

# Neurophysiological Evidence for a Cortical Contribution to the Wakefulness-Related Drive to Breathe Explaining Hypocapnia-Resistant Ventilation in Humans

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Spontaneous ventilation in mammals is driven by automatic brainstem networks that generate the respiratory rhythm and increase ventilation in the presence of increased carbon dioxide production. Hypocapnia decreases the drive to breathe and induces apnea. In humans, this occurs during sleep but not during wakefulness. We hypothesized that hypocapnic breathing would be associated with respiratory-related cortical activity similar to that observed during volitional breathing, inspiratory constraints, or in patients with defective automatic breathing (preinspiratory potentials). Nineteen healthy subjects were studied under passive (mechanical ventilation,  $n = 10$ ) or active (voluntary hyperventilation,  $n = 9$ ) profound hypocapnia. Ventilatory and electroencephalographic recordings were performed during voluntary sniff maneuvers, normocapnic breathing, hypocapnia, and after return to normocapnia. EEG recordings were analyzed with respect to the ventilatory flow signal to detect preinspiratory potentials in frontocentral electrodes and to construct time-frequency maps. After passive hyperventilation, hypocapnia was associated with apnea in 3 cases and ventilation persisted in 7 cases (3 and 6 after active hyperventilation, respectively). No respiratory-related EEG activity was observed in subjects with hypocapnia-related apneas. In contrast, preinspiratory potentials were present at vertex recording sites in 12 of the remaining 13 subjects ( $p < 0.001$ ). This was corroborated by time-frequency maps. This study provides direct evidence of a cortical substrate to hypocapnic breathing in awake humans and fuels the notion of corticosubcortical cooperation to preserve human ventilation in a variety of situations. Of note, maintaining ventilatory activity at low carbon dioxide levels is among the prerequisites to speech production insofar as speech often induces hypocapnia.

**Key words:** cerebral cortex; control of breathing; hypocapnia; respiration

## Significance Statement

Human ventilatory activity persists, during wakefulness, even when hypocapnia makes it unnecessary. This peculiarity of human breathing control is important to speech and speech-breathing insofar as speech induces hypocapnia. This study evidences a specific respiratory-related cortical activity. This suggests that human hypocapnic breathing is driven, at least in part, by cortical mechanisms similar to those involved in volitional breathing, in breathing against mechanical constraints or with weak inspiratory muscle, and in patients with defective medullary breathing pattern generators. This fuels the notion that the human ventilatory drive during wakefulness often results from a corticosubcortical cooperation, and opens new avenues to study certain ventilatory and speech disorders.

## Introduction

Spontaneous ventilation in mammals is driven by the automatic activity of brainstem neural networks (Feldman et al., 2013) gener-

ating the respiratory rhythm and adapting the ventilatory excretion of carbon dioxide ( $\text{CO}_2$ ) to metabolic needs (chemosensitivity). Consequently, hypercapnia (defined as an increase in  $\text{CO}_2$  in the arterial blood,  $\text{Pa}_{\text{CO}_2}$ ), acidosis, fever, and exercise automatically in-

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crease ventilation. Hypocapnia (low  $\text{Pa}_{\text{CO}_2}$ ) on the other hand, depresses the activity of pre-Bötzinger and Böttinger neurons within respiratory central pattern generators (Sun et al., 2001) with a breakdown of raphe-pontomedullary connectivity (Nuding et al., 2015), and induces apneas. In other words, there is no need to breathe when there is no  $\text{CO}_2$  to expel. In animals, hypocapnia arrests breathing during sleep and during wakefulness (with a higher apnea threshold). In humans during anesthesia (Hanks et al., 1961) and sleep (Henke et al., 1988; Datta et al., 1991), decreasing the arterial blood partial pressure of  $\text{CO}_2$  ( $\text{Pa}_{\text{CO}_2}$ ) by a few mmHg suffices to induce apneas. In contrast, human ventilation resists hypocapnia during wakefulness (so-called wakefulness drive to breathe) (Fink, 1961). This is true after active hyperventilation (Corfield et al., 1995) and after passive hyperventilation administered via a mechanical ventilator (Datta et al., 1991; Corfield et al., 1995). This observation is all the more surprising in that passive hyperventilation not only lowers  $\text{Pa}_{\text{CO}_2}$  but also exerts a nonchemical inhibitory influence on breathing control (Fauroux et al., 1998; Hopkinson et al., 2012). The mechanisms accounting for the resistance of human spontaneous ventilation to hypocapnia have not been clearly identified. Behavioral influences have been called upon (Datta et al., 1991; Corfield et al., 1995; Shea, 1996), and several hypotheses have been proposed (for review, see Shea, 1996). However, no recent data are available on this topic, and none of the proposed hypotheses has been tested experimentally. Yet this is a relevant issue insofar as maintaining ventilatory activity at low carbon dioxide levels is among the prerequisites to speech production insofar as speech often induces hypocapnia (Bunn and Mead, 1971).

In addition to the medullary automatic respiratory circuitry, cortical networks can also drive breathing activities. Voluntary breathing commands can arise from higher brain structures, as respiratory muscles are represented in the primary motor cortex and premotor structures (Smith, 1938; Gandevia and Rothwell, 1987; Macefield and Gandevia, 1991; Similowski et al., 1996; Hudson et al., 2016). Neuronal activities of the premotor cortex and supplementary motor area (SMA) are also involved when humans are exposed to inspiratory constraints, either experimental (Raux et al., 2007; Raux et al., 2013) or disease-related (Launois et al., 2015; Georges et al., 2016) and during speech breathing (Tremoureux et al., 2014b). In patients with deficient respiratory automatism due to a PHOX 2B mutation (congenital central hypoventilation syndrome or Ondine's curse) who must remain awake to breathe, a respiratory-related cortical activity is present during spontaneous breathing, with electroencephalographic signs of SMA activation (slow preinspiratory potentials resembling movement preparation potentials) (Tremoureux et al., 2014a). This finding has been interpreted in terms of cortico-subcortical cooperation to produce the wakefulness-related neural drive to breathe (Tremoureux et al., 2014a). It has also been demonstrated that the cerebral cortex, and more precisely the SMA, exerts a tonic respiratory facilitatory activity on breathing (Laviolette et al., 2013) because inhibitory conditioning of the SMA by repetitive transcranial magnetic stimulation depresses

the excitability of the corticospinal pathway to the diaphragm (Laviolette et al., 2013).

The present study was designed to review hypocapnic breathing in the light of recent progress in the understanding of cortical breathing control. We hypothesized that the maintenance of ventilation following deep hypocapnia induced by hyperventilation would be associated with activation of cortical respiratory networks. To test this hypothesis, we recorded EEGs in normal individuals during quiet normocapnic breathing, during sustained passively and actively induced hypocapnia, and after return to baseline  $\text{Pa}_{\text{CO}_2}$ . EEGs were processed with respect to ventilatory flow, as previously described (Raux et al., 2007), to identify pre-inspiratory potentials. For corroboratory purposes, EEGs were also processed by using a less specific but more robust time-frequency approach.

## Materials and Methods

### Main set of experiments (hypocapnia induced by passive hyperventilation)

**Subjects and ethical approval.** Ten healthy subjects (mean age 22 years; range 21–24 years; 5 men, 5 women; mean body mass index  $21.8 \text{ kg/m}^2$ , range  $20.8$ – $24.1 \text{ kg/m}^2$ ) participated in this study. None of them had previously participated in a respiratory physiology or neurophysiology protocol, and none of them performed any forms of activity involving voluntary control of breathing (e.g., yoga, swimming, acting, singing, wind instruments). The subjects had no history of respiratory disease, they were all nonsmokers and did not take any medications. Spirometry, performed in all subjects according to current guidelines, confirmed the absence of any obstructive or restrictive ventilatory disorders. All subjects had a Nijmegen score of  $<23$  of 64, making the presence of a chronic hyperventilation syndrome unlikely (van Dixhoorn and Duivenvoorden, 1985; van Dixhoorn and Folgering, 2015). On the day of the experiment, the subjects were not sleep deprived and had not consumed alcohol or any other psychoactive substances for at least 24 h.

The study had been previously approved by the appropriate regulatory and ethical authority (Comité de Protection des Personnes Ile-de-France 6 La Pitié-Salpêtrière, Paris). All subjects provided their written consent to participate after having been informed about the general study procedures, the methods used, and the absence of risk for their health or any residual effects. The precise objective of the study was only revealed after the study to preserve the naïveté necessary for the interpretation of the results.

**Ventilatory signals.** Ventilatory flow was recorded by means of a mouthpiece with occlusion of the nose by a pneumotachograph (New Single use Adult/Pediatric Flow Sensor PN 281637, Hamilton Medical) connected to a differential pressure transducer (Spirometer module, AD Instruments; RRID:SCR\_001620). Expired gas was collected at the mouth and transferred to an infrared gas analyzer (Servomex, La Plaine Saint-Denis) to determine the expired fraction of carbon dioxide and to calculate the end-tidal carbon dioxide pressure ( $\text{Pet}_{\text{CO}_2}$ ). Ventilatory flow and  $\text{Pet}_{\text{CO}_2}$  signals were digitized at a sampling frequency of 200 Hz and were recorded for subsequent analysis (PowerLab and LabChart 7.2 for PC, AD Instruments; RRID:SCR\_001620).

**Electroencephalographic signals.** The electroencephalographic signal was recorded by means of 12 equidistant scalp electrodes (ActiCap, BrainProducts), according to the conventional 10–20 topographic system (Rektor, 2002) at locations Fp1, Fp2, Fz, F3, F4, FCZ, Cz, C3, C4, Pz, P3, P4, on the earlobes at A1 and A2 with the ear at AFZ (ActiCAP, BrainProducts). The EEG signal was amplified, digitized at a sampling frequency of 2000 Hz, and recorded for subsequent analysis (V-Amp and Recorder software, BrainProducts).

**Experimental conditions and protocol.** During the experimental sessions, subjects were comfortably seated in an armchair with the head and neck maintained by a cushion, in a warm room in semidarkness. Their attention was captured by watching a film. Subjects were instructed to remain calm and relaxed without falling asleep and to avoid moving

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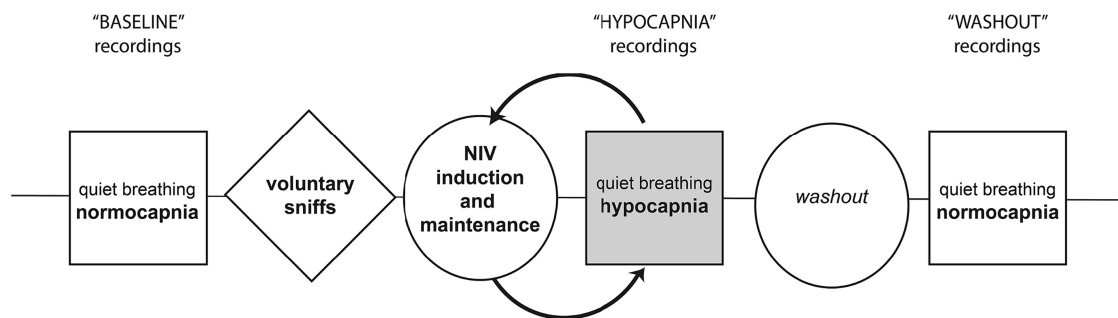
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\*M.D. and C.C. contributed equally to this study as first authors.

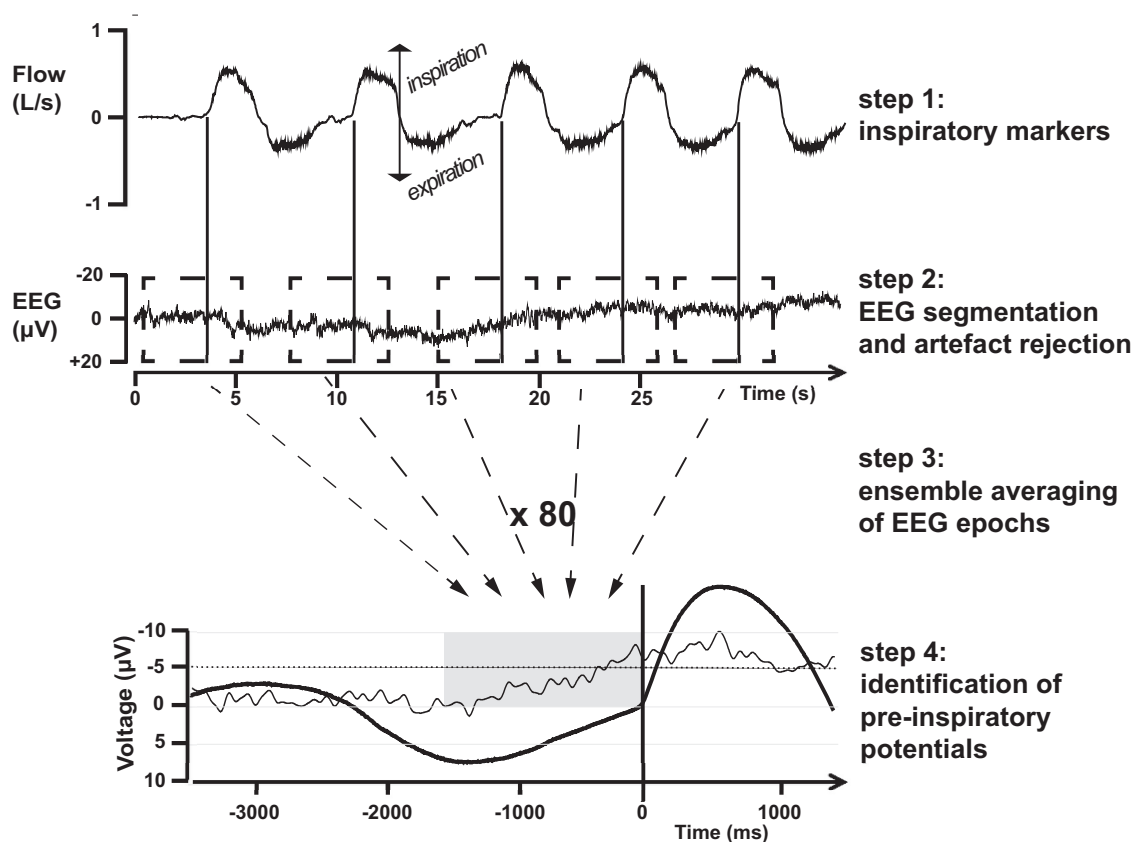
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**Figure 1.** Flow chart of the experimental protocol. All recordings were performed during calm normocapnic breathing (baseline recordings), during rapid voluntary inspirations (sniffs), then after induction of hypocapnia by passive hyperventilation ( $P_{\text{etCO}_2}$  20–30 mmHg) (hypocapnia recordings), and finally after return to  $P_{\text{etCO}_2} > 35$  mmHg (washout recordings).



**Figure 2.** Identification of preinspiratory potentials. Simultaneous ventilation recordings (first plot at the top; inspiration toward the top, expiration toward the bottom) and electroencephalography (second plot) were used to identify preinspiratory potentials based on the following: (1) detection of the start of inspiration; (2) segmentation of the EEG based on this marker, followed by rejection of artifactual segments (see Materials and Methods); (3) averaging of 80 EEG segments devoid of artifacts; and (4) visual identification of PIPs (see Materials and Methods) in the form of a negative wave (directed upwards) preceding inspiration.

the head, eyes, or limbs. No instructions were provided concerning breathing.

Recordings were performed under 4 successive experimental conditions (Fig. 1) as follows. Baseline spontaneous breathing recordings were initially performed under normocapnic conditions ( $P_{\text{etCO}_2} > 35$  mmHg). This session was designed to provide a negative control, as no breathing-related cortical activity is expected in this setting. Subjects then had to perform a series of rapid voluntary inspiratory maneuvers (sniffs). This session was designed to provide a positive control, as breathing-related cortical activity is expected in this setting (see below). Hypocapnia with  $P_{\text{etCO}_2}$  between 20 and 30 mmHg was then induced passively by means of noninvasive ventilation delivered by a Servo 900C ventilator (Siemens). This ventilator was adjusted to deliver pressure-controlled cycles, with a fixed respiratory rate of 16/min and an inspiration/expiration ratio of 0.33. Airway

pressure was gradually increased to achieve a  $P_{\text{etCO}_2}$  of 20 mmHg (median required value: 18 cmH<sub>2</sub>O [interquartile range: 14–21] c). The ventilator was then disconnected from the circuit, and recordings were performed during hypocapnic spontaneous breathing (hypocapnia condition), defined as a  $P_{\text{etCO}_2}$  between 20 and 30 mmHg. If  $P_{\text{etCO}_2}$  rose to  $>30$  mmHg during the recording following passive hyperventilation, controlled ventilation was resumed to lower this value to 20 mmHg. This cycle was repeated as many times as necessary to allow the recording of 120 ventilatory cycles under hypocapnia (in practice, 3–8 times depending on the subject, with a median duration of ventilation of 17 min 50 s [13 min 59 s to 22 min 52 s]). The median  $P_{\text{etCO}_2}$  during hypocapnia with this methodology was 26.9 mmHg (26.2–28 mmHg). The fourth and last condition consisted of recording during spontaneous breathing after  $P_{\text{etCO}_2}$  returned to a value  $>35$  mmHg (washout).

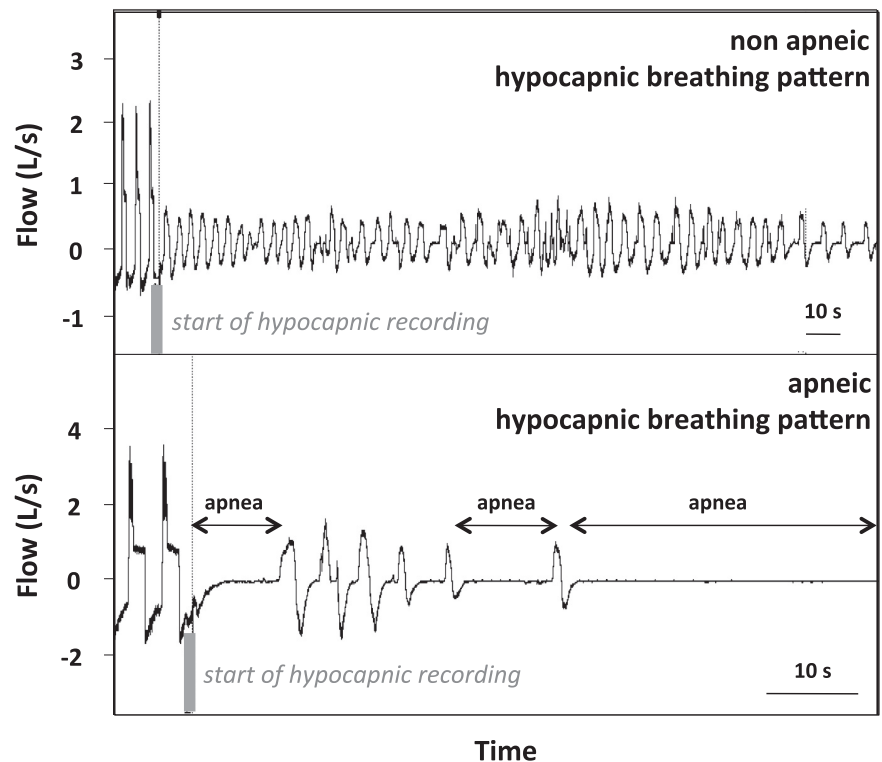
### Data processing

**Ventilation.** The ventilatory flow signal was inspected to count the number of ventilatory cycles performed during spontaneous breathing in hypocapnia and any posthypocapnia apneas. Apnea was defined by prolongation of the expiratory time ( $T_E$ ) by  $>3$  SDs compared with the  $T_E$  measured under baseline conditions (Chenuel et al., 2006). The ventilatory behavior related to hypocapnia was characterized graphically and expressed by the ratio of the number of apneas over the number of ventilatory cycles. The number of apneas and ventilatory cycles taken into account in the calculation of this ratio corresponded to the median overall periods of induced hypocapnia. The minute ventilation ( $\dot{V}_E$ ), respiratory rate ( $f_R$ ), and tidal volume ( $V_T$ ) were calculated from the ventilatory flow signal (MATLAB 7, The MathWorks; RRID:SCR\_001622).

**Preinspiratory potentials.** The reference EEG signal was defined as the linked ears reference (averaged between A1 and A2). The EEG signal was bandpass filtered between 0.05 and 10 Hz. The start of inspiration was visually marked on the ventilatory flow signal to segment the EEG signal into synchronous segments of ventilation. Each segment began 3500 ms before the start of inspiration and ended 1500 ms after the start of inspiration. Under each condition, 120 segments were therefore generated and selected. Segments containing artifacts were rejected, where artifacts were defined as a variation of the signal by  $>5 \mu V$  per ms or  $100 \mu V$  over 200 ms or signals exceeding the limits of  $100 \mu V$ . Artifacts were detected automatically on Cz, FCz, Fz, C3, and C4 electrodes and then controlled visually. Eighty segments were then averaged point by point under each condition. The EEG reference line was then individually redefined (Analyzer 2.0, Brain-Products) (Fig. 2). Following this EEG processing, the primary endpoint was the presence or absence of a preinspiratory potential (PIP). The presence of a PIP was investigated visually by a first examiner, then confirmed or invalidated by a second examiner blinded to the test conditions. In the case of discordance between the two examiners, a third examiner was asked to decide. Visual identification of PIPs was based on morphological criteria, requiring the presence of a slow negative wave during the 2500 ms preceding inspiration, and topographical criteria (potential present in Cz and/or adjacent to FCz, Fz, C3, or C4). The latency of the PIP, defined as the time between the start of the negative deflection and the start of inspiration, and the amplitude of the PIP, defined as the maximum value of the EEG signal on the PIP before the start of inspiration, were then determined.

### Statistical analysis (ventilatory data and preinspiratory potentials)

All statistical analyses were performed with SPSS version 19 for Macintosh software (IBM; RRID:SCR\_002865). The concordance between two examiners for identification of a PIP was evaluated by Cohen's  $\kappa$  test. As the normality of distribution was not verified for all quantitative variables, these values were therefore expressed by their median and interquartile range and nonparametric statistical tests were used. The presence or absence of a PIP during hypocapnia between subjects with a PIP during hypocapnia and those without a PIP during hypocapnia was compared by Fisher's exact test. Comparisons between experimental conditions were performed exclusively in the group of subjects presenting a PIP during hypocapnia by Wilcoxon's and Friedman's tests. The equality of distribution of PIP for paired series was tested between all ventilation conditions and then by pairs using Cochran's Q test. Differences were considered to be significant for a  $p$  value  $<0.05$ .



**Figure 3.** Examples of breathing patterns in hypocapnia in 2 subjects. Visual inspection of the top recording demonstrates the presence of breaths with prolonged expiration; these breaths were defined as apneas when the expiratory time was in excess of 3 SDs of the expiratory time measured during the baseline condition (normocapnic). This pattern is not present on the bottom recording.

### Time-frequency analysis

In addition to the PPI analysis described above, respiratory-related cortical activity was studied using time-frequency analysis for corroboratory purposes, as follows.

**Time-frequency maps.** For each  $s(t)$  signal, EEG recording were analyzed in the time-frequency domain by convolution with complex Gaussian Morlet's wavelets  $w(t, f)$  (Tallon-Baudry et al., 1997), thus providing a time-frequency power map  $P(t, f) = |w(t, f) \times s(t)|^2$ , where  $w(t, f)$  is a complex Morlet's wavelet as follows:

$$w(t, f) = A \cdot \exp(-t^2/2\sigma_t^2) \cdot \exp(2i\pi ft)$$

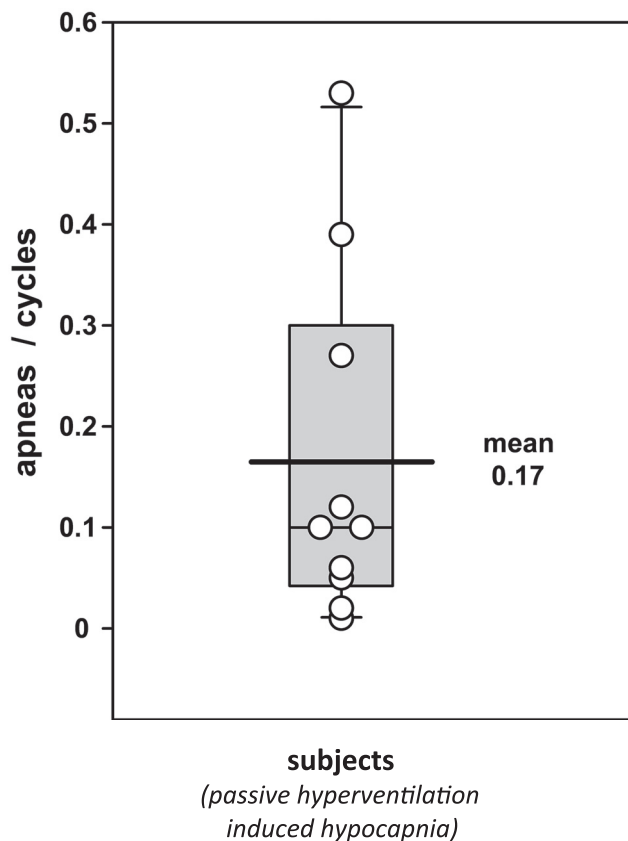
with  $A = (\sigma_t \sqrt{\pi})^{-1/2}$  and  $\sigma_f = 1/(2\pi\sigma_t)$  a function of the frequency  $f$ :  $\sigma_f = f/7$ .

Normalized time-frequency maps were computed for each EEG recording for visualization purposes. This normalization was performed separately for each frequency and consisted of (1) subtracting the mean power during a prestimulus baseline (−1900 ms: −1600 ms) and (2) dividing by the SD of the power during this same baseline. Time-frequency analysis was performed by computation of  $P(t, f)$  on the preinspiratory potential for time-locked activities linked to the respiration signal.

### Statistical analysis of time-frequency maps

Significance of the differences of all contrasts was established using a nonparametric cluster randomization test across spatial and spectral domains (Maris and Oostenveld, 2007). This test effectively controls the false discovery rate in situations involving multiple comparisons by clustering neighboring quantities that exhibit the same effect. For the power map  $P(t, f)$  analysis, the neighborhood was univariate across time (adjacent time-sample over the whole time-window) and frequencies (side-by-side frequency bins). The permutation method exhibits values for which  $t$  statistics exceeded a given critical value when comparing two conditions value by value. To correct for multiple comparisons, neighbor





**Figure 4.** Proportion of apneic cycles during the hypocapnic breathing phase of the protocol. The top 3 subjects exhibited a hypocapnia-related apneic pattern and did not present preinspiratory potentials during the hypocapnia period. The bottom 7 subjects did not exhibit hypocapnia-related apneas; 6 of them presented preinspiratory potentials during the hypocapnia period.

values exceeding the critical value were considered to be members of the same cluster. The cluster-statistic (CS) was taken as the sum of  $t$  values in a given cluster. The false discovery rate was controlled by evaluating the CS distribution on 1000 permutations (Pantazis et al., 2005). Each permutation represented a randomization of the data between the two conditions and across multiple subjects. For each permutation, the CSs were computed by taking the cluster with the maximum sum of  $t$  statistics. The threshold that controls the family-wise error rate was determined according to the proportion of the randomization null distribution exceeding the observed maximum CS (Monte Carlo test). We used a threshold critical value of  $3\sigma$ . Statistics were then corrected for both time and spectral dimensions, and the significance of difference of contrasts was determined for rejection of the null hypothesis and control of the false alarm rate at  $p = 0.01$ .

#### Complementary set of experiments (hypocapnia induced by active hyperventilation)

A second series of experiments was conducted on a separate set of 10 subjects (4 men, 6 women; median age 37 years, range 24–51 years) during a separate experimental campaign, for corroboratory purposes. The experimental setup was simplified, but globally similar to that used during the main set of experiments, with ventilatory measurements being performed either with small and unobtrusive nasal probes or with a pair of magnetometers (Mead et al., 1967) placed at the level of the umbilicus. The experimental protocol consisted of normocapnic ventilation and hypocapnic ventilation only; hypocapnia was induced by voluntary hyperventilation. Because of the methodological differences between the two sets of experiments, the EEG recordings were not pooled for ensemble averaging.

**Table 1.** Breathing pattern in the 7 subjects not exhibiting hypocapnia-induced apneas<sup>a</sup>

	Baseline	Hypocapnia	Washout	$p$
$V_T$ (ml)	397 (344–420)	386 (180–455)	328 (278–363)	0.0162*
$f_R$ (/min)	17 (15–18)	17 (16–21)	17 (13–20)	NS
$V_E$ (L/min)	6.14 (4.73–7.02)	5.33 (2.57–7.27)	4.73 (3.33–5.44)	NS

<sup>a</sup>Data are median (interquartile range).  $V_T$ , Tidal volume;  $f_R$ , respiratory rate;  $V_E$ , minute ventilation.

\*Significant difference between baseline and washout only, not between baseline and hypocapnia.

## Results

### Main set of experiments (hypocapnia induced by passive hyperventilation)

#### Breathing pattern in hypocapnia

Visual inspection of the ventilatory flow recordings indicated the presence of an apneic breathing pattern in 3 subjects and the absence of this pattern in 7 subjects (Fig. 3). According to the above definition (see Materials and Methods), the median apneic cycles/total cycles ratio in the 10 participating subjects was 0.10 (0.04–0.33), with a mean value of 0.17 that clearly distinguished the two groups of individuals (Fig. 4). The breathing pattern characteristics of the 7 subjects who did not exhibit hypocapnia-related apneas are described in Table 1. No significant differences were observed between the baseline breathing pattern and the hypocapnic breathing pattern in terms of tidal volume, breathing frequency, or ventilation.

#### Preinspiratory potentials

As expected, all subjects exhibited clear and typical preinspiratory potentials during the sniff condition. The interobserver  $\kappa$  coefficient for all aggregated conditions was 0.85.

In the 3 subjects who exhibited a hypocapnia-induced apneic breathing pattern, no preinspiratory potential was visible during baseline, hypocapnia, or washout. In the 7 subjects who did not exhibit a hypocapnia-induced apneic breathing pattern, a preinspiratory potential was visible during baseline in one case and during washout in one other case. A preinspiratory potential was present in 6 cases during hypocapnia ( $p = 0.033$  in comparison with the subjects exhibiting an apneic breathing pattern). The subject in whom no preinspiratory potential was present during hypocapnia did not exhibit any preinspiratory potential during baseline or washout (Figs. 5, 6).

In the 7 subjects who did not exhibit a hypocapnia-induced apneic breathing pattern, preinspiratory potentials were significantly more frequent during hypocapnia than during baseline or washout ( $p = 0.037$ ). Excluding the two subjects with preinspiratory potentials during baseline and washout did not modify this result.

The latencies and amplitudes of the preinspiratory potentials observed during hypocapnia were 1031 ms (767–1666) and 6.22  $\mu$ V (4.76–7.17), not significantly different from those observed during sniffs in the same subjects (latency 1572 ms [1070–1781],  $p = 0.184$ ; amplitude 9.28  $\mu$ V [8.13–15.2],  $p = 0.120$ ).

#### Time-frequency maps

In all subjects, voluntary inspirations induced an increase in  $\delta$  amplitudes in the frontal, frontocentral, and central regions (Fz, FCz, Cz, 0.6–1 Hz;  $\sim -1500$  ms to  $-510$  ms, average CS =  $-1030$ ,  $p < 0.01$ ) and also an increase in  $\beta$  rhythm in the central region (Fz, 20–23 Hz;  $-385$  ms to  $-350$  ms, CS =  $-443$ ,  $p < 0.01$ ) in comparison with quiet breathing (sniffs vs baseline).

In the 6 subjects who did not exhibit a hypocapnia-induced apneic breathing pattern and who exhibited a preinspiratory potential during hypocapnia, contrasting the time-frequency

maps showed a marked increase in the  $\delta$  band amplitude on the FCz electrode during hypocapnia compared with baseline (0.6–1 Hz; –1350 to 420 ms, CS = –1071,  $p < 0.01$ ) (Fig. 7).

### Complementary set of experiments (hypocapnia induced by active hyperventilation)

Nine of the 10 datasets from the subjects participating in this set of experiments were analyzed (one EEG recording was discarded because of continuous noise). None of these subjects exhibited preinspiratory potentials during normocapnic breathing. During hypocapnic breathing, 6 subjects maintained nonapneic ventilation and exhibited preinspiratory potentials, whereas the other 3 subjects exhibited apneas with no preinspiratory potentials.

Pooling the two sets of experiments confirmed a highly significant difference in the frequency of preinspiratory potentials between the 13 subjects without hypocapnia-induced apneas and the 6 subjects with hypocapnia-induced apneas ( $p < 10^{-3}$ ). Pooled analysis also confirmed that, in the 13 subjects without hypocapnia-induced apneas, preinspiratory potentials were significantly more frequent during hypocapnia than during normocapnia ( $p < 10^{-3}$ ).

## Discussion

By showing that subjects who maintain a ventilatory activity despite hypocapnia exhibit a respiratory-related cortical activity, while such activity is absent in subjects who exhibit hypocapnia-related apneas, this study provides a neurophysiological substrate for the peculiarity that constitutes the resistance of ventilation to hypocapnia in awake humans.

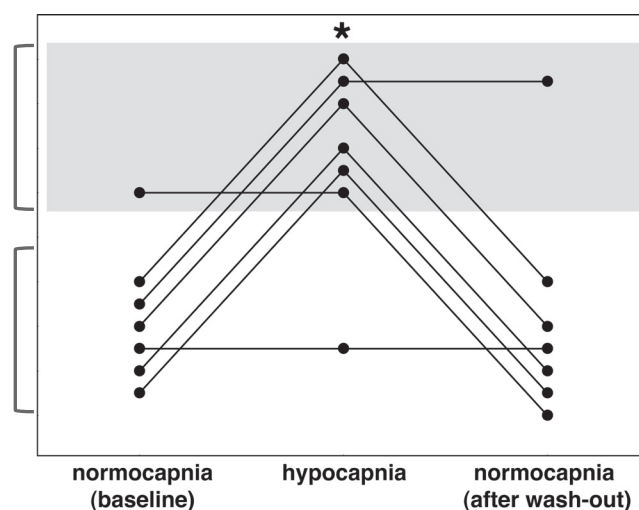
### Methodological considerations

Studies of hypocapnic breathing have placed considerable emphasis on the hypocapnia-inducing procedure. After active hyperventilation, hypocapnic breathing can be explained by posthyperventilation facilitation mechanisms involving the medulla, as they are present in decorticated animals (Eldridge, 1976). These mechanisms cannot explain our observations when hypocapnia was induced by passive hyperventilation, except if our subjects failed to actually relax under mechanical ventilation. In this case, it could be argued that the respiratory-related cortical activity observed after passive hyperventilation was a carryover of an activity elicited by the hypocapnia-inducing procedure itself. During preliminary experiments, we did observe preinspiratory potentials during mechanical ventilation (data not shown) that could have been related to either hypocapnia or incomplete relaxation. We therefore conducted control experiments during which passive hyperventilation was associated with  $\text{CO}_2$  administration to maintain isocapnia (data not shown): no preinspiratory potentials were observed. It has been postulated that the stimulus provided by the mouthpiece and nose clip used to measure ventilation could account for the persistence of ventilation after passive hypoventilation (Corfield et al., 1995). In our complementary set of experiments, ventilatory activity was assessed without a mouthpiece (see Materials and Methods), and the observations were identical. We found a strict parallelism between posthyperventilation preinspiratory potentials and apneas after passive and voluntary

pre-inspiratory potential present\*

pre-inspiratory potential absent\*

\* each dot represents one subject

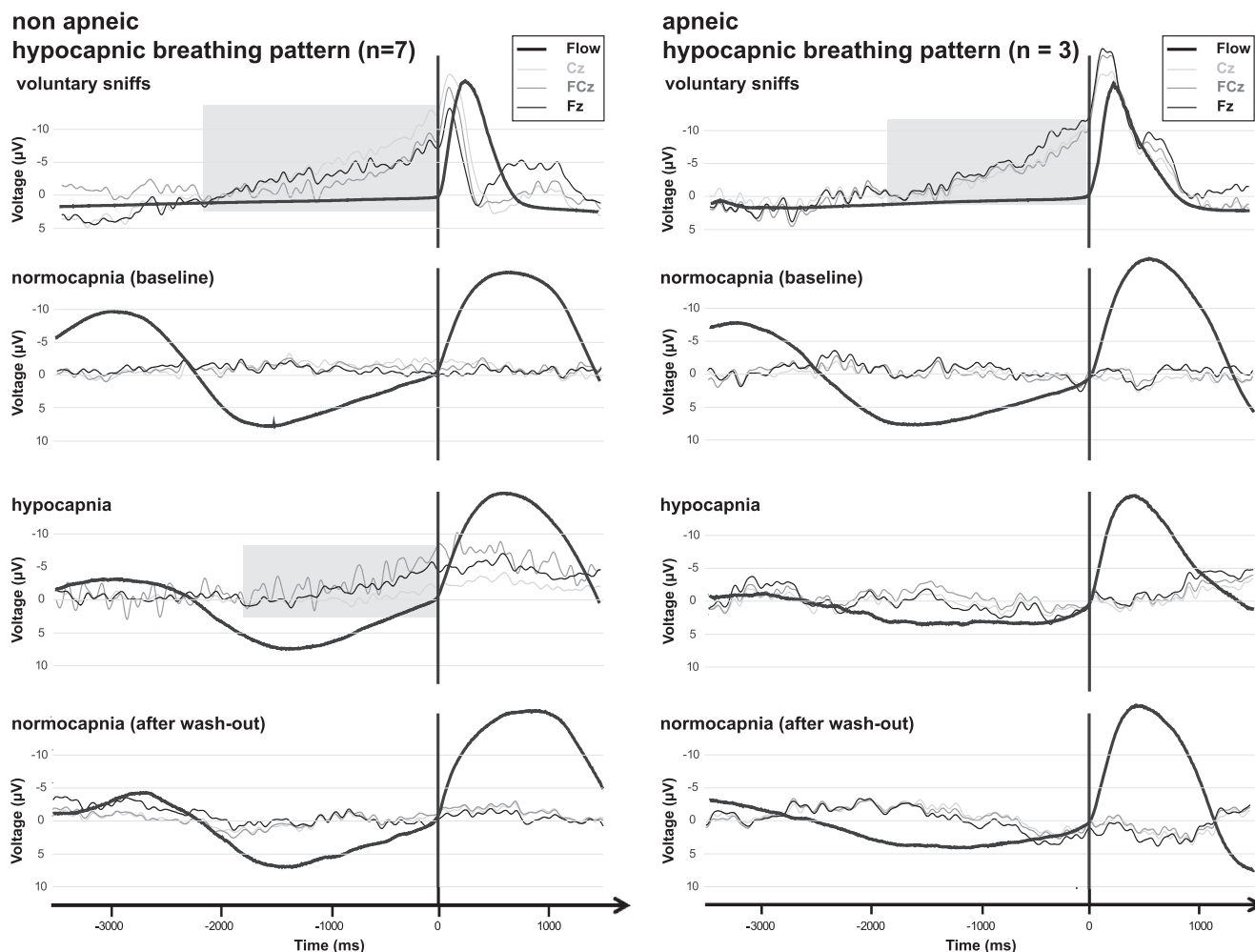


**Figure 5.** Preinspiratory potentials in the 7 subjects not exhibiting hypocapnia-induced apneas. Each dot indicates 1 subject. When the dot is in the white area, there is no preinspiratory potential; when the dot is in the gray area, a preinspiratory potential is present. Preinspiratory potentials are significantly more frequent during hypocapnia. \* $p = 0.037$ .

hyperventilation. Hypocapnic preinspiratory potentials consistently disappeared after return of  $\text{Pa}_{\text{CO}_2}$  to baseline, a strong argument against the potential biases described above that is also suggestive of causality. Of note, the first description of respiratory premotor potentials (Macefield and Gandevia, 1991) mentioned the absence of cortical activity in one subject breathing in hypocapnia after voluntary hyperventilation. This subject was recorded 3 min at a  $\text{Pa}_{\text{CO}_2}$  of 28–30 mmHg, versus 10 min at lower  $\text{Pa}_{\text{CO}_2}$  in our case, which might account for the difference. Electroencephalographic recordings are less precise measures of cortical activity than intracranial recordings or functional neuroimaging studies. However, cortical signals detected by scalp EEG by nature reflect intense activities; and by using event-related approaches with thorough preprocessing to eliminate artifacts, we optimized the signal-to-noise ratio and ensured that the activity detected was respiratory specific. In addition, we presented results averaged over the study population, which is a very conservative approach. Both the signal processing approaches (premotor potentials and time-frequency maps) that we used have been extensively validated before. The time-frequency analysis is particularly apt at identifying an event with a given frequency among artifacts of different frequencies, and does so with a high degree of precision allowed by the wavelet approach. In our subjects, the time-frequency analysis (Fig. 7) fully corroborated the preinspiratory potentials analysis by confirming that cortical activity changed during the preinspiratory period during hypocapnic breathing compared with normocapnic breathing, mostly in the  $\delta$  frequency band. These findings are similar to those obtained during experimentally loaded breathing in normal subjects (Hudson et al., 2016). All in all, we believe that our results are neurophysiologically robust and do demonstrate a cortical contribution to the neural respiratory drive to breathe in hypocapnia.

### Physiological significance

The contrast between sleep and wakefulness regarding hypocapnic breathing strongly contributed to the concept of human wakefulness drive to breathe. Identifying a cortical substrate to the hypocapnic breathing drive is therefore not unexpected. The cortical activity that we observed is compatible with SMA involvement, resembling that observed in reaction to inspiratory loading (Raux et al., 2007, 2013; Hudson et al., 2016). Of note, the paradoxical hyperventilation in response to inspiratory loading



**Figure 6.** Ensemble averaging of the signals obtained in the 4 different experimental conditions, in the 7 subjects not exhibiting hypocapnia-induced apneas (left) and the 3 subjects exhibiting hypocapnia-induced apneas (right). Vertical bar represents the beginning of inspiration as identified on the flow recording. Gray areas represent preinspiratory potentials when present. In each condition, the Cz (light gray), FCz (medium gray), and Fz (dark gray) electrodes are represented on the EEG recording.

(Yanos et al., 1990) also depends on wakefulness (Pillar et al., 2000). In both situations (hypocapnic breathing and inspiratory loading), the loss of an SMA influence on breathing during sleep could be due to a sleep-related breakdown in cortical connectivity (Massimini et al., 2005). Sleep depresses the excitability of the corticospinal pathway to the diaphragm (Mehiri et al., 2006), as does inhibitory conditioning of the SMA by repetitive transcranial magnetic stimulation (Laviolette et al., 2013).

Several mechanisms can be proposed to explain the respiratory-related cortical activation in hypocapnia. One would be the activation of a cortical oscillator when medullary oscillators are extinguished by hypocapnia. Haouzi and Bell (2009) and Haouzi et al. (2007) provided arguments in favor of this possibility by suggesting the existence of cortically set programs able to generate regulatory combinations of respiratory cycle duration and magnitudes to maintain  $P_{\text{etCO}_2}$  constant or almost constant during cortically driven breathing. Raux et al. (2013) suggested that cortically driven breathing can be automatized during inspiratory loading. Alternatively, activation of cortical structures could maintain hypocapnic breathing via corticosubcortical cooperation. Descending respiratory outputs (from medullary structures, limbic structures, or cortical structures) and afferent respiratory and nonrespiratory inputs converge onto respiratory spinal motoneurons and all contribute to determine their excitability, namely, their responsiveness to any type

of stimulus. As a result, the various components of the neural respiratory drive interact with one another and generally facilitate each other (e.g., enhanced diaphragm response to a corticospinal volley in conjunction with a  $\text{CO}_2$  enhanced bulbospinal outflow) (Straus et al., 2004). In the case of hypocapnic breathing, the cortical activation that we describe could sensitize respiratory spinal motoneurons to the hypocapnia-depressed medullary drive to breathe. This is the same hypothesis that has been elaborated to explain the wakefulness-related maintenance of ventilation in patients with congenital central hypoventilation syndrome who do exhibit a respiratory-related cortical activity (Tremoureaux et al., 2014a). Interindividual differences regarding hypocapnia-induced apneas could derive from a variable impact of hypocapnia on medullary respiratory generators. Of note, we observed preinspiratory potentials after both passive and active hyperventilation, which suggests that hypocapnic breathing after active hyperventilation could proceed from several, nonmutually exclusive mechanisms, including medullary after-discharge (Eldridge, 1973, 1974, 1976; Corfield et al., 1995) and cortical activation.

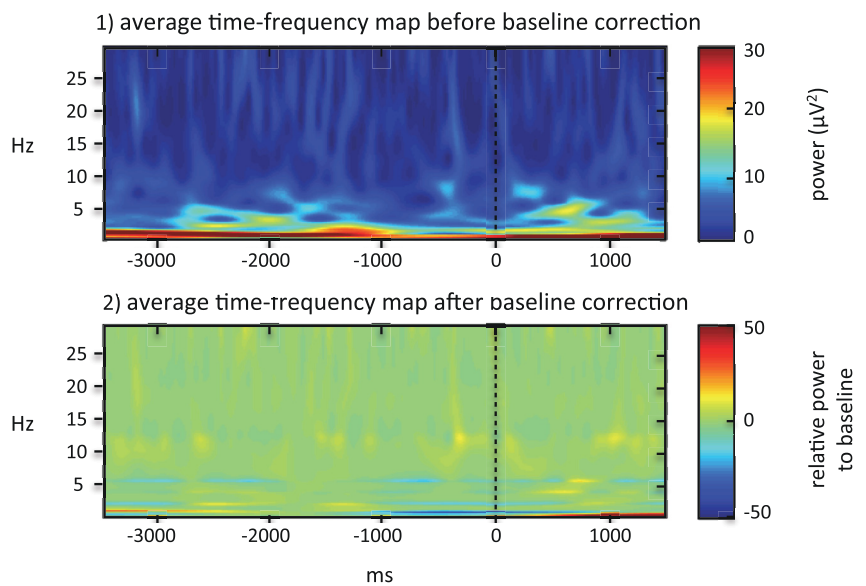
It has yet to be determined how the cortical network contributing to hypocapnic breathing becomes activated. Hypocapnia per se could be involved. The afferent stimulations associated with the hypocapnia-inducing procedure could also be involved. Jack et al. (2010) submitted patients with chronic hyperventilation to transient



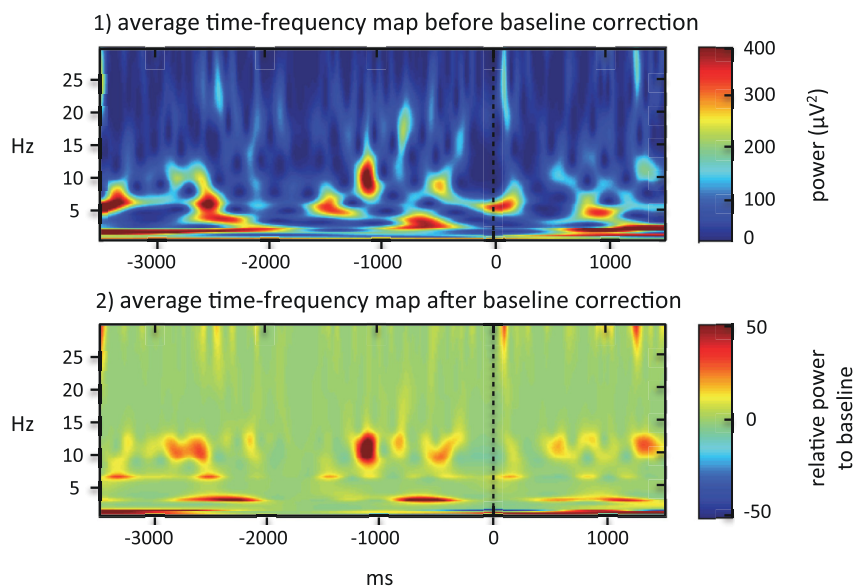
inspiratory occlusions and observed that the resulting cerebral activation was more marked in these patients than in control subjects. Not unexpectedly, this augmented activation was observed in the anterior insula that is known to be involved in the monitoring of respiratory-related afferent signals to the brain. Less expectedly, augmented activation was also present in premotor and motor areas, which is compatible with triggering of an (exaggerated) motor response by the afferent stimulus. Finally, the sudden cessation in respiratory movements at the end of the hypocapnia-inducing procedure could also be the trigger for the corresponding respiratory-related cortical activity, but the time resolution of the EEG approaches used in this study is insufficient to test this hypothesis. Examining hypocapnic breathing recordings available in the literature suggests that, after passive hyperventilation, a single brief apnea can precede the hypocapnic ventilatory activity (Bainton and Mitchell, 1966; Corfield et al., 1995). One indirect argument in favor of the role of respiratory afferents in activation of the cortical network responsible for hypocapnic breathing (as opposed to hypocapnia itself) derives from the observations by Bainton and Mitchell (1966) that repeating passive hyperventilation over several consecutive days results in attenuation of the hypocapnic breathing phenomenon (more frequent apneic responses), namely, an habituation dynamic similar to that observed during inspiratory loading (Gozal et al., 1995).

In conclusion, the respiratory-related EEG activity observed in this study resembles the respiratory-related activity that has been described during experimental inspiratory loading (Raux et al., 2007), during experimental expiratory loading (Morawiec et al., 2015), during speech (Tremoureaux et al., 2014b), in sleep apnea patients with intrinsically increased upper airway resistance (Launois et al., 2015), in amyotrophic lateral sclerosis patients with weak respiratory muscles (Georges et al., 2016), and in patients with defective automatic breathing control (Tremoureaux et al., 2014a). Together, these results suggest the existence of a general mechanism of corticosubcortical cooperation to generate the drive to breathe in awake humans, involving neural structures that source premotor potentials, including the SMA (Lee et al., 1986; Tarkka and Hallett, 1990; Ball et al., 1999). This hypothesis is in line with the known respiratory connections of the SMA, both efferent (Sharshar et al., 2004; Raux et al., 2010, 2013; Laviolette et al., 2013) and afferent (Logie et al., 1998; von Leupoldt et al., 2010). From an evolutionary perspective, the ability to supervene the automatic control of breathing by voluntary or automatized cortical mechanisms is a prerequisite for the full development of speech. Without this ability, ut-

## A normocapnic breathing (baseline) – FCz derivation



## B hypocapnic breathing – FCz derivation



**Figure 7.** Time-frequency maps of respiratory-related cortical activity in the FCz derivation. Ensemble averaging of the event-related time-frequency T-F maps obtained during (**A**) normocapnic breathing and (**B**) hypocapnic breathing. T-F maps highlight synchronized oscillations (across trials) with respect to inspiratory onset (vertical lines at  $t = 0$  s). **A1, B1**, Total average EEG power at different time-frequency pairs. **A2, B2**, Relative increase of power after correcting the baseline (see Materials and Methods). Red colored zones represent an increased cortical activity during hypocapnic breathing at a given time and frequency.

terance modulation and prosody would be very limited. This is well illustrated by the restricted speech capacities of mechanically ventilated patients who cannot control automatic breathing, as breathing rhythm is determined by an external machine. These patients can speak but have to adapt the length of their sentences to the set breathing frequency, and they cannot vary the loudness of their utterances (Hoit et al., 1994). Of note, regardless of the evolutionary driver of the cortical control of breathing, its establishment must predate the development of speech itself. From a clinical perspective, the present results are an additional incentive to study the cortical control of breathing during certain speech



disorders (Tremoureaux et al., 2014b). A dysregulation of the hypocapnia-induced respiratory-related cortical activity described here could be involved in patient suffering from idiopathic chronic hyperventilation (who do not hyperventilate during sleep) (Gardner et al., 1986). Identifying a respiratory-related cortical activity during spontaneous breathing could be of diagnostic value in this setting.

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