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**Impact of sleep deprivation on respiratory motor output and endurance: a physiological study**

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*Scientific knowledge on the subject:* It is known that sleep loss or sleep disruption triggers fatigue. Sleep deprivation alters endurance in cycling or treadmill exercises performed at high intensity. Only one study has suggested that sleep deprivation might cause respiratory task failure during a high intensity incremental inspiratory loading exercise, an experimental condition rarely experienced by patients in clinical conditions. Impact of one-night sleep deprivation on moderate-intensity constant-rate sustained exercise (more clinically relevant than incremental loading) and the neural pathways involved in respiratory endurance are unknown.

*What this study adds to the field:*

In healthy subjects with normal diaphragm function, one-night sleep deprivation precipitated task failure and dramatically decreased inspiratory endurance (-50%), independently of subjects' will. This deficit in inspiratory endurance was associated with progressive reduction of cortical contribution to

the respiratory motor output , and diaphragm contractile function was not affected. One night of sleep deprivation triggered severe brain dysfunction altering respiratory command. This endurance deficit should be considered and investigated in patients, especially when they experience rapid increases in inspiratory loads, for example during asthma/COPD exacerbations or during spontaneous breathing trials performed throughout the ventilatory weaning process.

This article has an online data supplement, which is accessible from this issue's table of contents online at [www.atsjournals.org](http://www.atsjournals.org)

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**ABSTRACT (249 words)**

**Rationale:** While sleep deprivation can alter endurance of skeletal muscles, its impact on respiratory command is unknown. We aimed to assess the effect of sleep deprivation on respiratory motor output and inspiratory endurance.

**Methods:** Inspiratory endurance was investigated twice in random order, following a normal sleep night and a sleepless night. Healthy subjects were asked to breathe as long as possible until task failure against a moderate inspiratory threshold constraint. Transdiaphragmatic pressure and diaphragm electrical activity were measured throughout the trial to assess pressure output of diaphragm and overall respiratory motor output. Cortical contribution to respiratory motor output was assessed by measuring pre-inspiratory motor potential amplitude and with cervical magnetic simulation.

**Results:** Twenty healthy males were studied. Time to task failure was significantly shorter after sleep deprivation than after normal sleep: (30min[17-41] vs. 60min[45-60],  $p=0.002$ ). At the beginning of the trial, pre-inspiratory motor potential amplitude was significantly lower in sleep deprivation condition ( $4.5 \mu\text{V}$ [2.5-6.4] vs.  $7.3\mu$ [4.3-10.4],  $p=0.02$ ) and correlated significantly with the duration of the endurance trial.

In sleep deprivation condition, pre-inspiratory motor potential amplitude, electrical activity of the diaphragm, pressure output of the diaphragm and tidal volume decreased and the respiratory rate increased significantly from the beginning to the end of the trial. Such decreases did not occur in normal sleep condition.

**Conclusion:** One-night sleep deprivation reduces respiratory motor output by altering its cortical component with subsequent reduction of inspiratory endurance by half. These results suggest that altered sleep triggers severe brain dysfunctions that could precipitate respiratory failure.

*Sleep deprivation and inspiratory endurance*

**Key words:** sleep deprivation, premotor potential, respiratory motor output, inspiratory endurance, healthy subjects.

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**INTRODUCTION (502)**

In animals, it has been shown that sleep deprivation alters physical and cognitive functions, and can lead to death if persisting over long periods of time (1). In humans, while sleep deprivation can reduce endurance of skeletal muscles in treadmill walking trials at high intensity (2), its influence on respiratory motor output is poorly known. In healthy subjects, it has been reported that sleep deprivation could precipitate inspiratory task failure in a high-intensity incremental inspiratory loading exercise (3). Chen and collaborators found that task failure occurred earlier in sleep deprivation condition. Furthermore, Chen and collaborators found that respiratory muscle strength was unaltered, a finding suggesting altered respiratory motor output in sleep deprivation. However, no study has as of yet investigated the effects of sleep deprivation on respiratory motor output during a sustained moderate and constant inspiratory loading trial. This type of exercise is more clinically relevant than incremental exercise insofar as it more closely resembles what can occur in patients suffering from respiratory failure.

Respiratory motor output is complex and relies on interplay between brainstem automatic control and corticospinal motor output. This respiratory motor output excites phrenic motoneurons which activate diaphragm and accessory inspiratory muscles. In normal respiratory conditions at rest, brainstem automatic respiratory motor output regularly activates the diaphragm muscle. In case of increased inspiratory load it has been shown, in healthy humans (4, 5) and in patients (6), that the premotor cortical regions are also activated (4) and that this supplemental cortical motor output increases inspiratory muscle activation (7), amplifies inspiratory muscle contractions (7) and allows maintenance of appropriate ventilation (5). If this inspiratory load is applied for several minutes, respiratory cycles can be performed for a certain time (depending on the load) (8), until task failure, a point at which inspiratory effort fails to overcome the load. Task failure might be due to insufficient respiratory motor output on phrenic motor neurons (i.e. central fatigue, (9)) or to reduced

contractile capacity of inspiratory muscles (i.e. contractile fatigue, (8)) or a combination of both (10).

Increased inspiratory load occurs in patients with severe acute respiratory failure, or during spontaneous breathing trials in intubated patients during the weaning process.

Patients with respiratory failure admitted in ICU are particularly exposed to altered sleep (11, 12). While the consequences of the sleep alterations experienced by ICU patients on inspiratory endurance are unknown, two recent papers have reported that, in mechanically ventilated patients, poor sleep quality was associated with a prolonged weaning process (13, 14). In order to better understand the relationship between sleep deprivation and respiratory performance, we decided to investigate the effect of one-night sleep deprivation on respiratory motor output and inspiratory endurance in healthy subjects. We hypothesized that experimental one-night sleep deprivation in healthy subjects would simultaneously alter respiratory motor output and reduce time to task failure during a moderate and constant sustained inspiratory loading task.

Some results of this study were previously reported in the form of an abstract at the 2017 meeting of the American Thoracic Society, Washington, USA (15).

## **METHODS (499)**

Detailed methods are provided in online data supplement.

### **Design and setting**

We conducted a single-center physiological study at University Hospital of Poitiers (France) between March 2016 and May 2017. Based on previous studies (5, 8), a minimum of 16 subjects was necessary to demonstrate a decrease of 20 min (-30%) of time to task failure in sleep-deprivation condition compared to normal sleep condition, in a bilateral hypothesis with a 5% alpha risk and a power of 80%. The study was approved by an independent ethics committee and registered at <http://www.clinicaltrials.gov> (#NCT02725190).



**Sleep protocol**

To assess endurance, exercise was performed after a night with normal sleep at home and after one night of sleep deprivation spent in the clinical investigation center. These two nights were randomly allocated and separated by at least 7 days. During sleep deprivation, subjects were forced to stay awake until the end of the experimentation.

**Endurance trial**

Endurance trials started at 10am in both conditions. Each session started by assessment of maximal inspiratory pressure using standard procedure (5). After that, subjects were asked to breathe as long as they could (maximum one hour) through a facial mask connected to an inspiratory threshold valve set to reach 30% of maximal inspiratory pressure.

**Assessment of respiratory motor output**

Overall respiratory motor output was assessed by continuous recording of diaphragmatic EMG (EMG<sub>dia</sub>) activity using a dedicated Gaeltec probe equipped with 3 pairs of electrodes.

Cortical contribution to the respiratory motor output was assessed by measuring the pre-inspiratory motor potential amplitude (PIP) obtained by continuously recording electroencephalogram (EEG) throughout the trial. Pre-inspiratory motor potential amplitude was calculated during the first 15 and last 15 min of each trial using a previously published method (4). (see supplemental Figure E1). We also investigated cortical respiratory motor output by assessing voluntary activation of the diaphragm. This technique consists in measuring the amplitude of a superimposed Pdi twitch elicited by bilateral phrenic stimulation (using cervical magnetic stimulation) during a maximal inspiratory maneuver.

**Ventilatory measurements**

Ventilatory variables including ventilatory flow, airway pressure, end-tidal CO<sub>2</sub>, pulse oximetry, were recorded continuously. Flow was recorded via a pneumotachograph connected to the mask. Esophageal (Pes), gastric (Pga), transdiaphragmatic (Pdi) pressures were continuously recorded to assess patient effort and pressure output of the diaphragm. Maximal inspiratory pressure was measured again immediately after the end of trial. The level of inspiratory effort was quantified by measuring pressure time index of the diaphragm (PTIdia). Spectral analysis of EMGdia was performed to search for contractile fatigue.

Dyspnea was assessed with a 10 cm-visual analog scale (VAS) at baseline (mean of 3 measures performed at 0, 2 and 4 min after trial initiation) and presented to the participants every 10 minutes and at the end of trial. The course of dyspnea was also assessed.

**Statistical analysis**

Continuous variables were expressed as median and [25<sup>th</sup>-75<sup>th</sup>] percentiles and compared using non-parametric paired test (Wilcoxon signed rank test). Correlation between continuous variables was assessed with Spearman correlation test. A P-value of less than 0.05 was considered as statistically significant.

**RESULTS (385)**

Twenty healthy males with a median age of 27 years [26-28] were included in the study. All subjects completed the protocol without side effects. Before endurance trials, maximal inspiratory pressure did not differ between one-night sleep deprivation and normal sleep conditions: 129 cmH<sub>2</sub>O [120-134] vs. 130 cmH<sub>2</sub>O [123-142], respectively ( $p=0.4$ ) and voluntary activation of the diaphragm was maximal (100%) in both conditions ( $p=0.4$ ).

Time to task failure was shorter after one-night sleep deprivation than after normal sleep and reduced by 50%: 30 min [17-41] vs. 60 min [45-60],  $p=0.002$  (**Figure 1**). The reduction of time to task failure was variable among individuals. While some subjects experienced a dramatic shortening of time to task failure (-85%), six of them had similar performance in sleep deprivation condition and normal sleep condition.

At the beginning of the trial, electrical activity of the diaphragm and pre-inspiratory motor potential amplitude was lower in one-night sleep deprivation condition than in normal sleep condition (4.5  $\mu$ V [2.5-6.4] vs. 7.3 $\mu$  [4.3-10.4],  $p=0.02$ ) (**Figure 2 and Table 1**). Pre-inspiratory motor potential amplitude correlated with the duration of the endurance trial ( $Rho=0.66$ ,  $p=0.003$ ) (**Figure 3**) in sleep deprivation condition. Other ventilatory parameters, including ETCO<sub>2</sub> values (36.2mmHg [34.4-39.7] vs 37.7 [30.8-40.7],  $p=0.88$ ), were not different in one-night sleep deprivation and in normal sleep condition (**Table 1**).

During trials performed in one-night sleep deprivation condition, pre-inspiratory motor potential amplitude (5.4 $\mu$ V [2.3-6.5] vs 1.1 $\mu$ V[0-2.9],  $p=0.02$ ), electrical activity of the diaphragm, pressure output of diaphragm (PTPdi) and tidal volume decreased and respiratory rate increased significantly from the beginning to the end of the trial (**table 2 and figure 4 and 5**). Such decreases did not occur in normal sleep condition.

Spectral analysis of diaphragmatic EMG (High frequency/Low frequency ratio) was not different prior compared to after trial in sleep deprivation and normal sleep condition.

*Sleep deprivation and inspiratory endurance*

Although baseline dyspnea was not different after sleep deprivation and after normal sleep (5.9 mm [4.6-7] vs. 4.3 [3.3-6.5], respectively,  $p=0.08$ ), dyspnea increased significantly from the beginning to the end of the trial in both conditions (**Table 2**). However, the course of dyspnea was steeper in sleep deprivation condition than in normal sleep condition and the maximal score was reached earlier in sleep deprivation condition than in normal sleep condition (16.8min [10.1-22.8] vs 33.5min [23.5-44], respectively,  $p=0.009$ ) (**supplemental figure E2**).

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**DISCUSSION (1586)*****Key findings***

In our study, time to task failure during an inspiratory endurance trial performed in healthy subjects was significantly shorter after one-night sleep deprivation than after normal sleep. Compared to normal sleep condition, sleep deprivation caused a significant reduction of pre-inspiratory motor potential amplitudes, which decreased throughout the trial, and a progressive decrease of diaphragm electromyographic activity. This suggests that the decreased respiratory motor output attested by the reduction in diaphragmatic activity might be due to the decline of its cortical component. In contrast, pre-inspiratory motor potential amplitudes and EMG diaphragmatic activity were maintained throughout the trial after normal sleep. In sleep deprivation condition, cortical respiratory motor output correlated with trial duration. Additional factors such as decreased motivation and increased dyspnea might be involved in endurance reduction associated with sleep deprivation. To our knowledge, this is the first study to show that sleep deprivation can alter respiratory motor output in general and its cortical contribution in particular.

**Impact of sleep deprivation on endurance**

Our results are in line with those of Chen and colleagues who found significantly decreased inspiratory effort tolerance (-25%) in a high intensity and brief inspiratory exercise study (3). However, we observed more markedly altered inspiratory endurance (reduced by 50%), suggesting that sleep deprivation might be more deleterious in low than in high intensity exercise.

Our results indicate a highly variable effect of sleep deprivation between subjects. Large and replicable, trait-like individual differences in performance vulnerability to acute sleep deprivation have been demonstrated repeatedly. Some individuals are highly vulnerable to neurobehavioral deficits when sleep-deprived, whereas others demonstrate remarkable resistance to sleep loss (16,

17). The underlying reasons are unknown but there are numerous arguments for a genetic contribution (16).

Several mechanisms can lead to reduced inspiratory endurance e.g. contractile fatigue of the diaphragm (18), altered cerebral command (9) or excessive perception of effort (19).

### ***Contractile fatigue of the diaphragm***

Reduction in contractibility of diaphragm is a mechanism that might occur during inspiratory endurance trials (8, 20). However, we reason that diaphragm contractile fatigue was not involved in shortened endurance induced by one-night sleep deprivation. First, the intensity of inspiratory effort (assessed with pressure time index [PTIdia]) was below 0.18. Several studies have reported that prolonged inspiratory effort with PTIdia below the critical threshold of 0.18 did not elicit contractile fatigue and could be maintained for several hours (8, 20). In addition, maximal inspiratory pressure measured after the end of the trial was not reduced compared to values before the trials. If contractile fatigue had been present, subjects would have been unable to produce maximal inspiratory pressure equivalent to prior trial measures(21). Finally, spectral analysis of EMGdia supports the absence of contractile fatigue (18).

### ***Overall respiratory motor output***

Electromyographic activity of the diaphragm relates to overall respiratory motor output (22-24). Our results showed that diaphragmatic EMG activity decreased from beginning to end in sleep deprivation conditions only. Indeed, reduced muscular activation is a hallmark of central motor fatigue in skeletal muscle as well as in diaphragm (25, 26). Thus, the significantly reduced EMG activity at the end of trial in sleep-deprived condition suggests alteration of the overall respiratory motor output. At the beginning of the trial in sleep deprivation condition, overall respiratory output was reduced but above the minimal respiratory motor output required to open the threshold valve

and overcome the load. As the trial was moving forward, the progressive decrease of cortical motor output brought the overall respiratory motor output closer to the minimal inspiratory effort required to open the valve. When overall respiratory motor output was below the minimal inspiratory effort required to overcome the load, the subject stopped the trial. This reduced motor output might be the cause of early task failure in sleep-deprived condition.

#### ***Brainstem respiratory motor output***

Locher and coll. have reported that brainstem motor output was not involved in inspiratory threshold loading (27). In our study, EtCO<sub>2</sub> remained in normal a range during trials in all our subjects but two, supporting the hypothesis that brainstem motor output might not have been overactivated. Furthermore, Spengler and coll. showed that one-night sleep deprivation does not alter brainstem ventilatory control (28).

#### ***Primary motor cortex of the diaphragm***

Our results show that during a voluntary maximal inspiratory maneuver, activation of cortical motoneurons was complete in sleep deprivation condition since bilateral phrenic stimulation did not elicit any superimposed twitch. These results are in line with the literature showing that a sleep-deprived brain is able to activate all cortical neurons of the primary motor cortex to produce an explosive maximal voluntary contraction (3, 29).

Evidence exists that motor cortex activity can also be modulated by sensory afferents from muscles (10, 25, 30), including noxious afferents (31). These afferents might be involved in some reflex mechanisms that could inhibit respiratory motor output under loaded conditions (10).

#### ***Supplementary motor cortex of the diaphragm***

To our knowledge, our study is the first to investigate the effect of sleep deprivation on supplementary motor area (SMA) cortical respiratory motor output. Pre-inspiratory motor potential

is thought to reflect the neuronal activity of the supplementary motor area required to ignite the primary motor cortex that will generate muscle contraction (32). High pre-inspiratory motor potential amplitude is associated with higher generated force (33, 34) and reflects higher neuronal activity (35). Then, the decrease of pre-inspiratory motor potential amplitude might reflect hypoactivity of the SMA. Indeed, direct projections from the SMA on phrenic motor neurons have been demonstrated in humans (36). Furthermore, the supplementary motor area exerts a tonic excitatory influence on corticospinal projection to phrenic motor neurons in awake humans (7). Since SMA has been shown to be hypoactive in sleep deprivation conditions (37), it is likely that sleep deprivation reduces SMA activity and its tonic influence on phrenic motor neurons, thereby reducing cortical respiratory motor output and precipitating task failure. The significant dose-effect relationship between pre-inspiratory motor potential amplitude and endurance capacities (subjects displaying higher respiratory motor output had longer endurance) further reinforces the hypothesis of a causal relationship between supplementary motor area hypoactivity and short endurance.

#### **Sleep-associated decreased motivation**

A study limitation could be our choice not to encourage volunteers during the inspiratory loading trial. We decided not to do so because such encouragements might have redirected attention on breathing and might have modified pre-inspiratory motor potential amplitudes (38). A lack of motivation could be implicated in early task failure(39). However, at the 15<sup>th</sup> minute of the trial, all but 4 sleep-deprived subjects were still breathing through the mask and had similar performances compared to normal sleep condition. Yet, a statistically significant reduction of respiratory motor output and a statistically significant decrease of the cortical motor output were observed during the first 15 minutes in sleep deprivation condition (figure 4). If reduced motivation existed at that time in sleep deprivation condition, it did not have any significant effect on performance. However, the



absence of reduced motivation at minute 15 does not exclude reduced motivation later in the trial. A loss of motivation could have contributed to the shortening of trials, likely as an additional factor. Reduced respiratory motor output and reduced endurance occurred independently of subjects' will.

### ***Repeated loaded breaths vs single breath***

Continuous inspiratory loading (i.e. load applied during several successive breaths) engages motor circuits in a very different way than single loaded breathing. While single loading activates many brain areas, continuous loading activates fewer phrenic motoneurons and fewer brain areas (40, 41). This might explain why continuous loading breathing is altered by sleep deprivation and why single breath (i.e. maximal inspiratory pressure and voluntary activation) seems not to be affected by one-night sleep deprivation. In fact, continuous loading engages the prefrontal cortices (27, 41), which are particularly affected by sleep deprivation (42, 43). Sleep deprivation might explain the reduction of pre-inspiratory motor potentials amplitude which are generated in the prefrontal cortex (32, 44). Our results suggest that sleep deprivation results in poor adaptation of cortical area during prolonged inspiratory loading (supplemental figure E3).

Sleep deprivation causes pronounced brain glycogen depletion (-40%) including motor cortex (45). During an exhausting motor exercise, cortical motoneurons consume the brain lactates provided by astrocyte glycogenolysis (46, 47). In our study, the depletion of brain glycogen induced by sleep deprivation might have prevented astrocytes from supplying energy sufficient to prolong exercise.

### ***Excessive dyspnea***

As an alternative to motor deficit, early exhaustion might be favored by higher sensory perception of dyspnea in one-night sleep deprivation condition. Sleep deprivation is known to increase effort perceptions (2, 19) and perception of pain (48). Sleep deprivation also increases brain responses to noxious stimuli (48). The steeper progression of dyspnea in sleep deprivation condition suggests that

sleep deprivation might have increased the sensation of noxious dyspnea. A higher noxious dyspnea sensation might have led volunteers to end the trial earlier when sleep-deprived and might have contributed to shortened endurance.

### **Clinical inferences**

Our results showed that sleep loss might have deleterious effects on capacity to cope with moderate additional inspiratory load and suggest that insufficient sleep could therefore precipitate respiratory failure in several clinical situations. Many patients with chronic respiratory diseases have supplementary inspiratory loads when experiencing COPD or asthma exacerbations, for instance. The supplementary inspiratory load requires additional inspiratory effort. In addition, many patients in ICU display serious sleep disruption (12), which might alter inspiratory endurance. In line with our results, two recent studies in mechanically ventilated patients have reported that poor sleep quality was associated with a prolonged weaning process (13, 14). The mechanisms involved in inspiratory endurance deficit induced by sleep deprivation need to be further investigated.

### **CONCLUSION**

Our results showed that one-night sleep deprivation decreased inspiratory endurance by half during a trial when breathing against a sustained inspiratory load, without affecting strength of respiratory muscles and without muscular fatigue. This deleterious effect may be due to altered cortical contribution to respiratory motor output as indicated by markedly decreased amplitude of pre-inspiratory motor potentials on EEG at the beginning of the trial. Increased perception of dyspnea and reduced motivation could also be involved. By extrapolation to patients, these results suggest that sleep deprivation could trigger real brain dysfunction that may have an impact on respiratory capacities in patients suffering from respiratory failure.

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## TABLES

**Table 1:** Comparison of variables measured at the beginning of the trial, after a normal sleep night and after a night of sleep deprivation

	Normal sleep (n=20)	Sleep deprivation (n=20)	p Value
<b>Time to time task failure (min)</b>	<b>60 [45 - 60]</b>	<b>30 [17- 41]</b>	<b>0.0018</b>
MIP (cm H <sub>2</sub> O) (prior trial)	130 [123 – 142]	129 [120 – 134]	0.40
Inspiratory load (cmH <sub>2</sub> O)	40 [37 – 41]	39 [36 – 40]	0.46
PTIdia	0.08 [0.07 – 0.13]	0.11 [0.09 – 0.13]	0.3
Minute ventilation (L/min)	19.4 [13.3-26.6]	20.9 [16.4-36.1]	0.08
Respiratory rate (n/min)	10 [6 – 13]	11 [7 – 14]	0.46
Tidal volume (L)	2.3 [1.7-3.2]	1.8 [1.4-3.7]	0.16
PTPes (cmH <sub>2</sub> O .s/cycle)	82.6 [59.7-102.8]	68.1 [55.5-111.7]	0.74
PTPdi (cmH <sub>2</sub> O .s/cycle)	92 [80-119]	88 [65-157]	0.84
EMGdia (%EMGmax)	43 [26-52]	27[18-31]	<b>0.03</b>
ETCO <sub>2</sub> (mmHg)*	36.2 [34.4-39.7]	37.7 [30.8-40.7]	0.88
<b>PIP (μV)*</b>	7.3 [4.3-10.4]	4.5 μV [2.5-6.4]	<b>0.02</b>
Dyspnea (VAS)	4.3 [3.3-6.5]	5.9 [4.6-7]	0.08
<b>Course of dyspnea § (min)</b>	33.5min [23.5-44]	16.8min [10.1-22.8]	<b>0.009</b>

Values are given in median [25th-75th percentiles].

Abbreviations: MIP = Maximal inspiratory pressure; Pes = Oesophageal pressure; Pdi = transdiaphragmatic pressure; PTP = Pressure time product; EMGdia = diaphragmatic electromyogram; ETCO<sub>2</sub> = End tidal CO<sub>2</sub> in the mask; PIP = Pre-inspiratory motor potential; VAS = Visual analog scale; \*: calculated on the 15 first min of the trial; §: measured through the trial.

## Sleep deprivation and inspiratory endurance

**Table 2:** Comparison of variables at the beginning vs the end of the endurance trial, after a normal sleep night and after a night of sleep deprivation

	Normal sleep		<i>p</i>	Sleep deprivation		<i>p</i>
	Beginning	End		Beginning	end	
MIP § (cm H <sub>2</sub> O)	137 [126-144]	128 [119-146]	0.07	137[123-141]	125[121-139]	0.22
Minute ventilation (L/min)	19.4[13.3-26.6]	15.8[12.6-24]	0.86	20.9 [16.4-36.1]	16.9[13.9-31.9]	0.74
Respi rate (n/min)	10 [6 – 13]	11[4-12]	0.52	11 [7 – 14]	15[8-17]	<b>0.02</b>
Tidal volume (L)	2.3 [1.7-3.2]	2.3 [1.4 -3.7]	0.87	1.8 [1.4-3.7]	1.6 [0.9 -3.2]	<b>0.03</b>
PTPdi (cmH <sub>2</sub> O.s/cycl)	92 [80-119]	93 [64-108]	0.09	88 [65-157]	77 [49-108]	<b>0.02</b>
EMGdia (%EMGmax)	43 [26-52]	39[21-60]	0.8	27[18-31]	20[14-29]	<b>0.05</b>
PTPdi/EMGdia	1506[883-2001]	1011[782-1583]	0.69	1286[981-1664]	1216[1028-1611]	0.93
ETCO <sub>2</sub> (mmHg)*	38.2 [30.4-40.7]	38[30.5-39]	0.3	38.0 [33.6-40.8]	39.7[35.2-41.5]	0.6
<b>PIP (μV)* (n=9)</b>	6.6 [3.9-10.4]	5.0 [3.7-8.6]	0.21	5.4 [2.3-6.5]	1.1[0-2.9]	<b>0.02</b>
Dyspnea VAS(mm)	4.3 [3.3-6.5]	9.2 [8.1-9.8]	0.0001	5.9 [4.6-7]	9.5 [9.2-9.7]	0.0002

Values are given in median [25th-75th percentiles].

Abbreviations: MIP : absolute value of maximal inspiratory pressure (§: measured at the mask, before and after the trial); Min ventil = minute ventilation (calculated on 5 first and 5 last minutes); Respi rate = respiratory rate; Tidal vol = tidal volume; Pdi = transdiaphragmatic pressure; PTP = Pressure time product; EMGdia = diaphragmatic electromyogram; ETCO<sub>2</sub> = End tidal CO<sub>2</sub> in the mask; PIP = Pre-inspiratory motor potential; VAS = Visual analog scale; \*: calculated on the 15 first or 15 last min of the trial, when possible (see online supplement).

**FIGURE LEGENDS****Figure 1: Endurance in normal sleep and sleep deprivation conditions.**

Individual data (squares) of endurance (delay to time failure, min) in 19 subjects in sleep condition (left) and in one-night sleep deprivation condition (right). Median endurance (vertical bars) is significantly shortened in sleep deprivation condition compared to normal sleep condition ( $p=0.0018$ , Wilcoxon signed rank test). To avoid superposition, the four subjects who had an endurance time of 60 min (maximal duration) in both conditions are positioned at 59.5, 60, 60.5 and 61min on the graph.

**Figure 2: Pre-inspiratory motor potentials amplitude at the beginning of the trial in sleep and sleep deprivation conditions.**

Individual data (squares) of pre-inspiratory motor potentials amplitude ( $\mu\text{V}$ ) in 19 subjects in sleep condition and in sleep deprivation condition. Median amplitude (vertical bars) is significantly lower in sleep deprivation condition (right black bar) compared to sleep condition (left white bar) ( $p=0.02$ , Wilcoxon signed rank test).

**Figure 3: Correlation between pre-inspiratory motor potential amplitudes at the beginning of the trial and endurance duration in sleep deprivation condition.**

Individual data (squares) of endurance time (time to task failure, min) depending on pre-inspiratory motor potential amplitude ( $\mu\text{V}$ ) measured in the first 15 min of trial in 18 subjects, in sleep deprivation condition. Endurance time and PIP amplitude are significantly correlated ( $Rho=0.66$ ,  $p=0.003$ , Spearman correlation test) suggesting that the higher the PIP amplitude, the longer the endurance.

**Figure 4: Diaphragmatic electromyogram at the beginning and at the end of trial, in normal sleep and sleep deprivation conditions.**

Individual data (squares) of diaphragmatic EMG ( $\mu\text{V}$ ) in 11 subjects in sleep condition (left) and in sleep-deprivation condition (right). Median amplitude (vertical bars) decreases significantly at the end of trial only in the sleep-deprived condition (right black bar) ( $p=0.04$ , Wilcoxon signed ranktest).

**Figure 5: Pre-inspiratory motor potential amplitudes at the beginning and at the end of trial, in normal sleep and in sleep deprivation conditions.**

Individual data (squares) of pre-inspiratory motor amplitude ( $\mu\text{V}$ ) in 9 subjects in sleep condition (left) and in sleep-deprivation condition (right). Median amplitude (vertical bars) decreases significantly at the end of trial only in the sleep-deprived condition (right black bar) ( $p=0.02$ , Wilcoxon signed rank test).



**Impact of sleep deprivation on respiratory motor output and endurance: a physiological study**

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**ON LINE DATA SUPPLEMENT****METHODS****Healthy volunteers**

Twenty healthy males (median body mass index 23kg/m<sup>2</sup>, 22–24 kg/m<sup>2</sup>) participated in this study. None of them had a history of respiratory disease or sleep disorder, were smokers or took any medication. All of them usually drank less than 3 espressos per day. All of them were good sleepers (Pittsburgh Sleep Quality Index <5), usually sleeping more than 6 hours per day, and had a neutral score on the morningness-eveningness questionnaire of Horne and Ostberg (ie score ≥ 31 and ≤ 69). None of them were shift-workers or had travelled across more than 4 time zones during the three weeks preceding their participation. Before each session, volunteers committed to keep their usual sleep schedules, to have at least 6 hours of sleep each night, and to refrain from consuming alcohol 48h before the session.

All subjects were informed regarding the sequence of events, but the precise objective of the study was revealed only after the study to preserve the naivety necessary for interpretation of the results.

The study was approved by the independent local ethics committee (Comité de Protection des Personnes Ouest-III, February, 22<sup>nd</sup> 2016, registration number: 16.01.04) and conducted according to the ethical principles of the Declaration of Helsinki and the current guidelines for good clinical practice. All subjects gave their written informed consent.

### **Statistical power calculation and minimal number of subjects**

Studies are quite consistent and reported shortening of 20 to 30% of time to task failure in sleep deprivation conditions, whatever the exercise (cycling or treadmill) or muscle groups involved (whole body or limb movement effort), without changes in data distribution (1). No study exists with moderate constant and prolonged (>40 min) exercise of the diaphragm. Considering the literature (2), a standard deviation of 20 minutes of time to task failure appeared realistic. Based on a previous study (3), we expected volunteers to be able to breathe around 60 minutes against moderate inspiratory loading (set at 30% of maximal inspiratory loading) in normal sleep condition.

In these conditions, a minimum of 16 subjects was necessary to demonstrate a decrease of 20 min (-30%) of time to task failure in sleep deprivation condition compared to normal sleep condition (40 min vs 60min), in a bilateral hypothesis with a 5% alpha risk and a power of 80%. An independent local ethics committee (Comité de Protection des Personnes Ouest III) authorized investigators to include 20 volunteers.

### **Sleep protocol**

Sleep deprivation session and normal sleep session were randomly allocated at inclusion. Group 1 (n=10) started with the sleep deprivation session and group 2 (n=10) started with the normal sleep session.

Subjects wore a wrist actigraph (Actiwatch<sup>®</sup> AW7, CamnTech, Cambridge, UK) three days before each session to check that they actually slept more than 6 hours per night.

### *Sleep deprivation*

Volunteers arrived at the Centre d'Investigation Clinique at 1pm. Quiet activities and ambulation were allowed, and conversations and games were organized. Exciting video-games or physical

activities, coffee and food were forbidden. Volunteers were watched over by a nurse throughout the night to check for sleep deprivation.

Calibrated meals (1050[985-1108] calories) were offered at 7pm and at 7am (median [25<sup>th</sup>-75<sup>th</sup>]).

Subjects filled in a visual analog score of fatigue every 2 hours. A miniEEG (DeltaWave, CamnTech, Cambridge, UK) with 3 EEG leads (F4-A1, C4-A1, O2-A1) and one right EOG lead were placed at 9pm to record possible micro-sleep episodes. Subjects were allowed to take a nap after the trial.

#### *Normal sleep night*

Volunteers slept at home for at least 6 hours and had their breakfast at home, without any coffee or tea. Correct sleep time was verified with actimetry.

#### **EEG acquisition**

EEG activity was recorded with a cup electrode at Cz (International 10-20 System) after gentle abrasion and cleaning of the scalp to ensure impedance below 5k $\Omega$ , with two reference electrodes placed on mastoids and a ground electrode placed at Fz. Electrooculograms (EOG) were recorded using two electrodes placed at the upper right eye canthus and at the lower left eye canthus and referenced to the opposite mastoid.

The electrodes were connected to an EEG preamplifier (EEG100C and EOG100C, Biopac System Inc, Goleta, CA, USA) with 2000- fold gain, a band-pass frequency of 0.05–1000 Hz digitalized at 1000 Hz (MP150, Biopac sytem Inc Goleta, CA, USA) and stored as computer files for offline analysis.



**EEG analysis: pre-inspiratory motor potential (PIP)**

Pre-inspiratory motor potential was built by averaging EEG epochs starting 2.5-sec before and 1sec after the onset of inspiration. The onset of inspiration was defined by zero crossing of the mask pressure signal. After deleting, EOG artifacts on EEG (using artifact denoising function, Acknowledge software, Biopac System Inc), epochs exhibiting movement artifacts were visually discarded.

Depending on the subjects, 5–20% of the recorded breaths were eliminated through this process. PIP amplitude was determined on the average signal as the voltage difference ( $\mu\text{V}$ ) between nadir and value when mask pressure signal crosses the zero line. Sixty to eighty respiratory cycles are required to obtain a reliable EEG average and reliable measurement of pre-inspiratory motor potential amplitude (3).

**Pressures and diaphragmatic recordings**

Esophageal (Pes), gastric (Pga) pressures and diaphragm electromyogram (EMGdia) were continuously recorded using a dedicated Gaeltec probe (CTO/7E-2, Gaeltec Devices Ltd, Dunvegan, Scotland, UK) inserted through the nose after topical anesthesia (5% lidocaine). Pressure captors were connected to a Validyne differential pressure transducer (Validyne, Northridge, CA, USA), digitalized (100Hz) and stored using a Biopac system (MP150, Biopac sytem Inc Goleta, CA, USA).

Transdiaphragmatic pressure (Pdi) was obtained on-line by subtracting Pes from Pga. EMGdia was recorded through 3 pairs of EMG bipolar electrodes (10mm long, separated by 10mm) mounted on the probe and placed between the esophageal and gastric pressure captors. EMGdia were amplified and digitalized at 1000Hz (MP150 Biopac system and Acknowledge software5.0, Biopac System Inc, Goleta, CA, USA) and stored for off-line analysis.

### Pressure analysis

Pes, Pgas and Pdi swings were identified automatically using a threshold recognition algorithm (Dataview<sup>o</sup> software, J. Heitler, Univ of Saint Andrews, Scotland, UK) and the area and peak of each respiratory cycle were calculated after visual inspection of the tracings to remove artifacted cycles.

We quantified inspiratory effort by calculating pressure time index of the diaphragm (PTIdia), which is the product of two ratios : inspiratory time divided by total cycle time and mean transdiaphragmatic pressure divided by maximal transdiaphragmatic pressure ratio. An inspiratory effort with PTIdia below the critical threshold of 0.18 will not trigger contractile fatigue and can be sustained for several hours(2, 4).

Specific and cautious measures were taken to position the EMG probe (a single specimen) at the same depth in both sessions. First, the probe has marks every ten cm. The last visible mark and the distance between the nostril and this mark was noted. Furthermore, we checked with Pdi twitch at rest that Pdi twitch amplitude was similar to the first session. If not the case, the probe was slightly pushed or pulled.

As results, Pdi twitch amplitudes between first and second sessions were not statistically different (18cmH<sub>2</sub>O [14 – 28] vs 19cmH<sub>2</sub>O [8 – 23], respectively, p=0.12).

### Diaphragmatic EMG analysis

Diaphragmatic electromyogram activity (EMGdia) was quantified on the derivation displaying the best EMGdia bursts during inspiratory effort. EMGdia raw signals were processed to extract ECG artifacts using processing adapted from previously published methods (5). Briefly, EMGdia raw signals were root-mean squared and high-pass filtered (50Hz) and then smoothed using a running median window of 0.03s (Acknowledge software5.0, Biopac System Inc, Goleta, CA, USA). Each

EMGdia burst was identified automatically using a threshold recognition algorithm (Dataview<sup>o</sup>, J. Heitler, Univ of Saint Andrews, Scotland, UK) and the peak of each EMGdia burst was calculated after visual inspection of the tracing to remove artifactual cycles, as previously reported (6).

Work of breathing was assessed by calculating pressure time product on esophageal signal, on a breath-by-breath basis, using a semi-automated algorithm of Dataview<sup>o</sup>. Diaphragm electrical activity was assessed by EMGdia and diaphragm electro-mechanical coupling by PTPdi/EMGdia ratio (7). Pressure output of the diaphragm was determined by calculating pressure time product on Pdi signal (PTPdi).

Spectral analysis of EMGdia was performed to search for contractile fatigue. EMGdia signals during three maximal inspiratory efforts prior and three after endurance trial were used to calculate high frequency to low frequency ratio. EMGdia signals were band-pass filtered (low frequency: 20 to 50Hz and high frequency: 130 to 250Hz), rectified and integrated (time constant of 100ms) as previously recommended (4).

### **Voluntary activation of the diaphragm**

Bilateral phrenic stimulation was delivered by cervical magnetic stimulation (CMS), using a Magstim 200 stimulator equipped with a 90mm circular coil placed over the 7<sup>th</sup> cervical process and manually triggered by experimenters. CMS pulse (intensity of 100% of stimulator output) was superimposed upon maximal voluntary inspiratory effort. Subjects were asked to produce voluntary inspirations from their functional residual capacity (FRC) against an occluded airway. The Pdi trace was displayed on screen, and cervical magnetic pulse was given by an experimenter when Pdi reached the maximal value (8). The absence of a superimposed Pdi twitch elicited by cervical magnetic pulse revealed that all cortical motor neurons had been recruited by the subject. In contrast, the amplitude of the superimposed Pdi twitch paralleled the additional motor neurons not recruited by voluntary activation but excited by cervical magnetic pulse (8, 9). Voluntary activation was carried out before endurance trial.

## Ventilatory signals

Ventilatory flow was recorded through a facial mask by a heated pneumotachograph (3700A series, 0–160 l/min; dead space volume 14mL Hans Rudolf, Kansas City, MO) attached to the main port of the mask, connected to a differential pressure transducer (Pneumotach Amplifier-1 Series 1110, Hans Rudolph, Kansas City, MO, USA). A very low resistance antibacterian filter was placed between mask and pneumotachograph (RT019; flow resistance of 1.08 +/- 0,18 cmH<sub>2</sub>O at 45 L/min; Fisher&Paykel Healthcare Ltd, Maidenhead, Berkshire, UK). Mask pressure was measured by connecting the mask to a pressure transducer (Pneumotach Amplifier-1 Series 1110, Hans Rudolph, Kansas City, MO, USA) with flexible tubing. End-tidal partial CO<sub>2</sub> pressure (ETCO<sub>2</sub>) was measured by connecting a flexible tube from the mask to a gas analyzer (Life sense LS1-9R, CO<sub>2</sub> pump flow 75 ml/min, Nonin Medical, Inc. MN, USA).

For statistical comparisons, we used ventilatory parameters measured during the 10<sup>th</sup> and 15<sup>th</sup> min of the trial. In addition to progressive application of the load by 10% steps, this ensured us that subjects reached a stable breathing state in loaded conditions (10).

## Endurance trial

The constant inspiratory load (using a threshold valve, Inspiratory Muscle Trainer, Philips) was chosen based on the literature (3, 4) to replicate the increase of inspiratory load experienced by intubated patients performing spontaneous breathing trials during their weaning procedure (4). Load intensity (30% of MIP) was chosen to allow endurance trial duration of approximately 1 hour in normal conditions (3). After a 5-minutes period of breathing through mask without any load, a load of 10% of MIP was applied during 5 minutes. Then, an inspiratory load corresponding to 20% of MIP was applied for 5 min. Finally, a 30%MIP load was applied constantly throughout the trial, until task failure. The start of the trial (time 0) was the time at which the 30% MIP inspiratory load was applied.

No instructions regarding the respiratory pattern were given to the volunteers. Subjects wore headphones and no time cues were displayed on the laptop screen. They were informed that the study investigated the effects of dyspnea on attention and that 2 questions regarding the scenario or characters would be asked at the end of the movie. No encouragements were provided but they received one thousand euros if they performed both sessions correctly with satisfactory cooperation and were aware that their recompense could be substantially reduced (150€) if they did not respect instructions. Volunteers were asked to indicate with a simple gesture when they wished to stop. They did not know that maximal duration was set at one hour. To control for attentional effect, subjects watched on a laptop a movie of their choice that they had already watched and opted to watch again (3).

### **Course of dyspnea**

Dyspnea was estimated with a 10 cm-visual analog scale (VAS) on which subjects were asked to quantify it. The scale was anchored by “no respiratory discomfort” on the left (VAS =0) and “intolerable breathing discomfort” on the right (VAS=10). The course of dyspnea was assessed by calculating the delay (in min) between the beginning of trial and time at which subjects reached 80% of their final VAS score (Figure E2 A and B).

All statistical analyses were performed using SAS statistical software (V9.4).

## RESULTS

### Voluntary activation of the diaphragm:

Bilateral phrenic stimulation using cervical magnetic stimulation was applied when the subject provided maximal voluntary diaphragm contraction and did not elicit any superimposed twitch either in sleep deprivation or in normal sleep condition, in all subjects (twitch amplitude [cm of water]: 0 [0-0] vs 0 [0-0],  $p=0,44$ ).

### Endurance trial

Inspiratory load therefore did not differ between the 2 conditions: 39 cm H<sub>2</sub>O [36-40] after one-night sleep deprivation vs. 40 cm H<sub>2</sub>O [37-41] after normal sleep ( $p=0.46$ ).

One subject (#17) was excluded from this analysis because he repeatedly fell asleep and was unable to stay awake 20 min after the onset of the endurance trial performed in sleep deprivation. Attention distraction was effective since all subjects responded correctly to questions on the movie they watched. Digital oxygen saturation and ETCO<sub>2</sub> remained in a normal range throughout the trial (above 97% and below 44mmHg respectively) in all subjects but two (in whom ETCO<sub>2</sub> reached 45 [subject #10, control condition] and 47mmHg [subject #12, sleep deprivation condition]).

### Cortical respiratory motor output assessment: Pre-inspiratory motor potential (PIP)

A median of 83 (59-173) breaths in SD and 124 (92-172) in sleep condition were used to calculate pre-inspiratory motor amplitude ( $p=0.03$ , Wilcoxon Signed rank test). One subject (#21) was excluded from this analysis because of blinking artifacts, preventing reliable PIP calculation.

Pre-inspiratory motor potential could not be measured at the end of the trial due to shorter duration of trial in 7 subjects (#4, #6, #10, #14, #15, #S19, #S20) and due to EEG artifacts in 3 subjects #9 #18 #22 and due to sleepiness in one subject (#17). This definitively eliminated these subjects from our paired statistical test (Wilcoxon signed rank test).

**Diaphragmatic EMG activity during loading trial.**

Some subjects had to generate 50% or more of EMG<sub>dia</sub>% to sustain a 30% inspiratory load. This incongruence might be explained by the tool used to measure diaphragmatic EMG<sub>dia</sub>%. EMG<sub>dia</sub> activity was measured as the peak of the EMG burst, on root mean squared EMG signals (6). Some subjects might have responded to the inspiratory load by having a respiratory cycle with short inspiratory time (= a high and narrow EMG<sub>dia</sub> burst), while others might have responded by taking prolonged breathes with long inspiratory time (= a smooth and flattened EMG<sub>dia</sub> burst). Such incongruence has been reported by other teams(11).

We could not evaluate diaphragmatic EMG in the last 6 subjects (#17, #18, #19; #20, #21; #22) included in our protocol because one EMG lead of the probe was damaged and not functioning properly. We were confronted with difficulties in EMG recordings in one session in subject #7 (sleep deprivation session) and #8 (sleep deprivation session). This eliminated these subjects from our paired statistical test (Wilcoxon signed rank test).

**Spectral analysis of EMG<sub>dia</sub> prior and after trials.**

H/L ratios were not different prior compared to after trial in sleep deprivation condition (1.02 [ 0.83–1.2] vs 0.86 [0.49 -1.5] respectively; p=0.88) and in normal sleep condition : 0.81 [0.65-1.35] vs 0.92 [0.67-1.14] respectively, p=0.80).

**Respiratory pattern during loading trial.**

As stated in the methods section, we did not give any instructions to volunteers regarding the respiratory pattern they should follow. Some subjects adapted their respiratory pattern by dramatically decreasing their respiratory rate (down to 3 per minute) and by markedly increasing their tidal volume (up to 3 liters per minute). Such respiratory patterns, with similar range, have also been reported by previous teams (3, 12).

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## SUPPLEMENTAL FIGURES

**Supplemental Figure E1: Illustration of method to calculate pre-inspiratory motor potential (PIP).**

EEG segments (2.5 s before and 1s after onset of inspiration) were selected (example in rectangles) and averaged. PIP amplitude (in  $\mu\text{V}$ ) was calculated from the nadir of the EEG segment to the point at which mask pressure crossed the zero line (double arrow).

**Supplemental Figure E2: Illustration of method to quantify the course of dyspnea during endurance trial.**

Curves of visual analog score during trial after one-night sleep deprivation (dash-line) and after a normal sleep night (continuous line) in one representative subject (#1). Note the different shapes of the two curves.

**Supplemental Figure E3: Impact of sleep deprivation on different pathways conducting respiratory motor output.**

Simplified illustration of the structures altered by sleep deprivation (in red) and the structures not altered in sleep deprivation (in green).

Decreased EMG<sub>dia</sub> electrical activity supported the hypothesis that overall respiratory motor output is weaker after one-night sleep deprivation compared to normal sleep.

Normal end tidal  $\text{CO}_2$  supported the hypothesis that brainstem component of respiratory motor output is not activated under any condition. Normal primary motor cortex excitability assessed by cervical magnetic stimulation supported the hypothesis that this cortical motor area is unaltered by sleep deprivation. Similar P<sub>di</sub>/EMG<sub>dia</sub> ratios and T<sub>Ti</sub> in sleep deprivation and normal sleep suggested that diaphragm contractility is unaffected in sleep deprivation condition.

In contrast, the premotor cortical component of respiratory motor output decreased after one-night sleep deprivation. A decrease in the tonic excitatory output exerted by premotor cortex might explain the reduced overall output to phrenic motoneurons.

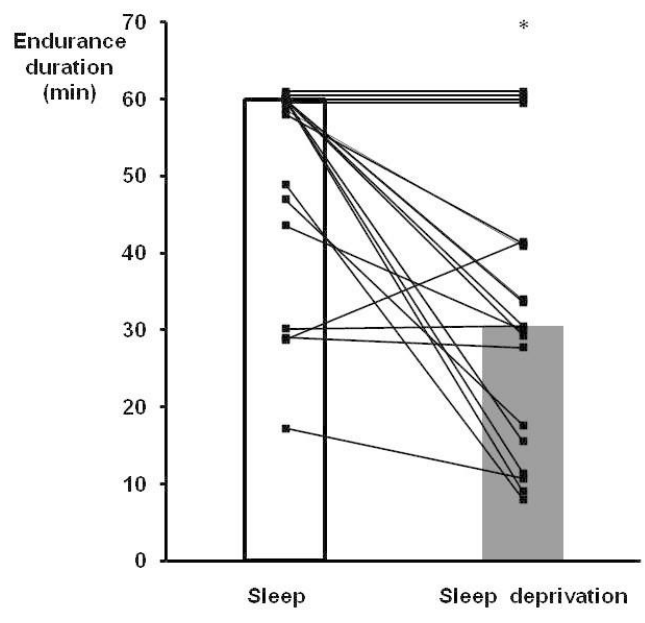


Figure 1: Endurance in normal sleep and sleep deprivation conditions.  
254x190mm (96 x 96 DPI)

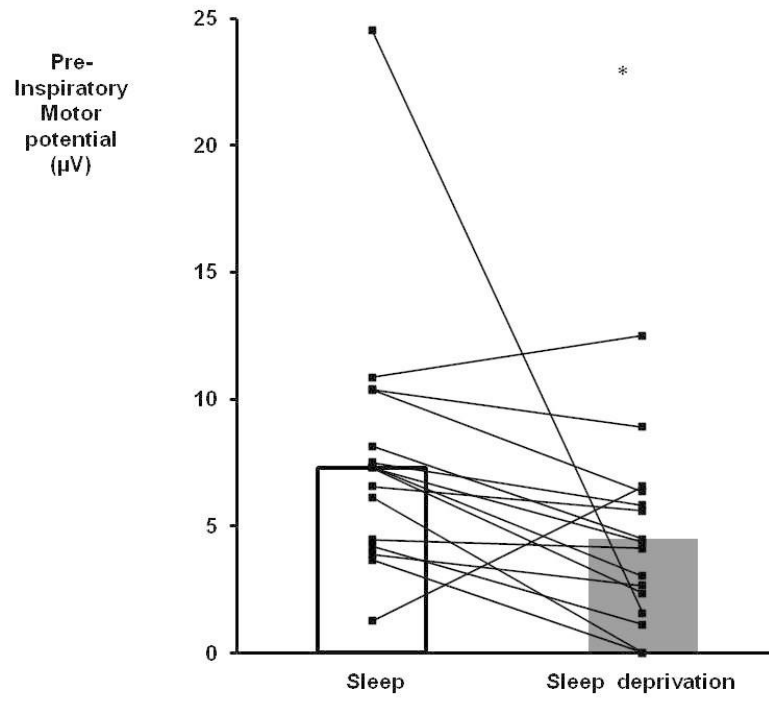


Figure 2: Pre-inspiratory motor potentials amplitude at the beginning of the trial in sleep and sleep deprivation conditions.

254x190mm (96 x 96 DPI)

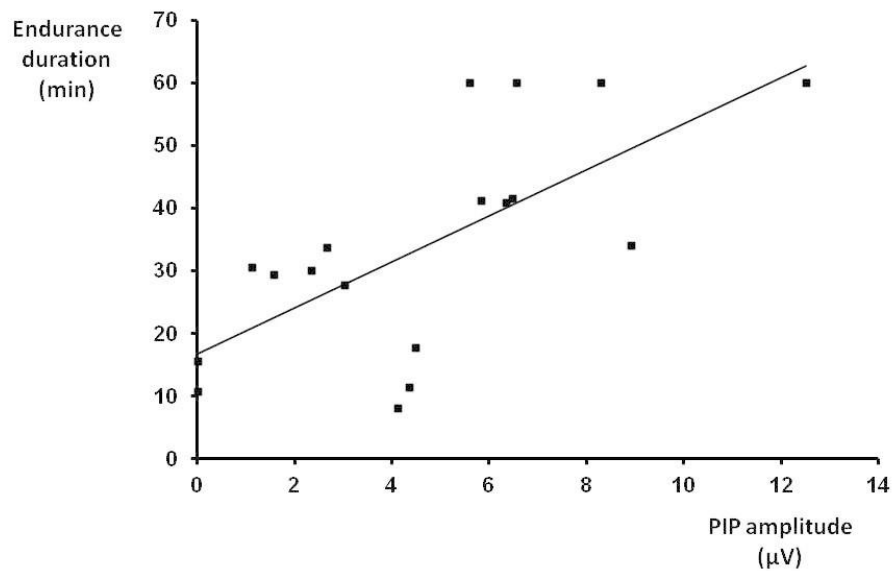


Figure 3: Correlation between pre-inspiratory motor potential amplitudes at the beginning of the trial and endurance duration in sleep deprivation condition.

254x190mm (96 x 96 DPI)

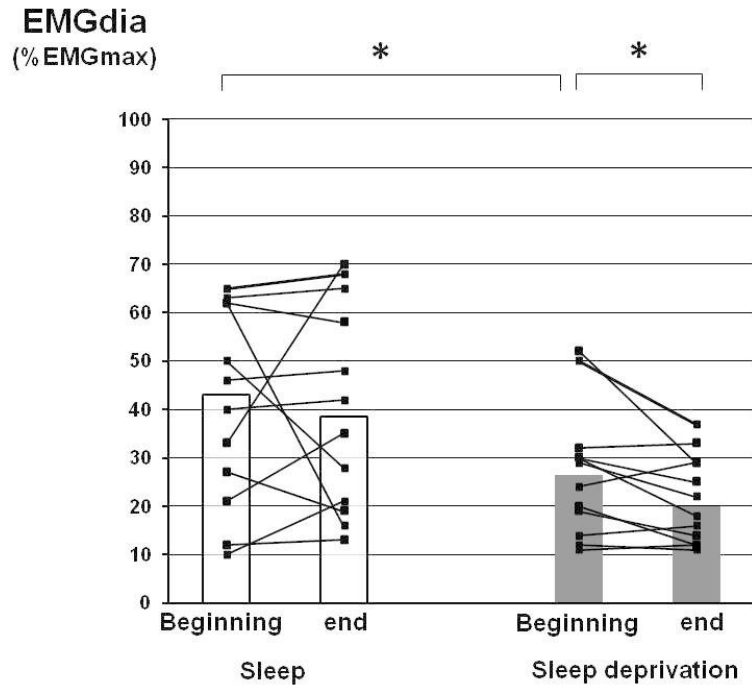


Figure 4: Diaphragmatic electromyogram at the beginning and at the end of trial, in normal sleep and sleep deprivation conditions.

254x190mm (96 x 96 DPI)

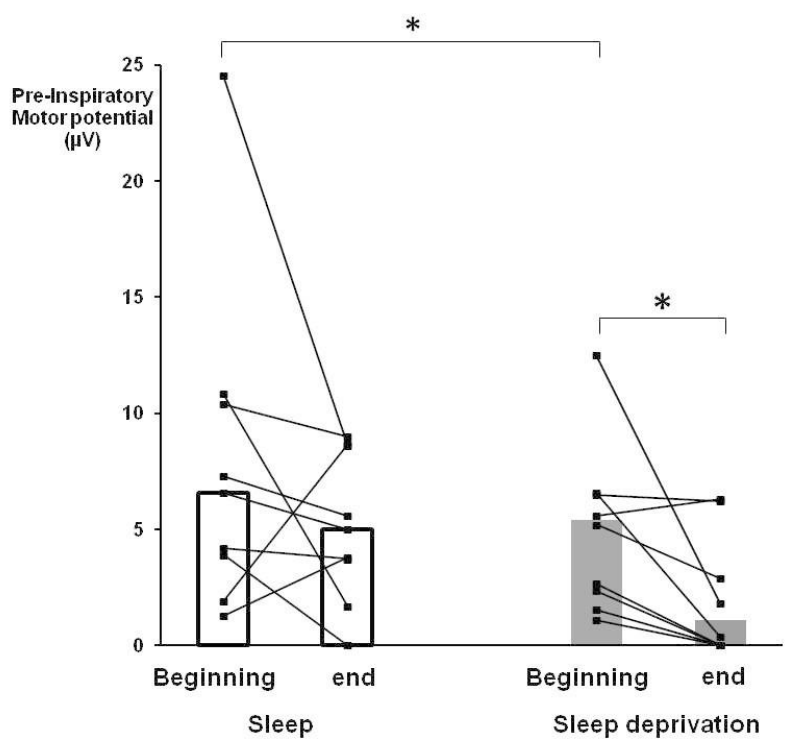
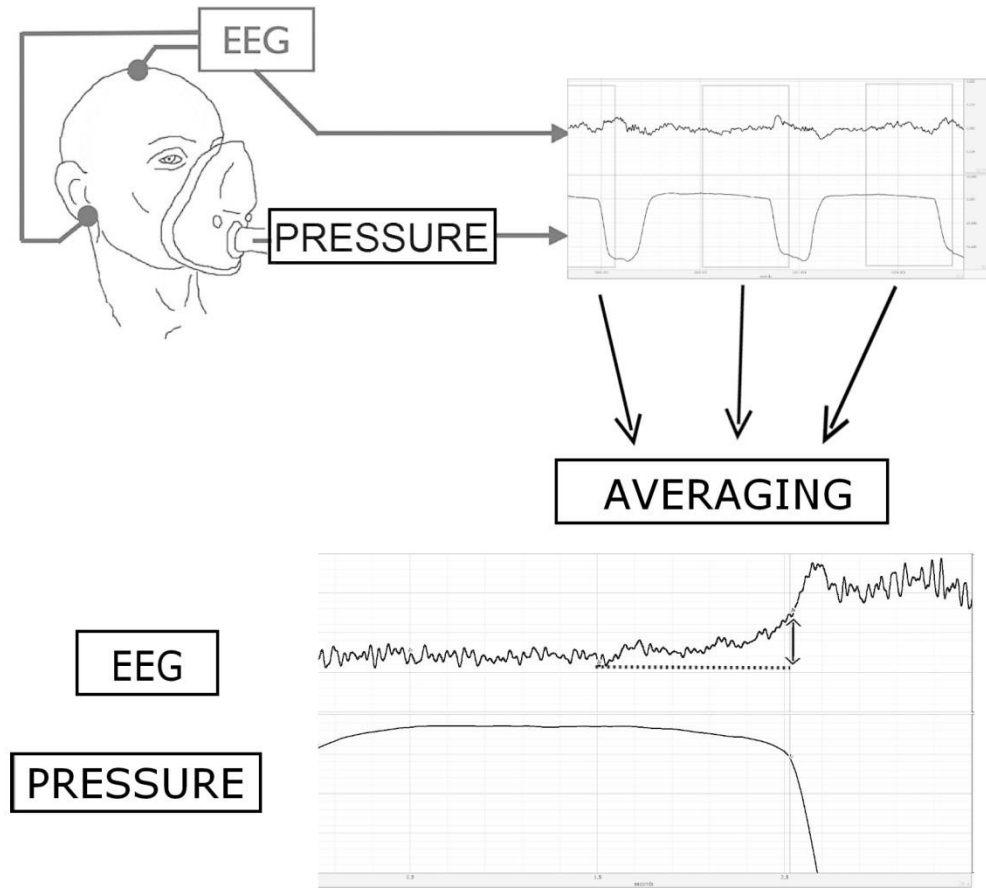


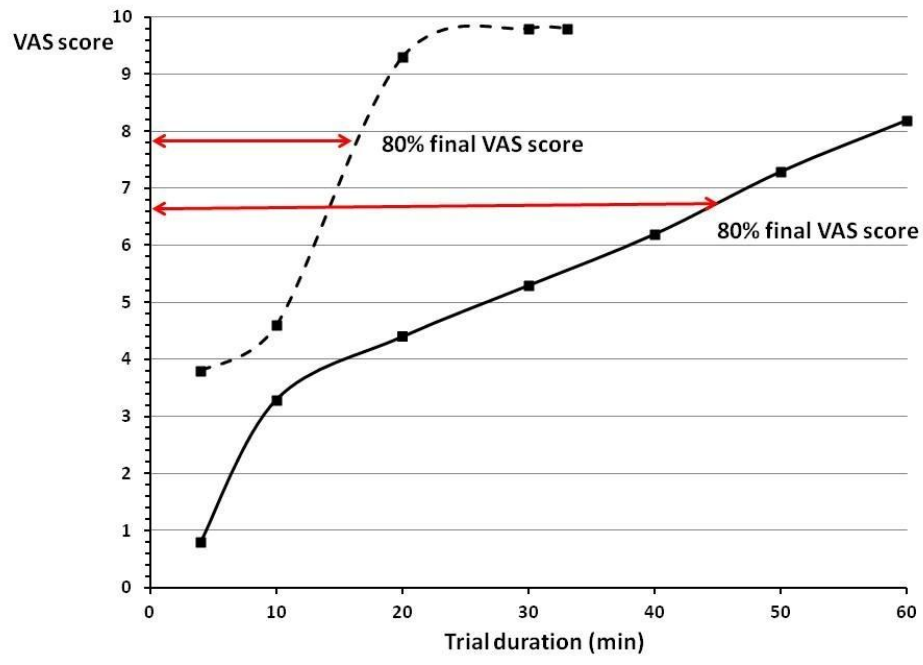
Figure 5: Pre-inspiratory motor potential amplitudes at the beginning and at the end of trial, in normal sleep and in sleep deprivation conditions.

254x190mm (96 x 96 DPI)



Supplemental Figure E1: Illustration of method to calculate pre-inspiratory motor potential (PIP).

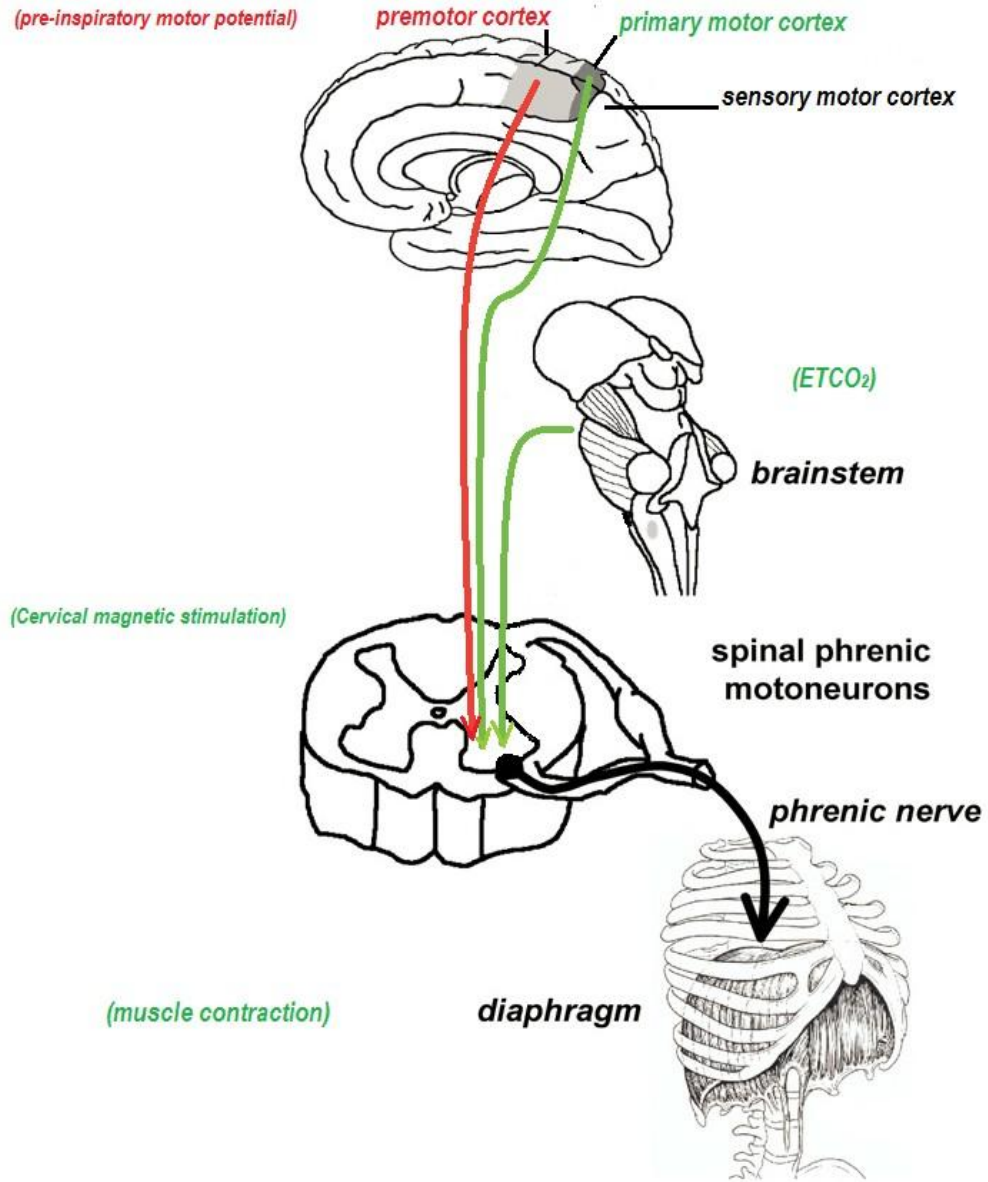
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Supplemental Figure E2: Illustration of method to quantify the course of dyspnea during endurance trial.

254x190mm (96 x 96 DPI)





Supplemental Figure E3: Impact of sleep deprivation on different pathways conducting respiratory motor output.