DEPRESSION OF DIAPHRAGM MOTOR CORTEX EXCITABILITY DURING MECHANICAL VENTILATION

Tarek Sharshar MD1,3, Ewen T Ross MD1, Nicholas S Hopkinson MD1, Raphael Porcher PhD2, Annabel H Nickol MD1, Sophie Jonville PhD1, Mark J Dayer MD1, Nicholas Hart MD1, John Moxham MD3, Frédéric Lofaso MD, PhD4, Michael I Polkey MD, PhD1

1 Respiratory Muscle Laboratory, Royal Brompton and Harefield NHS Trust
King’s College Hospital, Fulham Road, London, SW3 6NP, United Kingdom.
2 Département de Biostatistique et Informatique Médicale, Hôpital Saint-Louis, AP-HP, 75010 Paris, France.
3 Department of Respiratory Medicine, King’s College Hospital, Denmark Hill, London, SE5 9PJ. United Kingdom.
4 Service d’Exploration Fonctionnelles, Hôpital Raymond Poincaré, AP-HP, 92380 Garches, France.

Corresponding author: Dr Michael Polkey, Respiratory muscle laboratory, Royal Brompton Hospital, Sydney Street, London SW3 6NP United-Kingdom. Tel: (44) 207 351 80 29; Fax: (44) 207 351 89 39; E-mail: m.polkey@rbh.nthames.nhs.uk

Running head: Motor cortex and mechanical ventilation
The effect of mechanical ventilation on the diaphragm motor cortex remains unknown. We assessed the effect of mechanical ventilation on diaphragm motor cortex excitability by measuring the costal and crural diaphragm motor evoked potential (MEP) elicited by single and paired transcranial magnetic stimulation (TMS). In six healthy subjects, MEP recruitment curves of the costal and crural diaphragms were assessed at relaxed end-expiration during spontaneous breathing (VT_{baseline}) and isocapnic volume cycled ventilation delivered non-invasively (NIV) at three different levels of tidal volume (VT_{baseline}, VT_{baseline} + 5ml/kg L and VT_{baseline} + 10 ml/kg L). The costal and crural diaphragm response to peripheral stimulation of the right phrenic nerve was not reduced by NIV. NIV reduced the costal and crural MEP amplitude during NIV (P<0.0001) with the maximal reduction at VT_{baseline} + 5ml/kg. Response to paired TMS showed that NIV (VT_{baseline} + 5ml/kg) significantly increased the sensitivity of the cortical motor neurons to facilitatory (>9 ms) interstimulus intervals (P=0.002), suggesting that the diaphragm MEP amplitude depression during NIV is related to neuromechanical inhibition at the level of motor cortex. Our results demonstrate that mechanical ventilation directly inhibits central projections to the diaphragm.

**Key Words**: Transcranial magnetic stimulation ; mechanical ventilation ; diaphragm MEP depression
INTRODUCTION

Mechanical ventilation plays a key role in the treatment of acute and chronic respiratory failure. Effective mechanical ventilation reduces respiratory centre output whether measured as the diaphragm electrical activity (EMGdi) or as pressure generation (Pressure-time-product, PTPdi) (10, 11). Although neuromechanical feedback mediated by thoracic mechanoreceptors on brainstem structures is thought to contribute to this inhibition (11, 28, 34), the possibility exists that it may affect motor cortical control too.

Cortical control of the diaphragm can be assessed by measuring its electrical response, the motor evoked potential (MEP), to transcranial magnetic stimulation (TMS). TMS has previously been used to map the motor cortical area of different respiratory muscles as well as to determine excitability of their cortical neurons (7, 21, 23, 25, 33). Cortical excitability can be assessed by relating the amplitude of the MEP elicited by single stimulations to stimulus intensity (‘recruitment curve’) (27, 37) and by relating the amplitude of the MEP elicited by paired TMS to the time between the stimuli (‘intracortical inhibition/intracortical facilitation curves’, ICI/ICF curves) (4, 8, 18). The latter paradigm is a reproducible method that allows assessment of both inhibitory and facilitatory intra-cortical circuits that modulate the excitability of cortical motor neurons (22).

The hypothesis tested in the present study was that the excitability of the motor cortex supplying the diaphragm would be reduced during mechanical ventilation. Thus, we compared the recruitment and ICI/ICF curves of the diaphragm MEP in healthy subjects during spontaneous breathing and non-invasive mechanical ventilation set to produce increased tidal volumes and thus activate neuromechanical feedback.
METHODS

Subjects

Six healthy volunteers (five men) aged 25-45 (mean age 35) participated in the study. The subjects were members of the laboratory staff and all were free of neurological and respiratory disease. The study was approved by the ethics committee of the Royal Brompton and Harefield hospital and the subjects gave their informed consent. Five out the six subjects are coauthors of the manuscript.

Measurements

Throughout the study, subjects wore either a nasal or full-face mask. Flow was measured via a pneumotachograph head (Hans Rudolph 3700 series, Hans Rudolph Inc., Kansas City, MO) connected to a CS5T electrospirometer (GM instruments Ltd, UK). Minute ventilation (VE) and tidal volume (VT) were electronically derived. Airway pressure (Paw) was measured via a differential pressure transducer (MP 45 model, Validyne Corp., Northridge, CA). Esophageal (Pes) and gastric (Pga) pressures were measured using two air filled balloon catheters (Ackrad laboratories, inc., Crandford, NJ, USA.) positioned in a standard manner (2, 24). Both catheters were connected to differential pressure transducers (range ± 300 cm H₂O; Validyne corporation, Northridge, CA). Transdiaphragmatic pressure (Pdi) was derived electronically by subtracting Pes from Pga. End-tidal CO₂ (PetCO₂) was determined via a nasal catheter connected to a capnograph (PK Morgan Ltd, Gillingham, Kent, UK).
Surface recordings of the right diaphragm compound motor action potential (CMAP) and motor evoked potential (MEP) were obtained using Ag/AgCL electrodes whose optimal position was determined by using transcutaneous electrical stimulation of the right phrenic nerve (40). Abdominal muscle MEP was measured by positioning surface electrodes over the Rectus abdominis muscle (27).

Crural diaphragm CMAP, MEP and electromyographic (EMG) signals were obtained using a multipair esophageal electrode catheter, which consisted of five copper coils. The top coil was connected to earth and the lower four coils formed six sequential pair of electrodes. The optimal esophageal electrode position was characterized by opposite polarity and similar amplitude of the CMAP recorded from the two pairs of electrodes. The electrode was securely taped to the subject’s nose. The crural EMG signals recorded from the two optimal electrode pairs were amplified and then passed to a personal computer running LabView 4.1 software (National instruments). Electrocardiogram was simultaneously recorded using three chest electrodes. Mean peak-to-peak amplitude (µV) of the MEP first deflection was calculated.

**Transcranial magnetic stimulation and phrenic nerve stimulation**

Transcranial magnetic stimulation (TMS) was performed using a Magstim 200 stimulator (The Magstim Company, Whitland, UK) and paired TMS were delivered using two units linked via a Bistim clocking device. For both types of stimulations, a 110 mm double cone coil (The Magstim Company) oriented in the anterior-posterior direction was used. TMS was delivered at the best scalp position which was determined as previously described (31). In three subjects, this was over the vertex, in two subjects +1 cm forward and in one subject +1
cm forward and 1 cm laterally. Throughout the study, stimuli were delivered at resting end expiration and the interval between stimulations was at least 30 seconds. For TMS, surface and esophageal electrode signals were amplified with bandpass filters at 10Hz to 10kHz, digitized and acquired into a 5-channel EMG recorder (Medelec, Synergy, Oxford Instruments, Oxford, UK).

To assess the diaphragm recruitment curves to TMS, stimulus intensity was increased from 40% of maximal stimulator output in 10% increments up to 100% of stimulator output. The order in which stimuli from 40% to 100% were delivered was randomized. Five stimuli were delivered at each intensity level.

Motor threshold was defined as the lowest stimulator output (via the Bistim device) that produced an MEP peak-to-peak amplitude of at least 50µV in more than 5 out of 10 trials. Stimulator output was decreased from 100% by 5% increments until threshold was reached.

To assess the diaphragm ICI/ICF curves, intensity of sub-threshold conditioning stimulus (CS) and supra-threshold test stimulus (TS) was set at 80% and 125% of the motor threshold, respectively. The interstimulus interval, i.e. time elapsing between CS and TS, was set at 1, 3, 5, 7, 9, 11, 13, 15 and 20 ms in random order (8). Five stimuli were delivered at each interstimulus interval (ISI).

Unilateral anterolateral magnetic stimulation (UAMPS) of the right phrenic nerve was performed at 100% of stimulator output using a 45mm figure of eight coil (The Magstim Company). In cases (n=4) where the CMAP elicited by UAMPS yielded unacceptable stimulation artifact, we performed supramaximal transcutaneous electrical stimulation (EPS).
Non-invasive ventilation

Volume cycled non-invasive ventilation (Breas PV 403, Breas Medical AB, Sweden) was delivered via a commercially available nasal mask (Comfort classic Profile Therapeutics, Bognor Regis UK) in five subjects and a full-face mask (Mirage NV Oxfordshire UK) in one. Leaks were minimized and trigger sensitivity was reduced to minimize auto-triggering. Prior to the study, subjects had undergone practice runs to familiarize themselves with NIV. During ventilation the discomfort of breathing and overall discomfort was assessed using a visual analog score ranging from 0 (very comfortable) to 10 (very uncomfortable).

Study protocol and statistical analyses

The study protocol encompassed three studies performed on three separate occasions.

The objective of study 1 was to assess the effect of NIV on TMS recruitment curves of the diaphragm and abdominal muscles. It consisted of two periods of relaxed spontaneous breathing before and after three periods of assisted ventilation. During each of these, a tidal volume was delivered in a random order. During the two spontaneous breathing periods (SBbaseline and SBend), subjects breathed room air with the mask in position, but with the ventilator circuit disconnected. Baseline respiratory rate (RRbaseline), VT (VTbaseline) and PetCO2 (PetCO2baseline or isocapnia) were assessed for the last 90 seconds of a five minute control period. During assisted ventilation, the ventilator was set to deliver the same RR as during baseline spontaneous breathing. In each ventilator period the volume delivered was either equal to that during spontaneous breathing or VTbaseline + 5ml/kg or VTbaseline + 10ml/kg.
The diaphragm and abdominal muscle recruitment curves were assessed in each condition. In addition, the costal and crural diaphragm responses to peripheral right phrenic stimulation (UAMPS or EPS) were recorded during baseline spontaneous breathing and the two highest levels of NIV. MEP\textsubscript{100} and MEP\textsubscript{TS} referred to MEP elicited by 100% stimulator output. For recruitment curves, MEP amplitude and latency were normalized to MEP\textsubscript{100} response at baseline spontaneous breathing. MEP\textsubscript{100} amplitudes were compared between the two spontaneous breathing periods and the mechanical ventilation levels using mixed model analysis of variance with period fixed effect and random subject effects. MEP amplitude recruitment curves were analyzed using linear mixed effects models, with a fixed period effect, a linear intensity effect and their interaction. Random subject effects, slopes and interactions were also added to the model. The random effects variance structure was selected using Schwarz’ BIC model selection criterion (29). Post-hoc tests were performed to compare baseline spontaneous breathing to each of the other study periods, using least-squares means for MEP\textsubscript{100} amplitudes or estimated slopes for MEP recruitment curves, and Hochberg’s procedure for multiple testing (14).

Study 2 was an abbreviated version of study 1. It included only one period of relaxed spontaneous breathing and one period of NIV with set at VT\textsubscript{baseline} + 5ml/kg. The diaphragm MEP elicited by 100% of stimulator output, motor threshold and ICI/ICF curves were consecutively assessed in the final phase of spontaneous breathing and mechanically ventilated periods. Mean peak-to-peak amplitude (µV) of the MEP first deflection was calculated. MEP\textsubscript{TS} referred to MEP elicited by test stimulus alone. For the ICI/ICF curves, MEP amplitude was normalized to MEP\textsubscript{TS}. Diaphragm ICI/ICF curves were analyzed using linear mixed effects models, in which the ICI/ICF was considered to have a piecewise linear effect, with different slopes and intercepts before and after 9 ms.
In Study 3, we assessed the quadriceps response to paired-TMS in order to determine whether mechanical ventilation related changes in ICI/ICF curves were specific to the diaphragm. During VT_{baseline} and VT_{baseline} + 5ml/kg, quadriceps MEP amplitude was measured at 3, 9 and 15 ms interstimulus intervals, using the same paired-TMS paradigm.

Before each period of study 1, 2 and 3 an accommodation period of 20 minutes was allowed, and periods were separated by a 10 minutes rest, during which the mask was removed from the subject. PetCO2 was maintained at baseline level by bleeding in a gas mixture containing 15% CO2/ 85% air into the respiratory tubing proximal to the ventilator. HR, Paw, Pga, Pes, Pdi, flow, RR, inspiratory time, expiratory time and PetCO2 signals as well as crural diaphragm EMG activity were recorded over the final five minutes of each period. The peak Paw and inspiratory transdiaphragmatic pressure-time product (PTPdi, cmH2O/s/min) and, in study 1, inspiratory integrated EMG-time product (EMGdi, arbitrary units/s/min and percentage of baseline EMGdi) were calculated. Throughout the study 1, 2 and 3, the subjects were seated comfortably in an armchair and were unable to view either the various PC monitors, the settings on the ventilator, or the CO2 cylinder. Subjects were encouraged to maintain the same posture, with the upper and lower limbs and the head and neck in a relaxed position. The subjects were allowed to listen to music, but were not allowed to read or write. Subjects were checked regularly to ensure that they remained awake throughout the study. Importantly no information on the results obtained was given to the subject during the study, in order to not influence the subjects.
RESULTS

Effect of different level of mechanical ventilation on the diaphragm CMAP and on the diaphragm and abdominal muscle MEP recruitment curves (Study 1)

There was a significant decrease in PTPdi (p = 0.01) and EMGdi (p = 0.001) with mechanical ventilation (Table 1). There was no significant difference in end-expiratory Pes, Pga or Pdi measured at time of stimulation between each period of study 1 as well as in mean amplitude of diaphragm CMAP between spontaneous breathing and mechanical ventilation (Table 2 and Figure 2). Representative traces of the costal diaphragm, the crural diaphragm and abdominal MEP from one individual are shown in Figure 1.

There was a significant reduction with mechanical ventilation in MEP$_{100}$ amplitude of the costal and crural diaphragm but not of the Rectus abdominis (Figure 2). Changes with mechanical ventilation in MEP amplitude recruitment curves were also statistically significant for both the costal and crural diaphragm (fixed group by intensity interaction effect, p=0.005 and p=0.03, Figures 3 and 4) but not for the Rectus abdominis (p = 0.57, Figure 5). Post-hoc tests showed that the MEP amplitude recruitment curves were significantly steeper during SB$_{baseline}$ than during the second and third periods of mechanical ventilation, for both the costal and crural diaphragm. There was no significant change with mechanical ventilation in MEP$_{100}$ latency of the costal diaphragm, crural diaphragm and Rectus abdominis (p=0.17, p=0.71 and p=0.95).
Study 2 was conducted in five healthy subjects. The crural electrophysiological signals were interpretable in four of them. The effect of mechanical ventilation on breathing pattern and PTPdi was similar to that observed in study 1 (Table 3). There was also a reduction with mechanical ventilation in the costal and crural diaphragm MEP_{100} amplitude (Table 4). The motor threshold did not significantly change with mechanical ventilation (Table 4). For both the costal and crural diaphragm, there was a significant inhibition by 40% of MEP at interstimulus interval of 3 ms (p < 0.04) and facilitation by 200% at 9 ms (p < 0.04), during spontaneous breathing. The costal MEP amplitude was higher during mechanical ventilation than during spontaneous breathing (Figure 6), for interstimulus intervals longer than 9 ms (p = 0.007) rather than for interstimulus interval shorter than 7 ms (p = 0.06). The crural MEP amplitude was higher during mechanical ventilation than during spontaneous breathing at both short and long interstimulus interval (p = 0.02 and p = 0.0005). The greatest facilitation was at an interstimulus interval of 9 ms (Table 4).

Effect of mechanical ventilation on the quadriceps interstimulus interval curves (Study 3).

Three subjects were studied. There was no significant effect of mechanical ventilation on quadriceps motor threshold or MEP_{TS} amplitude (Table 5). During mechanical ventilation, quadriceps MEP amplitude increased at 3 ms but decreased at 9 and 15 ms of interstimulus interval.
DISCUSSION

The main finding of this study is that mechanical ventilation is associated both with a reduction in the diaphragm MEP and an increase in the response to paired TMS at facilitatory interstimulus intervals. By contrast, a similar trend was not observed for the quadriceps suggesting a specific effect on the diaphragm motor cortex. These phenomena demonstrate that firstly, mechanical ventilation specifically down regulates the cortical excitability and output of the cortico-spinal projections to the respiratory muscles; secondly, that mechanical ventilation induces a compensatory increase in the excitability of facilitatory intracortical neurons that modulate the excitability of the respiratory cortical motor neurons.

Critique of the Method

Our results raise several methodological issues. Firstly, we used chest wall surface electrodes to record the costal diaphragm MEP, rather than a needle electrode because of the risk of pneumothorax during mechanical ventilation. The risk of contamination of action potentials recorded using surface electrodes was minimized as their position was consistent with that previously validated (40) and was optimized with phrenic stimulation. In addition, a diaphragm origin of the MEP’s was supported by the use of esophageal electrode, which provided similar data as surface ones.

We acknowledge the concern that behavior might have contributed to the depression of the MEP as the subjects were coached to be relaxed and synchronized to the ventilator and blinding to the level of mechanical ventilation was not possible. In addition subjects were members of laboratory staff and most of them are coauthors of the manuscript. This is
potentially a major concern as they might have exerted a voluntary control influencing the response to single and paired transcranial magnetic stimulation (TMS). We are not able to rule out this hypothesis but attempted to prevent biasing of the results by performing data analyses only after data collection had been concluded. Therefore, each subject was not aware of the result found in the preceding subject and its own response. There are several additional arguments against the possibility of a behaviour effect. Firstly, one may argue that decrease in the diaphragm MEP amplitude to single TMS was foreseeable as mechanical ventilation reduces the diaphragm electromyographic activity. Our subjects knew of this effect. However, we did not find a correlation between reduction in the diaphragm MEP amplitude and electromyographic activity (data not presented). Moreover, we believe that the diaphragm responses to paired TMS were not foreseeable and various scenarios could have been preconceived with an equal plausibility. This study was the sequel of a previous one in which we confirmed the existence of a neuromechanical feedback inhibiting the respiratory centre output (30). Thus we hypothesized that this neuromechanical feedback might influence the motor cortex supplying the diaphragm, but we did not know how. Secondly, focusing attention on breathing should have induced in increase in amplitude of the diaphragm response to single TMS, as it has been shown that thinking about a movement alone facilitates MEP amplitude (16). We found the opposite result. Thirdly, it has also been shown that volitional inhibition of movements enhances the intra-cortical inhibition rather than facilitation (35). We found the opposite results. Therefore, we doubt that voluntary suppression of diaphragm activity would result in the observed data.

More importantly, MEP facilitation is known to occur during and after muscular contraction (12). Thus, if at end expiratory time, the diaphragm was more active and, accordingly, the diaphragm MEP more facilitated, during spontaneous breathing than during mechanical
ventilation, this might explain the MEP amplitude reduction during mechanical ventilation. However, there are some arguments against this possibility. The end-expiratory Pes, Pga and Pdi tended to be greater during the second and third levels of mechanical ventilation than during spontaneous breathing, indicating that, in term of pressure, the diaphragm was not more facilitated at baseline. In addition, the MEP latency and motor threshold remained constant throughout the present study whereas they are both known to be reduced by voluntary facilitation (31, 33). In addition, from our previous study (31), we can infer that to achieve the 50% ‘reduction’ from spontaneous breathing to second level of mechanical ventilation, there would need to be a end-expiratory Pdi greater than 20% Pdi_{max} during spontaneous breathing, which we should certainly have detected. Finally, it has previously been shown in various skeletal muscles that a voluntary contraction (of approximately 5 to 10% of maximum) induces a marked reduction in intracortical inhibition and abolition of intracortical facilitation (1). We found that, during spontaneous breathing, the diaphragm MEP was significantly inhibited and facilitated at short and long interstimulus intervals, respectively. This supports the premise that the diaphragm was not facilitated at end-expiration. An alternative hypothesis therefore is that the amplitude of MEP elicited at end-expiratory time was decreased because mechanical ventilation had reduced diaphragm activation during inspiratory phase. The neurophysiological mechanisms linking reduction in inspiratory effort and expiratory MEP amplitude are now addressed.

Significance of findings

The reduction in the diaphragm MEP amplitude during mechanical ventilation could be related either peripheral or central mechanisms. A peripheral mechanism seems unlikely as the CMAP amplitude of the costal and crural diaphragm was not altered during mechanical
ventilation, leading to the hypothesis of a central origin, theoretically at a spinal, medullary or cortical levels.

We are not able to rule out inhibitory mechanisms at the spinal level. In contrast to other nerves, it is impossible to assess motoneurone excitability by analyzing H-Reflex and F-waves for the phrenic nerve. Although a diaphragm response to magnetic stimulation at the brainstem level has previously been demonstrated (39), we and others (T. Similowski, personal communication) have been unable to elicit this response; nevertheless this perhaps represents a future direction for the study of the effects of mechanical ventilation.

The respiratory muscles are controlled via bulbo-spinal projections from the pontomedullary respiratory centers and also by motor cortical areas via the cortico-spinal pathways (13). Whether stimulation of the motor cortex acts via the brainstem respiratory centers remains unresolved and our results do not allow us to draw a clear conclusion. Nevertheless the short latencies observed would be consistent with an oligosynaptic corticospinal pathway bypassing medullary centers. This hypothesis is supported by data from Corfield et al (7) who found that diaphragm MEP amplitude is not altered during hypocapnia, when the respiratory ‘oscillator’ is presumed to be silent.

Our finding that mechanical ventilation related increase in MEP amplitude at facilitatory interstimulus intervals supports a cortical origin of the diaphragm MEP depression. It is well established that the paradigm of paired TMS that we used enables investigation of intra-cortical inhibitory and facilitatory circuits that modulate excitability of cortical motor neurons. Our findings indicate that mechanical ventilation made the motor neurons of the primary motor cortex more sensitive to facilitatory interstimulus intervals. Additional arguments
support our hypothesis of a cortical mechanism. First, in addition to the motor cortex, imaging studies using positron emission tomography and functional magnetic resonance have demonstrated that various supratentorial brain regions are involved in breathing control, including the supplementary motor area, premotor area, sensorimotor cortex, striatum and limbic system (6, 9, 26). Interestingly, it has been reported that these accessory regions are less active during passive mechanical ventilation with high tidal volume (1.3 L) than during active breathing (9). We can speculate that MEP amplitude depression was possibly related to the inactivation of these accessory areas. Second, the effect of sensory input on motor cortex excitability has been investigated in various skeletal muscle limbs. Muscle vibration augments MEP amplitude in the vibrated muscle (5, 17) but depresses it in ipsilateral and contralateral antagonist muscle (17, 32). Sensory electrical stimulation of the median nerve results in MEP inhibition as does electrical cutaneous stimulation (36, 38). Sensory deafferentation is also associated with an increase of motor cortex excitability (3). It is believed that MEP amplitude modulation by sensory afferents takes place at the cortical levels and involves GABAergic intra-cortical inhibitory circuits. Likewise, it has previously been shown that, independently of PaCO₂ levels, mechanical hyperventilation inhibits the neural drive to the diaphragm whether measured by PTPdi or EMGdi (11, 34). Such a phenomenon was observed in the present study. This inhibition is mediated by the thoracic mechanoreceptors via the vagal and other proprioceptive afferents to respiratory centres. It is, therefore, plausible that mechanoreceptor feedback accounts for the diaphragm MEP depression (11, 28, 34). Third, our data are consistent with and extend the data obtained from limb muscle by Lewis et al (19, 20). This group showed that passive movements of the limbs induced a reduction in MEP amplitude during the passive extension phase. Their data showed that sensory input from the target muscle could modify cortical sensitivity. Our data show that this might be so for the respiratory muscles.
Finally, we need to address whether mechanical ventilation related depression of the response to single TMS and enhancement of response to paired TMS of the diaphragm MEP are mediated by a common mechanism? In fact, these phenomena are not as contradictory as they may seem. Mechanical ventilation might have induced MEP depression indirectly by either stimulating intra-cortical inhibitory neurons or inhibiting facilitatory ones. The pattern of response to paired TMS suggests that intra-cortical inhibitory neurons cannot be further activated as short interstimulus intervals did not reduce MEP amplitude. By contrast, intra-cortical facilitatory neurons are more excitable as long interstimulus intervals resulted in a MEP amplitude increase. We reason that mechanical ventilation has modified the balance between intra-cortical inhibitory and facilitatory tone. There is an increase of intra-cortical inhibitory tone and a compensatory increase of facilitatory excitability. However, we acknowledge that this postulated mechanism is speculative and would require further studies to be proved. Specific pharmacological agents, especially those acting on gamma-aminobutyric acid (GABA) neurotransmission, could also be useful for elucidating the respective role of intra-cortical inhibitory and facilitatory circuits in the diaphragm MEP depression induced by mechanical ventilation (15, 41, 42).

In summary we found that mechanical ventilation was associated with a decrease of MEP amplitude and hyperexcitability of intra-cortical facilitatory neurons, suggesting that unloading the respiratory muscles reduces the excitability of the cortical motor areas representing these muscles. In addition to the physiological relevance of an observation of MEP depression in the absence of fatigue, drugs or movement, our findings may also have clinical implications for the understanding of patient-ventilator interaction.
REFERENCES


Legend of figure 1
The MEP\textsubscript{100} of the costal diaphragm (upper), the crural diaphragm (middle) and Rectus abdominis (lower) during baseline spontaneous breathing (left panel (i)), mechanical ventilation (VT\textsubscript{baseline} + 5 ml/kg; middle panel (ii)) and during spontaneous breathing following non-invasive ventilation (right panel (iii)). The MEP\textsubscript{100} morphology of each muscle was similar. The Rectus abdominis MEP\textsubscript{100} latency is longer than that of the costal and crural diaphragm. There is a decrease in MEP\textsubscript{100} amplitude of whole muscles during mechanical ventilation. There is no change in MEP\textsubscript{100} latency and morphology during mechanical ventilation.

Legend of figure 2
Change with mechanical ventilation in supramaximal CMAP (open symbols) and MEP\textsubscript{100} (plain symbols) amplitude of the costal diaphragm (diamond), crural diaphragm (square) and Rectus abdominis (triangle). Each point corresponds to mean size (± SEM) of CMAP or MEP in 6 subjects. There was an increase in costal and crural CMAP amplitude, but this difference did not reach the level of significance (p=0.47 and p=0.58). ANOVA showed a significant change in MEP\textsubscript{100} amplitude of the costal diaphragm (p=0.007), the crural diaphragm (p=0.0005) but not of Rectus abdominis (p=0.10). Abbreviation - SB: spontaneous breathing; MV: mechanical ventilation; SB\textsubscript{Baseline}: SB before MV; MV1: first level of MV (VT\textsubscript{Baseline}); MV2: second level of MV (VT\textsubscript{Baseline} + 5 ml/kg); MV3: third level of MV (VT\textsubscript{Baseline} + 10 ml/kg); SB\textsubscript{End}: SB after MV.

Legend of figure 3
Relationships between TMS intensity and MEP amplitude of the costal diaphragm during the five different periods: baseline (open square) and end (plain square) spontaneous breathing, mechanical ventilation with 0 ml/kg (plain diamond), mechanical ventilation with 5 ml/kg
(plain triangle), mechanical ventilation with 10 ml/kg (plain circle) above VT_{baseline}. The different levels of VT were applied in random order. In each test, TMS intensity was randomly delivered. Each point corresponds to mean size (+ SEM) of MEP in 6 subjects. There was a significant change in the costal diaphragm recruitment curve (p = 0.005), with its slope being significantly steeper during SB_{baseline} than during the second level and third level of mechanical ventilation (VT + 5 ml/kg, VT + 10 ml/kg).

**Legend of figure 4**

Relationships between TMS intensity and MEP amplitude of the crural diaphragm during the five different periods: baseline (open square) and end (plain square) spontaneous breathing, mechanical ventilation with 0 ml/kg (plain diamond), mechanical ventilation with 5 ml/kg (plain triangle), mechanical ventilation with 10 ml/kg (plain circle) above VT_{baseline}. The different levels of VT were randomly applied. In each test, TMS intensity was randomly delivered. Each point corresponds to mean size (+ SEM) of MEP in 6 subjects. There was a significant change in the recruitment curve (p = 0.03), with its slope being significantly steeper during SB_{baseline} than during the second level and third level of mechanical ventilation (VT + 5 ml/kg, VT + 10 ml/kg).

**Legend of figure 5**

Relationships between TMS intensity and MEP amplitude of the Rectus abdominis during the five different periods: baseline (open square) and end (plain square) spontaneous breathing, mechanical ventilation with 0 ml/kg (plain diamond), mechanical ventilation with 5 ml/kg (plain triangle), mechanical ventilation with 10 ml/kg (plain circle) above VT_{baseline}. The different levels of VT were randomly applied. In each test, TMS intensity was randomly delivered. Each point corresponds to mean size (+ SEM) of MEP in 6 subjects. There was no
significant effect of mechanical ventilation on the Rectus abdominis MEP amplitude recruitment curves \( (p = 0.57) \).

**Legend of figure 6**

Relationships between interstimulus interval and MEP amplitude of the costal diaphragm during baseline spontaneous breathing (plain square) and mechanical ventilation with 5 ml/kg (plain triangle) above \( VT_{\text{baseline}} \). In each test, interstimulus intervals were varied in random order. Each point corresponds to mean size \((+ \text{ SEM})\) of normalized MEP \((\%)\) in 5 subjects. Mechanical ventilation induced a significant change of the interstimulus interval curves, notably for interstimulus interval longer than 9 ms \((p = 0.007)\).
Table 1. Mean values of HR, comfort of breathing, RR, flow, PTPdi, EMGdi during each period of the study.

<table>
<thead>
<tr>
<th></th>
<th>SBBaseline</th>
<th>MV1</th>
<th>MV2</th>
<th>MV3</th>
<th>SBEnd</th>
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<tr>
<td>HR (Beat/min)</td>
<td>68 ± 10</td>
<td>68 ± 7</td>
<td>68 ± 8</td>
<td>74 ± 5</td>
<td>65 ± 8</td>
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<td>Discomfort of breathing</td>
<td>0.1 ± 0.2</td>
<td>2.4 ± 1.0</td>
<td>0.6 ± 0.2</td>
<td>1.1 ± 0.2</td>
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<td>PetCO₂ (kPa)</td>
<td>5.45 ± 0.34</td>
<td>5.69 ± 0.43</td>
<td>5.48 ± 0.33</td>
<td>5.51 ± 0.33</td>
<td>5.55 ± 0.36</td>
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<td>Ti/Ttot (%)</td>
<td>0.41 ± 0.01</td>
<td>0.43 ± 0.08</td>
<td>0.42 ± 0.07</td>
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<td>RR (cycle/min)</td>
<td>13 ± 2</td>
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<td>14 ± 2</td>
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<td>VT (mL)</td>
<td>560 ± 90</td>
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<td>1250 ± 180</td>
<td>1830 ± 190</td>
<td>500 ± 190</td>
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<td>VE (mL/sec)</td>
<td>7.3 ± 0.9</td>
<td>10.7 ± 2.6</td>
<td>17.1 ± 3.5</td>
<td>23.6 ± 3.9</td>
<td>6.2 ± 1.9</td>
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<td>Peak Paw (cm H₂O)</td>
<td>1.9 ± 1.7</td>
<td>4.7 ± 2.5</td>
<td>9.7 ± 5.4</td>
<td>16.9 ± 10.8</td>
<td>2.1 ± 1.7</td>
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<td>PTPdi (cm H₂O.sec/min)</td>
<td>213 ± 105</td>
<td>178 ± 70</td>
<td>125 ± 104</td>
<td>117 ± 94</td>
<td>148 ± 61</td>
</tr>
<tr>
<td>EMGdi (%)</td>
<td>100.0 ± 0.0</td>
<td>87.0 ± 50.0</td>
<td>50.67 ± 34.1</td>
<td>49.1 ± 28.1</td>
<td>76.2 ± 39.7</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD. Abbreviation - SB: spontaneous breathing; MV: mechanical ventilation; SBBaseline: SB before MV; MV1: first level of MV (VTBaseLine); MV2: second level of MV (VTBaseLine + 5 ml/kg); MV3: third level of MV (VTBaseLine + 10 ml/kg); SBEnd: SB after MV. HR = heart rate; Ti/Ttot = inspiratory time/ total respiratory time ratio; RR = respiratory rate; VT = tidal volume; VE: flow; PAW = airway pressure; PTPdi = transdiaphragmatic pressure time product; EMGdi: normalized electromyographic activity of the crural diaphragm. PTPdi and EMGdi significantly decreased with ventilation (ANOVA, p = 0.01 and p = 0.001).
Table 2. Mean values of Poes, Pga and Pdi preceding end-expiratory time stimulation at during each period of the study 1.

<table>
<thead>
<tr>
<th></th>
<th>SBBaseline</th>
<th>MV1</th>
<th>MV2</th>
<th>MV3</th>
<th>SBEnd</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pes (cm H2O)</td>
<td>- 2.3 ± 1.4</td>
<td>0.4 ± 4.2</td>
<td>- 0.6 ± 5.0</td>
<td>- 0.8 ± 4.9</td>
<td>- 1.1 ± 2.1</td>
</tr>
<tr>
<td>Pga (cm H2O)</td>
<td>9.9 ± 5.3</td>
<td>14.2 ± 5.9</td>
<td>16.8 ± 4.6</td>
<td>15.2 ± 3.4</td>
<td>11.3 ± 2.6</td>
</tr>
<tr>
<td>Pdi (cm H2O)</td>
<td>12.4 ± 5.3</td>
<td>14.0 ± 2.3</td>
<td>18.0 ± 7.3</td>
<td>16.2 ± 5.6</td>
<td>13.3 ± 2.7</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD. Abbreviation - SB: spontaneous breathing; MV: mechanical ventilation; SBBaseline: SB before MV; MV1: first level of MV (VtBaseline); MV2: second level of MV (VtBaseline + 5 ml/kg); MV3: third level of MV (VtBaseline + 10 ml/kg); SBEnd: SB after MV. There was no significant change in Poes, Pga and Pdi with ventilation (ANOVA, p = 0.31, p = 0.08 and p = 0.19).
Table 3. Mean values of discomfort of breathing, PetCO₂, RR, VT, PTPdi during spontaneous breathing and second level of isocapnic non-invasive mechanical ventilation (Study 2).

<table>
<thead>
<tr>
<th></th>
<th>SB Baseline</th>
<th>VT + 5 ml/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discomfort of breathing</td>
<td>0.2 ± 0.3</td>
<td>0.2 ± 0.3</td>
</tr>
<tr>
<td>PetCO₂ (kPa)</td>
<td>5.04 ± 0.34</td>
<td>4.97 ± 0.76</td>
</tr>
<tr>
<td>Ti/ Ttot (%)</td>
<td>0.37 ± 0.1</td>
<td>0.37 ± 0.1</td>
</tr>
<tr>
<td>RR (cycle/min)</td>
<td>13 ± 1</td>
<td>14 ± 3</td>
</tr>
<tr>
<td>VT (mL)</td>
<td>524 ± 76</td>
<td>1170 ± 217</td>
</tr>
<tr>
<td>PTPdi (cm H₂O.sec/min)</td>
<td>150 ± 50</td>
<td>64 ± 13</td>
</tr>
</tbody>
</table>

Abbreviations - HR = heart rate; Ti/Ttot = inspiratory time/total respiratory time ratio; RR = respiratory rate; VT = tidal volume; VE: flow; PAW = airway pressure; PTPdi = transdiaphragmatic pressure time product; PTPdi significantly decreased with ventilation (ANOVA, p = 0.04).
Table 4. Mean values of the costal and crural diaphragm MEP amplitude elicited at 100% of stimulator output and interstimulus interval of 3, 9 and 15 ms during spontaneous breathing and second level of isocapnic non-invasive mechanical ventilation (Study 2).

<table>
<thead>
<tr>
<th></th>
<th>SBBaseline</th>
<th>VT + 5 ml/kg</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Motor threshold</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diaphragm (%)</td>
<td>92 ± 7</td>
<td>91 ± 8</td>
<td>0.65</td>
</tr>
<tr>
<td><strong>MEP$_{100}$ amplitude</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Costal MEP$_{100}$ (%)</td>
<td>100 ± 0.0</td>
<td>62.8 ± 12.3</td>
<td>0.04</td>
</tr>
<tr>
<td>Crural MEP$_{100}$ (%)*</td>
<td>100 ± 0.0</td>
<td>74.6 ± 8.1</td>
<td>-</td>
</tr>
<tr>
<td><strong>Costal MEP$_{100}$</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ISI 3 ms (% of TS)</td>
<td>62.0 ± 25.9</td>
<td>91.1 ± 51.5</td>
<td>0.08</td>
</tr>
<tr>
<td>ISI 9 ms (% of TS)</td>
<td>193.5 ± 58.4</td>
<td>378.0 ± 139.8</td>
<td>0.04</td>
</tr>
<tr>
<td>ISI 15 ms (% of TS)</td>
<td>530.9 ± 333.9</td>
<td>848.2 ± 456.3</td>
<td>0.04</td>
</tr>
<tr>
<td><strong>Crural MEP$_{100}$</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ISI 3 ms (% of TS)</td>
<td>60.3 ± 20.4</td>
<td>87.7 ± 34.2</td>
<td>0.05</td>
</tr>
<tr>
<td>ISI 9 ms (% of TS)</td>
<td>165.4 ± 82.1</td>
<td>244.9 ± 95.0</td>
<td>0.05</td>
</tr>
<tr>
<td>ISI 15 ms (% of TS)</td>
<td>415.7 ± 211.0</td>
<td>548.9 ± 213.2</td>
<td>0.008</td>
</tr>
</tbody>
</table>

Crural electrophysiological signals were available in four subjects. * Comparisons were tested by Wilcoxon signed rank test. Abbreviations. TS: test stimulus; ISI: interstimulus interval.
Table 5. Mean normalized values quadriceps MEP amplitude elicited at test stimulus intensity and 3, 9 and 15 ms of interstimulus interval during spontaneous breathing and second level of isocapnic non-invasive mechanical ventilation (Study 3).

<table>
<thead>
<tr>
<th></th>
<th>SB_{Baseline}</th>
<th>VT + 5 ml/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motor threshold (% of SO)</td>
<td>82 ± 8</td>
<td>83 ± 6</td>
</tr>
<tr>
<td>MEP_{TS} (µV)</td>
<td>119.1 ± 4.0</td>
<td>121.3 ± 33.2</td>
</tr>
<tr>
<td>ISI 3 ms (% of TS)</td>
<td>87.3 ± 256</td>
<td>94.7 ± 25.8</td>
</tr>
<tr>
<td>ISI 9 ms (% of TS)</td>
<td>222.5 ± 70.9</td>
<td>164.0 ± 72.2</td>
</tr>
<tr>
<td>ISI 15 ms (% of TS)</td>
<td>299.3 ± 73.4</td>
<td>220.2 ± 142.4</td>
</tr>
</tbody>
</table>

Abbreviations. SO: stimulator output; TS: test stimulus whose intensity was 25% above motor threshold; ISI: interstimulus interval.
Figure 1
Figure 2

CMAP or MEP amplitude (% of baseline values)

SBbaseline  MV1  MV2  MV3  SBend

Periods
Figure 3

Costal diaphragm MEP amplitude (% of baseline MEP100) vs. Stimulus intensity (%)

0 20 40 60 80 100 120 140
40 50 60 70 80 90 100
Stimulus intensity (%)

Costal diaphragm MEP amplitude (% of baseline MEP100)
Figure 4

Crural diaphragm MEP amplitude (% of baseline MEP100) vs. Stimulus intensity (%)
Figure 6

![Graph showing MEP amplitude (% of MEPTS) against interstimulus interval (ms). The graph plots MEP amplitude on the y-axis and interstimulus interval on the x-axis. The data points are represented with error bars, indicating variability. Two distinct lines are visible, one with a solid line and the other with a dashed line, each with different markers. The x-axis labels are at intervals of 1, 3, 5, 7, 9, 11, 13, 15, and 20 ms, while the y-axis ranges from 0 to 1200.](image-url)