
<tr>
<td><strong>Amplitude$_{\text{max}}$, μV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subject 1</td>
<td>770.8</td>
<td>1606.6</td>
<td>2344.4</td>
<td>2881.1</td>
<td>477.1</td>
</tr>
<tr>
<td>Subject 2</td>
<td>770.1</td>
<td>1788.9</td>
<td>1975.5</td>
<td>2527.6</td>
<td>960.6</td>
</tr>
<tr>
<td>Subject 3</td>
<td>851.1</td>
<td>1788.9</td>
<td>1975.5</td>
<td>2527.6</td>
<td>960.6</td>
</tr>
<tr>
<td>Subject 4</td>
<td>851.1</td>
<td>1788.9</td>
<td>1975.5</td>
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<td>851.1</td>
<td>1788.9</td>
<td>1975.5</td>
<td>2527.6</td>
<td>960.6</td>
</tr>
<tr>
<td><strong>Latency$_{\text{min}, \text{ms}}$</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subject 1</td>
<td>16.3</td>
<td>15.5</td>
<td>15.3</td>
<td>13.7</td>
<td>15.5</td>
</tr>
<tr>
<td>Subject 2</td>
<td>16.4</td>
<td>15.3</td>
<td>14.8</td>
<td>14.7</td>
<td>16.6</td>
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<tr>
<td>Subject 3</td>
<td>16.4</td>
<td>15.3</td>
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<td>14.7</td>
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<td>16.4</td>
<td>15.3</td>
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<td>16.4</td>
<td>15.3</td>
<td>14.8</td>
<td>14.7</td>
<td>16.6</td>
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</table>

Threshold is expressed as lowest %stimulator output eliciting a diaphragm motor-evoked potential $>50$ μV.

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because of the possibility of signal contamination, de-
spite the use of a needle electrode (8). We are not able,
on the electrophysiological traces, to rule out such
contamination. However, several arguments support
our view that contamination from intercostal muscles
is minimal. First, the position of the surface electrodes
was optimized by using electrical stimulation of the
phrenic nerve. Their position was similar to that pre-
viously reported and that known to provide largely
uncontaminated EMG signals from the relaxed costal
diaphragm during cervical magnetic stimulation (31)
and TMS (5). Second, the active and reference elec-
trodes were set 2 cm apart, thus reducing the risk of
cross talk (8). Third, Pes and Pdi recordings demon-
strate that, during facilitation, the diaphragm was
preferentially activated, particularly at the highest
level of inspiratory effort. In fact, there was signi-
ificant correlation between the increase in MEP elic-
titated at 100% of TMS and the magnitude of the Pdi
generated during the facilitatory effort as a propor-

Fig. 4. Relationships between TMS intensity
and diaphragm MEP amplitude in 4 different
conditions: unfacilitated baseline at beginning
(●) and end (○) and facilitation of 20% (●), 40%
(○), and 60% (■) of the maximal inspiratory
Pes. The order of facilitation was randomly
determined. In each test, TMS intensity was
randomly delivered. Each point corresponds to
mean size (±SD) of MEP in 10 subjects. By
using two-factors ANOVA (TMS intensity and
test), increase of facilitation induced a signi-
ficant change in the recruitment curves (P <
0.0001).

Table 4. Fitting of Boltzmann sigmoid, second-order polynomial, sigmoid, and linear models

<table>
<thead>
<tr>
<th>Model</th>
<th>Goodness of Fit ($r^2$)</th>
<th>Comparison of Fit ($F$-test)</th>
<th>Comparison of Fit, $P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boltzmann sigmoid</td>
<td>0.77–0.82</td>
<td>4.1–16.77</td>
<td>0.04 to $&lt;10^{-3}$</td>
</tr>
<tr>
<td>Second-order polynomial</td>
<td>0.76–0.81</td>
<td>33.3–149.1</td>
<td>$&lt;10^{-3}$ to $&lt;10^{-4}$</td>
</tr>
<tr>
<td>Sigmoid model</td>
<td>0.59–0.72</td>
<td>6.1–34.5</td>
<td>$&lt;0.03$ to $&lt;10^{-3}$</td>
</tr>
<tr>
<td>Linear</td>
<td>0.66–0.79</td>
<td></td>
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</table>

Comparison of fit was done between Boltzmann sigmoid model and each other model. For each model, the ranges of $r^2$, $F$, and $P$ values for the recruitment of each condition (relaxed baseline and end, and 20, 40, and 60% facilitation) are shown.

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tion of maximal inspiratory Pdi ($r^2 = 0.27; P = 0.003$; Fig. 3).

The diaphragm response to TMS has previously been investigated with circular or figure-of-eight coils (13, 25). After some preliminary pilot work, we opted to use a double-cone coil during this study to elicit a cortical response, in preference to a circular or figure-of-eight coil. In this study, in the relaxed diaphragm, the motor threshold was $70\%$ in four of our subjects, whereas it was $75\%$ in all nine subjects previously studied with a circular coil (27). The double-cone coil, therefore, allows a more detailed characterization of the stimulus-response curves of both the relaxed and facilitated diaphragm.

The effect of relaxation and facilitation on diaphragm response to TMS has been previously investigated (11, 25–27, 34). Recruitment curves have been previously presented (27) over a very limited range of intensities due to the high-motor thresholds with TMS by using a circular coil. The present study is the first to comprehensively investigate recruitment curves over wide ranges of stimulation intensity and at a different level of underlying inspiratory activity. The recording of Pdi allowed objective assessment of diaphragm contraction. This is of interest because the TMS field inevitably activates areas of the cortex controlling other muscles, including the extra-diaphragmatic inspiratory muscles and the expiratory muscles. During the facilitation maneuvers, the mean Pes/Pdi was between 84.6 and 95.3%, suggesting a predominantly isolated diaphragm contraction (as opposed to a combined inspiratory-expiratory maneuver). The twitch Pes-to-twitch Pdi ratio was $0.86:0.91$ during unfacilitated maneuvers but ranged between $-0.38$ and $-0.58$ during facilitated maneuvers. In a previous study, our laboratory found the twitch Pes-to-twitch Pdi ratio to lie between $-0.53$ and $-0.62$ for four different modes of peripheral phrenic nerve stimulation (18). These data confirm that the responses obtained during facilitation are substantially more similar to the responses obtained by isolated diaphragm contraction than those obtained during unfacilitated stimulation. It could be argued that the MEPs obtained in the unfacilitated state, in fact, simply originate from the abdominal muscles. However, we believe that this is unlikely to be the case, both because we have validated our recording site (31) and because we have previously shown that action potentials recorded after abdominal muscle contraction elicited by magnetic stimulation at T10 have a different morphology from those produced by phrenic nerve stimulation (19). Finally, we caution that, because of twitch potentiation (32), the quantitative interpretation of interpolated twitch to TMS is difficult and does not allow the assessment of the level of voluntary corticospinal output in the same way as interpolated peripheral stimuli.

When determining motor threshold, a precise definition is required, in terms of stimulus intensity, amplitude of MEP, minimal number of MEP above a predetermined amplitude, and the global number of stimuli applied (22). In previous studies examining diaphragmatic responses to TMS, a precise definition for threshold was not provided (3, 4, 11, 17, 27) or was partially provided (34). In the present study, motor threshold was defined as the lowest stimulation intensity (expressed in percentage of maximal stimulator output) that evoked a diaphragm MEP peak-to-peak amplitude of 50 $\mu$V in one-half of seven or more stimulations. The minimal MEP amplitude of 50 $\mu$V required at thresh-
old in the present study was within the range previously reported (i.e., from 20 to 100 μV) (16, 22).

Earlier studies have used different methods of diaphragm facilitation, including making a forced deep breath (34), breathing 1–3 liters above FRC (4), and achieving various degrees of static diaphragm contraction, judged from Pdi (25) or from diaphragmatic EMG (27). In the present study, the level of inspiratory effort was assessed by measuring inspiratory Pes, which is closely related to the force generated by diaphragm contraction. The stepwise increment of Pes and Pdi was similar and ~20% of Pes max and maximal inspiratory Pdi, respectively. Therefore, levels of facilitation were effectively 20, 40, and 60%, irrespective of the use of either Pes or Pdi as the index of diaphragm activation. We opted to use the Pes, rather than Pdi, as inspiratory Pes is closely related to inspiratory mouth pressure (10). The measurement of mouth pressure, a noninvasive measurement, is likely to make TMS studies of the respiratory muscles better tolerated in patients with neurological and respiratory disease.

Interindividual variability of MEP recruitment curves has long been appreciated. In the diaphragm, interindividual variability has been described for MEP amplitude at a single stimulus intensity (17, 27). However, there has been no previous systematic study of the TMS stimulus-response curve for the diaphragm. Despite interindividual variability, intrasubject diaphragmatic stimulus-response curves were highly reproducible. Figure 1 shows that, in test 3, the motor threshold was higher than in tests 1 or 2, for reasons that are not clear cut but that are not statistically significant.

Our results confirm that facilitation modifies the overall diaphragm response to TMS, with a decrease in motor threshold and MEP latency and an increase in MEP amplitude. Interestingly, this effect is positively correlated to the intensity of facilitation. The gradient of the stimulus-response curve during facilitation is closer to that found in proximal limb muscles (in which an increase in MEP amplitude continues up to 75% of motor voluntary contraction) than to the results in hand muscles, in which MEP increases little >10% of motor voluntary contraction (9). In addition, facilitated recruitment curves plateaued at high-TMS intensity, indicating that supramaximal TMS for the diaphragmatic motor cortex is possible. This observation may be of use in future clinical studies. We also found that relaxed and facilitated recruitment curves best fitted with the Boltzmann sigmoid model, as shown in some other muscles. Facilitation induced significant changes in V50 and slope. This finding raises a physiological issue regarding the type of motoneurons involved during facilitation. Indeed, the facilitation-related changes of both V50 and slope might have been due to the stimulation of a new group of neurons rather than a simple recruitment of a larger number of pyramidal tract neurons in the motor cortex. In the latter case, the recruitment curve would have been shifted to the left, without any change in the slope. In the former case, voluntary facilitation could have involved facilitatory intracortical neurons, whose activation would have modulated the motoneurons’ responses. It is also conceivable that facilitation took place at either the brain stem or spinal level. In addition, we found that the full effect of facilitation on the recruitment curve shape occurred for a level of 40% of maximal contraction. This finding may be useful for the design of further studies on facilitated diaphragm recruitment curves in healthy subjects or patients.

Age may influence the skeletal muscular response to TMS, notably through changes in the intracortical circuits (14). However, the influence of age on diaphragm response to TMS has not yet been studied. We found that the effect of facilitation on the diaphragm recruitment curves did not differ between young and elderly subjects. This suggests that age has, at most, minor influence on the excitability to TMS of cortical motoneurons controlling the diaphragm.

Finally, it is well established that submaximal or maximal contraction of skeletal muscle is followed by a MEP potentiation, which usually disappears after 10 min, and then a MEP depression, which can last at least 30 min. Our laboratory has previously demonstrated that a depression in diaphragm MEP amplitude can be induced by exercise (30), and it is conceivable that repeated facilitating efforts could also induce a MEP depression. We did not systematically assess the effects of submaximal or maximal diaphragm contraction on diaphragm MEP amplitude; however, we found that repeated facilitatory maneuvers at 60% of maximal diaphragm voluntary contraction had no carryover effect on the subsequent unfacilitated diaphragm recruitment curve. However, these data should not be regarded as sufficiently strong to refute the hypothesis that central diaphragm fatigue could be induced by strenuous voluntary efforts.

We conclude that, in healthy subjects, the motor threshold and stimulus-response curve after TMS of the motor cortex are altered by voluntary activation of the diaphragm, in a similar fashion to that reported for proximal upper limb muscles. Subsequent responses in the resting diaphragm were not altered by prior facilitatory maneuvers. The response to TMS in the resting diaphragm was also highly reproducible. These observations may be helpful to determine physiological and pathophysiological changes in cortical excitability and to assess plasticity of the motor cortex controlling the diaphragm.

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