Sustained preinspiratory cortical potentials during prolonged inspiratory threshold loading in humans

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Humans can program and control movements, including breathing-related movements. On the electroencephalogram (EEG), this preparation is accompanied by a low-amplitude negativity starting ~2.5 s before inspiration that is best known as a Bereitschaftspotential (BP). The presence of BPs has been described during the compensation of mechanical inspiratory loading, thus identifying a cortical involvement in the corresponding ventilatory behavior. The pathophysiological interpretation of this cortical involvement depends on its transient or enduring nature. This study addressed this issue by looking for BPs during sustained inspiratory loading (1 h). Nine healthy male volunteers were studied during unloaded quiet breathing and inspiratory threshold loading (with unloaded expiration). Analyses of EEG signal and ventilatory variables were used to compare beginning and end of sessions. Inspiratory threshold loading caused ventilatory modifications that persisted, unchanged, for an hour. The presence of a BP at the beginning and end of a session was the most frequent occurrence (6 of 9 cases with a 17-cmH2O threshold load; 8 of 9 cases with a 23-cmH2O load). These observations support the hypothesis that the cerebral cortex is involved in the compensation of sustained experimental inspiratory loading. How this translates to respiratory disease involving acute or chronic inspiratory loading in humans.

Ventilation of the lungs requires that respiratory muscles overcome a mechanical load that is determined by the physical properties of the respiratory system. In healthy humans at rest this load is very low, but it can increase considerably and rapidly during certain respiratory disorders. Without compensation by the neuromuscular respiratory system, this causes ventilatory failure. This does not occur in awake healthy humans, who generally do not develop hypoventilation when faced with experimental acute inspiratory loading. The contrary tends to happen during sleep or anesthesia, both states that are characterized by interrupted functioning of many intracerebral connections (9). As a result, the absence of hypoventilation in awake humans faced with inspiratory loading points to the involvement of cortical structures to compensate. Higher cortical involvement has indeed been evidenced electroencephalographically during experimental inspiratory loading (12), in the form of preinspiratory low-amplitude negativities (a few microvolts) similar to the cortical potentials described during the preparation of voluntary movements. These potentials, best known as Bereitschaftspotentials (BPs), begin ~2.5 s before the movement onset in the rostral part of the presupplementary motor area (pre-SMA) and in the anterior SMA and shortly thereafter in the lateral premotor cortex bilaterally (1, 16). About 400 ms before the onset of the movement, BPs exhibit a steeper negative slope that arises in the contralateral primary motor cortex and lateral premotor cortex (5, 22). Of note, in contrast to mechanical inspiratory loading, ventilatory stimulation by carbon dioxide does not give rise to BPs (11).

In disease, the cortical compensation of sustained increases in mechanical inspiratory loads would help explain—through the corollary requirement for wakefulness—why patients with severe exacerbations of asthma or of chronic obstructive pulmonary disease (COPD) commonly report “not sleeping for days” during such episodes. For this reasoning to hold, the cortical involvement in inspiratory loading compensation must be not transitory but, on the contrary, sustained over a prolonged period. To date, respiratory-related BPs have only been evidenced during short experiments involving <15 min of inspiratory loading (11, 12). Whether or not the cortical activity persists if the load is maintained over prolonged periods of time (as it would be in the case of disease) is unknown. There is not much data in the literature describing ventilatory behavior after more than a few minutes of inspiratory loading in healthy humans, but Yanos et al. (21) have shown that the ventilatory adaptation following the application of moderate inspiratory threshold loads to healthy humans was unchanged after 30 min. From this observation, we hypothesized that the cortical response to inspiratory loads would be durable over time. To test this hypothesis, we performed EEG recordings during the prolonged (1 h) application of inspiratory threshold loads in normal volunteers, while keeping expiration unloaded. We compared the results of inspiratory pretriggered averaging of the EEG signal during the first and the last 15 min of loaded breathing.

Materials and Methods

Subjects

Nine healthy volunteers (9 men, age 21–26 yr, height 178.5 ± 6.2 cm, weight 74.7 ± 6.3 kg) participated in the study. The study was one part of a more general experimental program that had received appropriate legal and ethical clearance (Comité de Protection des Personnes se pretant à des Recherches Biomédicales, Ile-de-France 6, Pitié-Salpêtrière, France). The participants received detailed information about the methods used but not about the actual purpose of the study. They gave their written consent. None of the subjects had a significant history of disease. They were all naive concerning physiological experiments. They were requested to refrain from alcohol...
consumption and psychotropic treatments during the 24 h preceding the experiment and to avoid sleep deprivation.

**Experimental Conditions**

In the laboratory, the subjects were placed in a warm setting with subdued lighting. They were studied while sitting in an easy chair providing support for their legs, arms, neck, and head and were insulated from extraneous sounds. To distract their attention from their respiration, they watched without interruption throughout the experiments a movie of their choice already known to them. Both the experimenters and the recording equipment were hidden from the view of the subjects.

**Respiratory Measurements**

The subject breathed through a face mask (Ultra Mirage, ResMed, Poway, CA) permitting application of inspiratory threshold loading and measurement of the ventilatory variables of interest.

Pressure inside the mask was measured with a differential pressure transducer (DP-45-18, ±100 cmH2O; Validyne, Northridge, CA) connected to the mask with flexible tubing.

Ventilatory flow was measured with a heated pneumotachograph attached to the main port of the mask (3700A series, 0–160 l/min; Hans Rudolf, Kansas City, MO). This low-dead-space pneumotachograph (14.2 ml) had an airflow resistance of 0.02–0.04 cmH2O·l−1·s−1. During application of inspiratory loading, the pneumotachograph was connected to a nonrebreathing device with one-way valves permitting separation of inspiratory flow from expiratory flow (2600 series; Hans Rudolph).

Tidal volume was calculated from integration of flow onset signal during a ventilatory cycle (Chart v5.2, AD Instruments, Castle Hill, Australia).

End-tidal partial CO2 pressure (PETCO2) was measured with an infrared gas analyzer connected to the mask that ensured rapid response (CO2 pump flow 150 cm3/min, IR1505; Servomex, Plaine Saint Denis, France).

During experimental sessions, the subjects self-evaluated the degree of respiratory discomfort induced by the loading (dyspnea) on a 10-cm visual analog scale (VAS) (from 0, “no respiratory discomfort” to 10, “maximum respiratory discomfort”) every 10 min.

At the end of each experimental session, the subjects were asked to fill out a French version (10) of the questionnaire developed by Simon et al. (17), which provides a choice of descriptors for dyspnea and respiratory discomfort.

**Electrophysiological Measurements**

**EEG activity.** EEG activity was recorded with a sterile single-use subcutaneous needle electrode inserted into the scalp at Cz (International 10-20 System) (12 mm, diameter 0.35 mm, ref. MF3OEIS3512; Comega, Bagnolet, France), with two reference electrodes on the mastoids. The impedence of the EEG electrodes was regularly checked during the experiments and remained between 2 and 5 kΩ. The ground was an EEG electrode (48 × 340 mm, ref. 31.1925.21; Tyco Healthcare) placed on the forehead after skin abrasion and cleaning (pumice and alcohol).

These electrodes were connected to an EEG preamplifier (2000-fold gain, band-pass frequency 0.05–500 Hz; Electronique du Mazet, Le Mazet St Voy, France) out of which the signals were fed to an analog-to-digital converter, digitized at a sampling frequency of 2,000 Hz (ML 780 PowerLab 8s, 16-bit resolution, maximum range ±10 V, recording range set on ±1 V; Chart v5.2 software, AD Instruments), and then stored as computer files for subsequent analysis.

**Electromyogram.** Electromyogram (EMG) signals from the right scalene muscle were recorded with two electrodes (48 × 34 mm, ref. 31.1925.21; Tyco Healthcare) placed over the middle body of the muscle and separated by 2 cm.

The electrodes were connected to an EMG-specific preamplifier (Electronique du Mazet) for signal filtering (third-order anti-aliasing Butterworth filter, cutoff frequency 1,000 Hz) and preamplification (300-fold gain). This signal was fed to an analog-to-digital converter, digitized at a sampling frequency of 2,000 Hz (ML 780 PowerLab 8s, 16-bit resolution, maximum range ±10 V, recording range set on ±1 V; Chart v5.2 software, AD Instruments), and then stored as computer files for subsequent analysis.

**Experimental Protocol**

After a 15-min control period during which the subjects breathed freely through the measurement system, two inspiratory loading thresholds—17 or 23 cmH2O—were used (Spring-to-Stretch, Threshold Inspiratory Muscle Trainer no. 730; Health Scan) depending on the experimental session. They were applied for 60 min, after which a washout period of 15 min took place.

**EEG Data Processing**

This was conducted according to the method described by Raux et al. (12) and summarized in Fig. 1. Briefly, onset of inspiration was detected on the flow signal, and the EEG signal was split into 80 segments, each corresponding to a breath and starting 2.5 s before the start of inspiration and lasting 1.5 s after, which were ensemble averaged point by point (Excel, Microsoft). Data analysis was performed on the first 15 and last 15 min of the recordings. To ascertain the presence of a BP within the period of interest, the averaged EEG was visually inspected for a departure from baseline in the negative

![Fig. 1. Summary of the signal processing used to look for inspiratory Bereitschaftspotentials within the raw electroencephalographic signal (adapted from Ref. 11). The sequential steps are as follows. 1) Identification of the onset of “mechanical” inspiration from the flow signal. 2) Definition of electroencephalographic (EEG) epochs starting 2.5 s before inspiration and ending 1.5 s after. 3) Averaging of 80 epochs devoid of obvious artifacts, spurious EEG activity exceeding 20% of the baseline signal, or electrooculographic activity {solid line box at bottom right shows the result of this averaging [Cz EEG, top; rectified scalene muscle electromyogram (EMG), middle; flow, bottom]}. 4) Visual inspection of the signal for a negative departure from baseline within the time window where a Bereitschaftspotential is expected (0.5–2 s before inspiration), in such instance fitting of a first-order least-square regression over the corresponding region of the averaged signal; Bereitschaftspotential is considered present if the slope of this regression is positive and significantly differs from 0 according to the F-test for equality of variances; the latency of the Bereitschaftspotentials is measured from the first departure of the EEG signal from baseline to the onset of inspiration identified on the scalene EMG signal (“neural” inspiration). 5) Application of a first-order least-square regression over the region of the averaged signal where a motor potential is expected; a motor potential is considered present if the slope of this regression significantly differs from the slope of the “preinspiratory regression,” also according to the F-test for equality of variances.](http://jap.physiology.org/doi/fig/10.1152/jappl.00210.2009)
direction within the time window where the potential is expected (0.5–2 s before inspiration). In such instance, the signal was then fitted to a linear regression of order 1. A BP was deemed present if the slope of this regression was significantly greater than 0 (F-test for equality of variances). The latency of the BPs was measured as the time elapsed between the first departure of the electroencephalographic signal from baseline to the onset of inspiration identified on the rectified and averaged scalene electromyographic signal (“neural” inspiration). In some cases in which identifying inspiration from the scalene EMG signal proved difficult, the start of “mechanical” inspiration, determined from the mouth pressure signal, was used. The amplitude of the BPs was defined as the value reached by the EEG signal at this time. Their slope was defined as the order 1 coefficient of the linear regression used to ascertain the potentials, when significant. Motor potentials, defined as increases in negativity synchronous with onset of inspiration, were identified in a similar manner (Fig. 1).

Statistical Analysis
All statistical analyses were performed with Prism 4 (GraphPad Software) or Excel (Microsoft) software. The normality of data distributions was first tested with the Shapiro-Wilk test. Normality not being consistent, data are described by their median values with indication of interquartile range, and statistical comparisons were performed with the Friedman test followed by Dunn’s test to check for differences between conditions. For data organized in contingency tables with the conditions “presence or absence of BP,” Fisher’s exact test was applied to determine whether the occurrence of BPs and motor potentials was more frequent under loading than under control conditions. Differences were considered to be significant when the probability $P$ of a type 1 error was <5% ($P < 0.05$).

RESULTS
Bereitschaftspotentials

Control situations. In eight of the nine subjects, during unloaded breathing, no preinspiratory negativity was observed. In one of the nine subjects, a preinspiratory negativity was observed during the first 15 min of a control session, but not during the last 15 min of this session or during the other sessions (Fig. 2).

Inspiratory threshold loading at 17 cmH$_2$O. In six of the nine subjects, a preinspiratory negativity was observed, both at the beginning and at the end of the experimental session. In one of the nine subjects, the BP identified at the beginning of the session disappeared at the end of the hour when a load of 17 cmH$_2$O was applied. In two of the nine subjects, this load never provoked the appearance of potentials.

Inspiratory threshold loading at 23 cmH$_2$O. In eight of the nine subjects, a preinspiratory negativity was observed with this load, at the beginning and the end of the experimental session. In one of the nine subjects, this load was not associated with a preinspiratory negativity at the beginning or the end of the session. This subject was one of those in whom no potentials were observed with a load of 17 cmH$_2$O.

Overall, the presence of a BP both at the beginning and the end of a session was significantly more frequent with a load of 17 cmH$_2$O and 23 cmH$_2$O than under control conditions ($P = 0.0069$ and $P = 0.0004$, respectively). The presence of a BP at the beginning and the end of a session was statistically more frequent than its presence only at the beginning with a load of 17 cmH$_2$O and with 23 cmH$_2$O ($P = 0.04$ and 0.0004, respectively).

Table 1 summarizes the latencies, amplitudes, and slopes of the potentials. No significant differences were detected.

Motor Potentials

Each and every identified BP was followed by a motor potential, and there was no occurrence of a motor potential not preceded by a BP (Fig. 2). The results of the statistical analysis

Fig. 2. EEG responses (top) to inspiratory threshold loading [mouth pressure (negative pressure downward), middle: rectified and averaged scalene EMG, bottom] as recorded in 1 subject. Top: application of lower load (17 cmH$_2$O). Bottom: application of higher load (23 cmH$_2$O). Depicted from left to right are the control period, the average EEG over the first 15 min of loaded breathing, the average EEG over the last 15 min of loaded breathing, and the washout period. Bereitschaftspotentials are only present during loading and are not visible during the control and the washout periods. They seem more marked during the 23-cmH$_2$O loading.

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are therefore exactly the same for the motor potentials as for the BPs.

**Respiratory Pattern**

The effects of inspiratory threshold loading on the respiratory pattern are described in Figs. 3 and 4. With both loads, the modifications detected at the end of the first 15 min persisted at the end of the hour of loaded breathing.

**Respiratory Sensations**

With inspiratory threshold loading at 17 cmH2O, the VAS dyspnea measure was 3.4 ± 1.7 cm at the end of the first 15 min (against 0 under control conditions). This value was 3.6 ± 1.9 cm at the end of the last 15 min (P = 0.46).

With loading set at 23 cmH2O, the VAS dyspnea measure was 4.6 ± 1.7 cm at the end of the first 15 min (against 0 under control conditions). This value was 5.2 ± 1.7 cm at the end of the last 15 min (P = 0.057).

The most frequent descriptors of the respiratory sensations evoked by threshold loading were “my breathing requires more work” (7 of 9 subjects for the lower load, 8 for the higher load) and “my breathing requires more concentration” (6 of 9 subjects for the lower load, 7 for the higher load).

**DISCUSSION**

This study confirms that inspiratory threshold loading in healthy subjects is the most often associated with cortical activity characterized by the presence of BPs preceding inspiration (12). In addition, it shows that this activity is not transient but, on the contrary, endures.

**Methodological Aspects**

Having to breathe against inspiratory loading, particularly if the load is relatively high, can cause modifications in muscle activation with, for example, rhythmic movements of the shoulder muscles or of orofacial muscles [see the animal study by Song and Pae (18)]. The inspiratory nature of the EEG activity recorded may thus be considered with caution. We specifically asked the participants to try to relax and minimize muscle activity during the experiments. In addition, assigning the averaged EEG signals to a respiratory signal provides some guarantee as to the respiratory nature of the BPs.

One subject exhibited a preinspiratory negativity during the first 15 min of a control session, which is surprising. It is possible that despite the measures used to distract the participants from their breathing, this subject did focus on his respiration because of the installation of the face mask and recording devices. Another subject presented a preinspiratory negativity at the beginning of a threshold loading session but not at its end. In this case, it is possible that loading was not sufficient to elicit a cortical response in this subject, the initial preinspiratory negativity being due to an attention temporarily focused on respiration. In this view, Fig. 2 seems to indicate that BPs and motor potentials are more visible with the higher inspiratory load. There were, however, no statistically significant differences between the 17- and 23-cmH2O loads (Table 1).

**Table 1. Characteristics of Bereitschaftspotentials in subjects in whom they were present at beginning and end of session**

<table>
<thead>
<tr>
<th>Inspiratory Threshold Loading</th>
<th>17 cmH2O</th>
<th>23 cmH2O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beginning of Session (n = 7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>End of Session (n = 6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latency, s</td>
<td>1.2 (1.1–1.8)</td>
<td>1.4 (1.3–1.8)</td>
</tr>
<tr>
<td>Amplitude, µV</td>
<td>5.7 (4.5–8.2)</td>
<td>70.6 (4.9–8.6)</td>
</tr>
<tr>
<td>Slope, µV/s</td>
<td>3.6 (1.7–5.4)</td>
<td>3.3 (2.3–4.5)</td>
</tr>
</tbody>
</table>

Values are medians with interquartile range (in parentheses) for n subjects.
1), possibly because of an insufficient power in view of the known intra- and interindividual variability of the BPs (2).

The length of the experimental sessions with inspiratory loading was fixed at 1 h. This duration was chosen arbitrarily, and we cannot affirm with certainty that the phenomena observed would persist over a very long period. It did not, however, seem likely that after an hour the preinspiratory cortical activity associated with an inspiratory load causing moderate respiratory discomfort would still indicate a transient state.

Load Compensation

The initial finding that led to the demonstration of preinspiratory EEG activity in the presence of inspiratory loading (11, 12) was the observation by Locher et al. (8) of a facilitation of diaphragm response to transcranial stimulation by the application of inspiratory resistive loading, even when this loading did not bring about a significant increase in the automatic ventilatory command. Combined, the two observations—resistance-associated facilitation and load-induced BPs—support the hypothesis of activation of corticospinal output originating in or near the SMA (14) and exerting a facilitating action on phrenic motoneurons. It has indeed been demonstrated that manipulating the excitability of the SMA with repetitive transcranial magnetic stimulation could modify the response of the diaphragm to magnetic stimulation applied over its motor representation (13). According to this concept, the role of a cortical response to inspiratory loading would be to optimize transmission of an unchanged or increased ventilatory command to the effector respiratory muscles. Contrarily, this could explain the development of alveolar hypventilation with inspiratory loading observed in patients with locked-in syndrome (23), a situation characterized by cortical disconnection. Likewise, sedation decreases lung volumes (4) and increases upper airway resistance (3) and thus increases the mechanical impedance of the respiratory system. If or when spontaneous ventilation occurs or resumes in such a context, the neuromuscular respiratory system is faced with an augmented mechanical load. In the absence of cortical compensation, this can theoretically contribute to the sedation-related hypoventilation. Indeed, in patients comatose after the voluntary ingestion of barbiturates and in whom cortical compensation is absent, it has been shown that mechanical factors accounted for at least part of the observed depression of the ventilatory response to carbon dioxide (6). Similarly, in patients comatose after the voluntary ingestion of benzodiazepines and who are not intubated, upper airway obstruction (3) is present and treatment with the antidote flumazenil both corrects it and improves minute ventilation. This does not occur in patients who are intubated and therefore protected against upper airway collapse, in whom ventilation is neither low nor modified by flumazenil (3).

It should be noted that threshold loading allows for very few possibilities of ventilatory pattern adaptation, denoting its "obligatory" nature. Initial ventilatory pattern modifications (which depend on the load: hyperventilation is possible in response to low loads but is not observed with high loads) tend to persist with time (21). Our findings correspond to the general description in the literature. In fact, the ventilatory modification induced by inspiratory threshold loading during the first 15 min, quite variable between individuals, remained unchanged during the last 15 min (Figs. 3 and 4) whatever the load considered. Significant hyperventilation with hypocapnia was noted with the lower load (Fig. 3), as in the study by Yanos et al. (21). This phenomenon was not statistically significant with the higher load (Fig. 4), even though some subjects presented marked hypocapnia. The persistence of a BP
throughout the application of the load, noted in the large majority of subjects, suggests that persistence of the stimulus sustained, to some extent, the cortical response. This observation supports the cortical facilitation hypothesis elaborated in the previous paragraph. However, because it is necessary to average many EEG epochs to extract the respiratory BPs from the raw EEG signal, it is impossible to know whether the respiration-related cortical response is continuous or intermittent. We are inclined to think that it is continuous, because in our experience the number of epochs that must be averaged to observe BPs is roughly similar during loaded breathing (as in this study) and during self-paced sniff maneuvers. This remains speculative, but the answer to this question does not change the implications of our results: be it intermittent or continuous, the cortical response elicited by inspiratory loading does persist over an hour. As is the case for BPs in general, the significance of this cortical response in terms of awareness and voluntariness is complex (see, for example, Ref. 7), keeping in mind that a cortical activity can persist when a voluntary movement becomes automatic (20). However, we wish to emphasize that we do not think that during inspiratory loading our subjects voluntarily controlled their breathing on a breath-by-breath basis. They were ignorant of the final objective of the study and were recorded while watching a movie to distract them from their breathing. In children suffering from congenital central hypoventilation syndrome (CCHS) and devoid of automatic breathing, Shea et al. (15) showed that intense mental concentration did not produce hypoventilation. They concluded that their patients did not rely on a breath-by-breath cortical command to breathe, and that “cortically controlled breathing has become so well programmed in CCHS patients that other cortical tasks do not interfere with it.”

Of note, all the subjects participated in two separate sessions, one with a load of 17 cmH2O and the other with a load of 23 cmH2O. After the first session the patients were thus no longer naive concerning the protocol and the sensations evoked by inspiratory loading. There was no difference, however, between the two sessions concerning the presence and persistence of BPs and motor potentials. The involvement of the cerebral cortex in the compensation of inspiratory threshold loading thus not only is persistent during a given session but also is a reproducible phenomenon from one session to another (absence of arguments for a learning process).

Pathophysiological Perspectives

Certain respiratory disorders can radically modify respiratory mechanics over a short period of time and are associated with increased inspiratory loads of various sorts. This is the case for exacerbations of asthma and COPD. If the participation of higher cortical structures is indeed necessary to maintain ventilation in such circumstances, it follows that patients experiencing an asthma attack or a COPD exacerbation would have to remain awake to prevent hypoventilation. This would turn in turn expose them to the negative vital and cognitive effects of sleep deprivation. It will therefore be interesting to test for the presence of BPs in patients with acute exacerbation of asthma and COPD. If they are indeed present, it will be all the more important to better understand the nature of the cortical respiratory motor control in sustained load compensation. For example, the nature of the actual stimulus for cortical involvement will have to be studied (frequency and duration of the load giving rise to the BPs, relationship between perceptual and motor processes, etc.), and specific paradigms to study automaticity will have to be designed (19, 20).

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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