Validation of improved recording site to measure phrenic conduction from surface electrodes in humans

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MAGNETIC STIMULATION of the phrenic nerves, first introduced in 1988–1989 under the form of cervical magnetic stimulation (CMS) (34), has become an established tool to study diaphragm function in health and disease. CMS is painless and easy to perform, thus alleviating some of the pitfalls of electrical phrenic stimulation (ES), although the two techniques bring complementary information. Of note, several techniques are now available, including unilateral magnetic stimulation (UMS) (24), bilateral anterior magnetic phrenic stimulation (23), and anterior magnetic stimulation (27, 28, 35). The mechanical response of the diaphragm to those stimulations is well described (15, 27, 36), and there have been various pathophysiological and clinical applications.

Besides giving access to a nonvolitional measure of the diaphragm contractile efficiency, phrenic nerve stimulation is also a unique tool to assess the function of the phrenic nerve itself. This is achieved by measuring the latency of the diaphragmatic motor response after stimulation, or phrenic nerve conduction time (PNCT), and in fact has been the primary application of the technique (8, 25). The PNCT derived from electrical stimulation in the neck (ES-PNCT) is known from numerous studies (see Table 1 in Ref. 35) and revolves around a 7-ms figure. Measuring ES-PNCT is necessary to make the diagnosis of many types of phrenic nerve damage and is instrumental to their follow-up. The absence of diaphragm response to ES of the phrenic nerve should be the only rigorous criterion to attribute diaphragm abnormalities to phrenic nerve paralysis, strictly speaking (10). However, ES can be painful and difficult to achieve because of the morphology of the subject or for anatomic reasons. Indeed, the phrenic nerve is small and not always fully constituted at the base of the neck (14) and hence can be difficult to locate. Therefore, ES carries a risk of falsely negative results. In addition, ES cannot stimulate the share of motor fibers destined to the diaphragm that constitute the accessory phrenic nerve (29).

Because magnetic stimulation gives easier access to the nerve, it should have become popular for phrenic nerve conduction studies. This has not been the case, and several teams using magnetic stimulation mentioned difficulties in collecting the related diaphragm
electromyogram (EMG) signals. Our finding of CMS-PNCTs shorter by an average of 1 ms than ES-PNCTs (35), which we attributed to a more distal depolarization of the nerve with CMS, has been ascribed by others to a contamination of the EMG signal by the unavoidable contraction of coactivated extradiaphragmatic muscles (17). This phenomenon has also been reported for UMS (16) and has led some to deem unreliable the measurement of PNCTs using surface recordings in response to magnetic stimulation. Of note, contamination of the signal picked up by electrodes aimed at recording a diaphragmatic response (from top to bottom and right to left, pectoral major, serratus anterior, latissimus dorsi). It can be seen that these muscles are relatively far from the Sant pair of electrodes, which lies in a window free of muscles possibly activated by a stimulation of cervical roots or the corresponding nerves. In both A and B, bold vertical lines mark the anterior axillary line and the midclavicular line, from left to right. Artwork by Merri Scheitlin-Nordman.

Because intramuscular electrodes have a very limited sampling volume and hence carry a low risk of signal contamination through volume conduction, we designed this study to test the above hypothesis. We compared surface recordings of diaphragm contractions induced by CMS and ES with concomitant recordings obtained from needles inserted in the diaphragm, considering contamination present if a surface PNCT was markedly shorter than the corresponding needle one.

MATERIALS AND METHODS

Subjects

Five healthy volunteers participated in the study (Table 1), after completion of the French legal procedure for studies in human volunteers. The study was approved by the Comité Consultatif de Protection des Personnes se prêtant à des Recherches Biomédicales, Pitié-Salpêtrière. All subjects were informed in detail of the purpose of the study and methods used and gave written consent. They were studied sitting on a chair equipped with headrests, abdomen unbound. In each subject, the right and the left phrenic nerves were studied sequentially, providing 10 sets of data for analysis.

Methods

Abdominal displacements. Changes in abdominal circumference were monitored with a strain gauge consisting of a piezoelectric sensor encased in a molded box 3 cm × 3 cm

Table 1. Physical characteristics of the subjects studied

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age, yr</th>
<th>Height, m</th>
<th>Weight, kg</th>
<th>BMI, kg/m²</th>
<th>Neck Morphology</th>
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<td>34</td>
<td>1.83</td>
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<tr>
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<td>68</td>
<td>21.0</td>
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<tr>
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<td>26.4</td>
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</tr>
<tr>
<td>5</td>
<td>45</td>
<td>1.95</td>
<td>100</td>
<td>26.3</td>
<td>Thick-short</td>
</tr>
</tbody>
</table>

All subjects were male. BMI, body mass index.
In all subjects, two needle electrodes were inserted in each 25 mm, sampling surface 0.03 mm²; Medelec, Old Woking, UK). Bipolar concentric needle electrodes (diameter 0.3 mm, length on the muscle mass of the pectoralis major, on the midclavicular line or adjacent to Sax. Electrode insertion and positioning were thus kept roughly constant (5, 34) in that the subjects kept the neck in a neutral position instead of bending it forward. The handle of the coil was directed caudally and held either parallel to the vertebral column or at a 45° angle. Two different coil positions were used, centered over the spinous process of the seventh cervical vertebra (C₇-CMS) (“traditional” technique) or lower, over the spinous process of the second thoracic vertebra (T₂-CMS). Stimulation was delivered by using the maximal output of the stimulator, after building simplified recruitment curves (stimulations at 60, 85, 90, and 95% of maximal output).

Conventions for data analysis. The term “M-wave,” abbreviated as “M-w,” will henceforth be used to designate motor responses to phrenic stimulation. M-w latencies were measured as the time elapsed between the stimulus and the onset of the action potential, namely, the first departure of the signal from baseline. Data were rejected if the return of the signal to baseline after the stimulation was ambiguous or if there was evidence of contamination by an electrocardiogram complex. M-w latencies provided thereafter correspond to the average of five accepted stimulations. M-w amplitudes were measured from peak to trough. For S_ant and S_ax, signal contamination was deemed present when, in two or more of the five stimulations analyzed, the surface M-w latency was shorter than the corresponding needle M-w latency by more than 0.75 ms or closer to the latency measured in S_post or S_up than to the latency measures at the relevant needle electrode. The first of these criteria stemmed from the fact that needle electrodes are known to possibly provide longer latencies than surface electrodes for a given muscle (13, 22) with the second criterion stemming from the experience and common practice in routine clinical neurophysiology at our institution. Statistical analysis. Analysis was performed by using the SuperAnova software (Abacus Concept, Berkeley, CA) on an Apple Macintosh computer. Differences between M-w latencies were assessed by using an analysis of variance for repeated measures followed by a Fisher’s protected least-square difference test. Differences were considered significant when the probability P of a type I error was 0.05 or less. Mean ± SE values correspond to the results from 10 phrenic nerves.

RESULTS

The M-w latencies measured at different sites with different stimulation techniques are depicted by Fig. 2. No right-to-left difference could be detected.
Fig. 2. Phrenic nerve conduction times (y-axis) measured at the different recording sites (x-axis) with different stimulation techniques (z-axis). C7-CMS, cervical magnetic stimulation (CMS) at the level of the 7th cervical vertebra; T2-CMS, CMS at the level of the 2nd thoracic vertebra; ESphren, specific electrical stimulation of the phrenic nerve; ESbrach, electrical stimulation of the phrenic nerve with brachial plexus costimulation. Vertical columns represent mean values from 10 phrenic nerves studied and are topped by a representation of 1 SE. With CMS (both C7 and T2) and ESphren, no significant difference was detected between PNCTs measured at Sant vs. Nant or at Sax vs. Nax. Only with ESbrach did contamination occur often enough to make such differences appear.

Surface Latencies

The latencies measured at Sant and Sax were in the normal range, not significantly different from one another (6.8 ± 0.25 ms vs. 6.7 ± 0.35 ms, respectively; \( P = 0.678 \)) and similar to data obtained in our laboratory with the same technique (35). Analogous observations were made for C7-CMS (6.0 ± 0.25 ms vs. 5.7 ± 0.35 ms, \( P = 0.201 \)). Moving the coil down (T2-CMS) did not significantly modify the latencies measured at Sant and Sax.

At both Sant and Sax, the C7-CMS-related latencies were significantly shorter than the ES-related ones (Sant: 6.8 ± 0.25 ms vs. 6.0 ± 0.25 ms, respectively, \( P < 10^{-4} \); Sax: 6.7 ± 0.35 ms vs. 5.7 ± 0.35 ms, \( P < 10^{-4} \)). Again, this finding is usual in our hands, and the values are similar to previous ones (35).

The responses at Sup and Spost were always of shorter latencies than at other sites (Fig. 2).

Needle Latencies

At Nant, the M-w latency of the response to ESphren averaged 7.8 ± 0.7 ms vs. 6.2 ± 0.13 ms in response to CMS (\( P < 10^{-4} \)). At Nax, those figures were 7.2 ± 0.35 and 6.2 ± 0.16, respectively (\( P < 10^{-4} \)). Again, there was no difference between C7-CMS and T2-CMS.

Contamination

According to our convention (see MATERIALS AND METHODS), contamination of the signal picked up by Sax occurred in 1 of 10 phrenic nerves with ESphren, 5 with ESbrach, 2 with C7-CMS, and 1 with T2-CMS. At Sant, contamination was never observed with ESphren, C7-CMS, or T2-CMS and was clearly present in two cases with ESbrach. Figure 3 gives examples of uncontaminated recordings obtained with Sant in response to ESphren, ESbrach, and CMS.

With CMS, the average latency at Sant was not significantly different from the one at Nant (\( P = 0.19 \)). This was also the case for the difference between Sax and Nax (\( P = 0.20 \)). With ESphren, the average latency at Sant was significantly shorter than that at Nant (\( P = 0.04 \)), with a similar finding for Sax and Nax (\( P < 10^{-4} \)) (Fig. 2).

The latencies measured at Spost and Sup were always significantly shorter than those measured at all the other sites with C7-CMS, T2-CMS, and ESphren (\( P < 10^{-4} \)). With ESbrach, the M-w latency measured at Sax when present was significantly shorter than that measured at Nax (\( P < 10^{-2} \)) and not significantly different from that measured at Sup (\( P = 0.14 \)) and Spost (\( P = 0.48 \)), illustrating contamination of the axillary electrode by signals arising from extradiaphragmatic muscles. Conversely, the latency measured with ESbrach at Sant was, on average, longer than at Sup and Spost (\( P < 10^{-4} \) in both cases, Fig. 2). Only in two phrenic nerves was the latency at Sant clearly shorter than Nant and close to the latency at Spost or Sup, showing that contamination is possible but not systematic.

In cases in which contamination was considered likely with a given stimulation technique at maximal intensity, decreasing stimulation intensity did not modify the observed pattern.

Supramaximality

Expectedly according to the chosen experimental design, ESphren provoked supramaximal responses at Nax and Nant in all subjects. This was also the case at Sax and Sant when only uncontaminated data were considered. Simplified recruitment curves showed that this
was also the case for C7-CMS and T2-CMS, again when only those $S_{ant}$ and $S_{ax}$ responses that were deemed uncontaminated were taken into account.

**DISCUSSION**

Our results show that it is possible to record an uncontaminated diaphragm response to electrical and magnetic phrenic nerve stimulation with the use of surface electrodes, which should make CMS a reasonable means of determining PNCTs in the clinical setting. In addition, the comparison of needle ES-PNCTs and needle CMS-PNCTs supports the notion that ES and CMS depolarize the phrenic nerve in a different manner or at a different site.

**Comparison With Previous Data From Our Laboratory**

The PNCTs obtained in the present study are comparable to those reported by our laboratory several years back with the same techniques (35). As noted by Luo et al. (17), the PNCT values reported in earlier studies from our laboratory were longer (32, 33). This is likely accounted for by the lower power of the stimulator used in these studies. Indeed, increasing the power of a magnetic pulse applied to a peripheral nerve results in a distal shift of the site of depolarization and hence in a shortening of the latency of the evoked motor potential (19, 30), independently of the supramaximal nature of the stimulus.

**Signal Contamination**

**Relevance.** With CMS and UMS, the coactivation of extradiaphragmatic muscles is unavoidable (15, 17, 33). With ES, it can be difficult to dissociate the stimulation of the phrenic nerve from that of the brachial plexus (2, 18). If this coactivation perturbs the signal picked up by “diaphragm” electrodes, then this signal cannot be used as an index of the function of the phrenic nerve. Reviewing the literature, Luo et al. (18) remarked several studies possibly affected by this problem. The issue is thus worth clarifying.

**Reality of the risk of signal contamination.** In response to nerve stimulation, a pair of surface electrodes principally records the activity of the muscle that lies underneath it. It can also record activity arising from distant, coactivated muscles, because of volume conduction of the signal through the adjacent tissues. This can occur for the diaphragm, as shown by several elegant studies by Luo et al. (16–18). In a first paper, they compared CMS and ES in terms of M-w recorded by a pair of surface electrodes placed in the sixth to eighth intercostal space on the anterior axillary line and by a special esophageal electrode (17). They noted shorter latencies in response to CMS than to ES at both sites. With CMS, the surface tracings consistently showed small short-latency waves preceding the main action potential, which were attributed to contamination from extradiaphragmatic activity (the...
exact source of contamination was not looked for). In addition, the amplitudes of the CMS-related M-w at the esophageal site were never bigger than their ES-related counterparts, whereas this was common at the surface site, which was also considered as a sign of contamination. In a study focused on ES, stimulating the brachial plexus purposely instead of the phrenic nerve shortened the M-w latency and changed its shape (18). This was also interpreted as a sign of signal contamination. Luo et al. quoted a study (2) mentioning that surface electrodes could pick up a small amount of diaphragm activity during contralateral phrenic stimulation and another one reporting signal contamination arising from the serratus anterior in response to ES (21).

Possibility to record an uncontaminated diaphragm signal. Before the present study, several arguments already supported this possibility. With ES, this is not disputable, and although the possibility to pick up a contralateral diaphragm activity during unilateral phrenic stimulation has been mentioned, seemingly in a single study (2), this is very rare. Over many years and among several hundreds of subjects and patients, we encountered it only twice, in extremely thin subjects with small thoraxes. Laghi et al. (15) mentioned that electrodes placed over the sternocleidomastoid, trapezius, parasternal, and pectoralis muscles did not always pick up activity in response to CMS. Because slight changes in stimulation technique over time can influence the degree of activation of contaminant muscles and because this is generally not controlled for, signal contamination is likely a source of a low reproducibility. We recently reported a case of demyelinating phrenic neuropathy (12) in which CMS and ES latencies varied together over 2 yr, which seems to us an argument against contamination. Another argument is the observation of an abolished response to CMS in patients with phrenic nerve palsy (6). We used this argument in a previous study [complete absence of response from surface electrodes in six patients with amyotrophic lateral sclerosis and complete diaphragm paralysis diagnosed on mechanical grounds (1, 35)]. Luo et al. (17) rebutted this position by postulating that, in our patients, the disease could have affected extradiaphragmatic muscles as well as the diaphragm. This is not likely to have been the case. Indeed, in this particular set of patients, the muscles of the upper rib cage did not appear atrophied, as illustrated by their ability to expand the rib cage in response to CMS (1). In a subsequent study, we described amyotrophic lateral sclerosis patients with diaphragm dysfunction and attenuated but persistent M-w in response to CMS (31). The latencies of these M-w could exceed 10 ms, probably in line with denervation atrophy (9). It is difficult to imagine which upper rib cage muscle activated from CMS could respond with such a delay.

The present study brings several additional arguments. First, the latencies determined from S_ant and S_ax were in the vast majority of cases clearly longer than the latencies measured at S_up and S_post (Fig. 3). This observation means that the amplitude of the corresponding signal did not incorporate a contaminant component, all the more so that, second, the M-w recorded at S_post and S_up tended to have higher amplitudes than that recorded at N_ant and N_ax. Third, and principally, the latencies measured by intramuscular diaphragmatic electrodes were, as a rule, closer to the latencies measured by their surface counterparts than to the latencies measured at S_post and S_up. These criteria (see MATERIALS AND METHODS) were not met in only a limited number of cases with CMS (see RESULTS) at S_ax and never at the S_ant site. By contrast, an important difference between surface and needle latencies was noted with ES, in the cases in which the S_ax or, less frequently, S_ant signals had latencies close or equal to the S_up or S_post ones. This substantiates the fact that needle diaphragm electrodes are hardly sensitive, if at all, to contamination. Of note, Zifko et al. (37), using CMS and needle electrodes, already reached conclusions similar to ours.

Mechanisms of the discrepancies between studies. The reasons why our conclusions differ from that of Luo et al. (17, 18) probably lie in the recording techniques. In our setup, the electrodes are set <2 cm apart, compared with the 3- to 5-cm figure mentioned by Luo et al. in one of their studies (18) and the average 5-cm figure generally encountered in the literature. Moving the electrodes closer to one another should make them much more likely to record near-field potentials than far-field ones (4), as observed, for the diaphragm, by Markand et al. (21). The second major difference between our setup and commonly used ones is the location of the electrodes. The most classical placement is the anterior axillary line, used in all the studies by Luo and co-workers (16–18). The intercostal space over which the electrodes are placed varies from the sixth to the eighth one. It is clear from anatomy (Fig. 1) that this implies a high probability for the electrodes to directly overlay muscle fibers from the serratus anterior, particularly so when the electrodes are wide apart (21). The higher the electrodes sit on the thorax, the closer they will be to the muscle mass of the pectoralis major. This is well described in the ES study by Markand et al. (21), in which contamination was frequent when the electrodes were either high or posterior. For instance, contamination was apparent in a derivation comprising an active electrode in the eighth intercostal space 5 cm behind the axillary line but was absent when the active electrode was in the same intercostal space, close to the costochondral junction. Low and median recording sites were free of contamination. Our results with CMS fit the observations of Markand et al. (21). In the way our electrodes are positioned at S_ant, the diaphragm should be closer to them than the most anterior insertions of the serratus anterior and latissimus dorsi and than the lowest insertions of the pectoralis major. The only remaining source of cross talk should be insertions of abdominal muscles intermingled with the diaphragm at the inner face of the lower ribs, but these cannot be activated by CMS.
Differences Between Surface and Intramuscular Recordings

The PNCTs from needle electrodes tended to be longer than their surface counterparts (Fig. 2). Similar observations are traditional in other muscles (13), although not constant (22). A needle electrode can provide falsely long latencies if it samples slow motor units (the risk being greater with muscles comprising many slow fibers, such as the diaphragm) or if its tip is distant from the motor plate of the sampled units (13). By contrast, surface electrodes give a more global idea of the muscle response (compound action potential) and should not miss the fastest motor units. It is interesting to note that the smallest needle-surface difference was observed with CMS (Nant vs. Sant 0.2 ms, not significant; Nan vs. San 0.5 ms, not significant). This supports the notion that CMS activates fast myelinated fibers more easily than does ES (6, 7).

Differences Between ES and CMS

In a previous study (35), our laboratory reported that CMS provided PNCTs shorter than ES and attributed this finding to either the preferential activation of fast fibers by magnetic stimulation or a more distal nerve depolarization site. Signal contamination would invalidate these interpretations. A major argument for the reality of the ES-CMS difference comes from the results obtained here with the needle electrodes. Indeed, both at Nant and Nan, the CMS-PNCTs were shorter than the ES-PNCTs by 1–1.5 ms, which exactly fits the difference between surface CMS and ES PNCTs found in this study and the previous one. It also fits the latency difference reported for other nerves [1.4 ms for Ono et al. (26); 1.2 ms for Schmid et al. (30)]. The underlying mechanism is considered to be a more distal site of depolarization with magnetic stimulation than with electrical stimulation. Therefore, the present study consolidates our conviction that CMS activates the phrenic nerve more distally than ES. Although artifacts of electronic source cannot be ruled out to explain the differences in shape that can at times be observed between M-w obtained with ES and CMS (Fig. 3), alternative mechanisms and site of depolarization probably account for most of these differences, which have been reported for other muscles as well.

From the results of the present study, which was designed specifically in “latency” terms, going further carries the risk of overinterpretation. Nevertheless, we have noted that the amplitude of uncontaminated CMS diaphragm M-w tended to be greater than that of ES M-w. This was also true for needle electrodes, ruling out signal contamination as an explanation. The number of phrenic fibers depolarized by ES could thus have been inferior to that depolarized by CMS. If our contention of a more distal depolarization by CMS is true, the stimulation of an accessory phrenic nerve would explain the higher M-w amplitude, because ES cannot reach this contingent of fibers. An accessory phrenic nerve is common (29) in humans and can be sufficient to sustain ventilation, as illustrated by the recovery of a diaphragmatic respiration described after phrenic nerve crushing in the neck for the treatment of tuberculosis (see Ref. 14). Such a hypothesis would imply that combining ES and CMS could allow one to study, in humans, the function of the accessory phrenic nerve, otherwise not accessible.

Practical Consequences

From their conclusion that surface electrodes are unreliable in response to magnetic phrenic nerve stimulation, Luo et al. (16) have advocated the use of esophageal recordings to measure PNCTs. Such recordings are not, however, without inconveniences, beyond their practical limitations. An esophageal recording cannot separate the responses of the two hemidiaphragms during bilateral stimulation. Signal contamination is not impossible (16, 17). The crural diaphragm is preferentially sampled, as opposed to the costal diaphragm with surface electrodes. Given that the crural diaphragm is predominantly innervated by the upper contingent of the cervical phrenic motoneurons (C3–C4) (20) whereas the costal diaphragm is innervated by the lower contingent, CMS coupled with surface recordings could be the safest way to activate and record the greatest possible number of phrenic fibers, which is important for strength and activation studies.

We wish to emphasize that our current technique for electrode positioning is the result of a slow evolution over years. From this trial and error process, we propose that, in routine clinical practice, a safe procedure to verify that signal contamination is not a factor in a given patient could include 1) the placement of the electrodes in the lowest accessible intercostal space, close to the chondrocostal junction; 2) keeping the two electrodes as close as possible to one another, as many electrodes repositioning relative to each other as needed to obtain a clear and steady return of the signal to baseline after the stimulation artifact; and 3) simultaneous recordings from electrodes placed on the muscle mass of the pectoralis major or of the latissimus dorsi. An EMG response providing a PNCT shorter than 5 ms and not clearly longer than the concomitant latency of the response of the pectoralis major or of the latissimus dorsi to stimulation should be strongly suspected to be troubled by signal contamination.

In conclusion, our study shows that it is possible to study the EMG of the diaphragm in response to CMS and ES with surface electrodes, hence to diagnose and follow up phrenic nerve conduction abnormalities. In this regard, the study, small in size, must be taken as preliminary: the frequency of signal contamination in the relevant population, which is patients with suspected phrenic nerve problems, remains to be determined. Our results also indicate that CMS-PNCTs cannot be interpreted by using normal values derived from ES studies (we consider 6.5 ms as the upper limit of normal for CMS-PNCT). In this view also, large-
scale studies of CMS-PNCTs with comparisons with ES are required to better determine normal values. Meanwhile, every laboratory wishing to implement the technique should perform exploratory studies to optimize its setup.

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REFERENCES


