Expiratory load compensation is associated with electroencephalographic premotor potentials in humans

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Morawiec E, Raux M, Kindler F, Laviolette L, Similowski T. Expiratory load compensation is associated with electroencephalographic premotor potentials in humans. J Appl Physiol 118: 1023–1030, 2015. First published February 6, 2015; doi:10.1152/japplphysiol.00201.2014.—In normal humans during quiet breathing, expiration is mostly driven by elastic recoil of the lungs. Expiration becomes active when ventilation must be increased to meet augmented metabolic demands, or in response to expiratory loading, be it experimental or disease-related. The response to expiratory loading is considered to be mediated by both reflex and cortical mechanisms, but the latter phenomenon have not been neurophysiologically characterized. We recorded the EEG in 20 healthy volunteers (9 men, 11 women; age: 22 to 50 yr) during unloaded breathing, voluntary expirations, and in response to 50 cmH2O expiratory resistive load (ERL), 20 cmH2O expiratory threshold load (high ETL), and 10 cmH2O expiratory threshold load (low ETL). EEGs were processed by ensemble averaging expiratory time-locked segments and examined for pre-expiratory potentials, defined as a slow negative shift from the baseline signal preceding expiration, and suggestive of cortical preparation of expiration involving the supplementary motor area. Four subjects were excluded because of technical EEG problems. Pre-expiratory potentials were present in one subject at baseline and in all subjects during voluntary expirations. They were present in eight subjects during low ETL, in 15 subjects during high ETL, and in 13 subjects during ERL (control vs. low ETL, P = 0.008; control vs. high ETL, P < 0.001; and control vs. ERL, P < 0.001). Respiratory discomfort was more intense in the presence of pre-expiratory potentials (P < 0.001). These results provide a neurophysiological substrate to a cortical component of the physiological response to experimental expiratory loads in humans.

Control of breathing; cerebral cortex; supplementary motor area; expiration; expiratory loading; dyspnea.

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In normal humans during quiet breathing, expiration is mostly driven by elastic recoil of the lungs, although it may involve a certain amount of expiratory muscle activity (1). In this setting, expiratory flow and expiratory duration are not neurally controlled or only loosely so. Expiratory muscles are readily recruited (active control of expiration) when ventilation must be increased to meet augmented metabolic demands. This is the case during exercise (see Ref. 2) or in response to experimental chemostimulation by hypercapnia or hypoxia (12, 42). Active expiration also occurs in response to experimental expiratory loads (5, 15–17). From the animal observations, it is generally considered that the expiratory activity that follows application of experimental expiratory loads involves a reflex component (see review in Ref. 28). Medullary expiratory neurons are inhibited or disfacilitated when lung volume is sufficient to allow passive lung recoil to generate expiratory flow (review in Ref. 20). However, as transpulmonary pressure falls with decreasing lung volume, this inhibition is lifted and is replaced by excitatory inputs (see review in Ref. 20). Symmetrically, Remmers and Bartlett (37) have observed a sudden decrease in abdominal activity in response to abrupt expiratory unloading that was obtained by suddenly diverting expiratory flow from the upper airway. All expiratory muscle groups appear to be involved in the reflex responses to expiratory loading that might involve the activation of muscle spindles (39). Thus, it has been shown that expiratory threshold loading induces reflex recruitment of expiratory intercostal muscles, including triangularis sterni (10, 38). The excitatory effect of low lung volumes on inspiratory neurons, the inhibitory effect of unloading on abdominal expiratory activity, and the activation of intercostal muscles in response to expiratory threshold loading are generally considered that the expiratory activity that follows application of experimental expiratory loads involves a reflex component (see review in Ref. 28). Medullary expiratory neurons are inhibited or disfacilitated when lung volume is sufficient to allow passive lung recoil to generate expiratory flow (review in Ref. 20). However, as transpulmonary pressure falls with decreasing lung volume, this inhibition is lifted and is replaced by excitatory inputs (see review in Ref. 20). Symmetrically, Remmers and Bartlett (37) have observed a sudden decrease in abdominal activity in response to abrupt expiratory unloading that was obtained by suddenly diverting expiratory flow from the upper airway. All expiratory muscle groups appear to be involved in the reflex responses to expiratory loading that might involve the activation of muscle spindles (39). Thus, it has been shown that expiratory threshold loading induces reflex recruitment of expiratory intercostal muscles, including triangularis sterni (10, 38). The excitatory effect of low lung volumes on inspiratory neurons, the inhibitory effect of unloading on abdominal expiratory activity, and the activation of intercostal muscles in response to expiratory threshold loading are all vagally mediated (3, 37, 38).

In conscious healthy humans, experimental expiratory loading also tends to generate active expiratory patterns (5, 15–17). This response exhibits a wide interindividuality variability that contrasts with the relatively stereotyped pattern observed during anesthesia (review in Ref. 28). In addition, the short-term response to expiratory loads can be altered by previous instructions to the subject. These observations suggested that, to a large extent, the response to expiratory loading could be cortically modulated (see reviews in Refs. 28 and 40). Cortical control of expiration has been mostly described during voluntary exhalation paradigms. In this setting, Ramsay et al. (33) described expiration-related increases in regional cerebral blood flow “in the right and left primary motor cortices dorsally just lateral to the vertex, the right and left primary motor cortices more ventrolaterally, the supplementary motor area, the right lateral premotor cortex, the ventrolateral thalamus bilaterally, and the cerebellum6”. Macefield and Gandevia (25) described electroencephalographic premotor potentials preceding voluntary expiratory maneuvers in normal subjects. These premotor potentials are the signature of prepared voluntary movements and suggest activation of the premotor complex, including the supplementary motor area (SMA) (4). During prolonged (but not forced) exhalations, Loucks et al. (24) found activated cortical areas overlapping
with cortical areas involved in certain types of sound production. A considerable amount of overlap is also observed between “expiratory” cortical areas and “inspiratory” areas (33).

Our group has previously shown that respiratory-related cortico-subcortical circuits are activated not only during voluntary ventilatory maneuvers, but also during sustained mechanical inspiratory loading (35, 44). This activation seems to be the result of “cortical automatization” (36). It might correspond to the “cooperation” of cortical and subcortical processes in inspiratory load compensation. Indeed, in normal humans, inspiratory loading induces hypoventilation during sleep (where respiration-related cortical activation should by nature be absent), but hypoventilation lacks when loads are applied during wake (47). An extreme illustration of this concept has been provided by EEG recordings performed in patients suffering from congenital central hypoventilation (43) and in whom the automatic breathing control is not sufficient to ensure adequate ventilation during sleep. These patients differed markedly from control subjects in that, when awake, they exhibited preinspiratory cortical potentials during quiet, unloaded breathing (43). It was hypothesized that this cortical activation contributed to maintain their ventilation at an adequate level during wakefulness, possibly through facilitation of the bulbospinal drive. In the present study, we tested the general hypothesis that similar “cooperation” mechanisms are involved in the compensation of expiratory mechanical loads. More specifically, we tested the hypothesis that sustained expiratory loading triggers a cortical drive to inspiratory muscles that is associated with an electroencephalographic pattern, suggestive of SMA activation.

MATERIALS AND METHODS

Subjects. Twenty healthy subjects (9 men, 11 women, age: 22–50 yr; BMI: 18.0 to 28.4 kg/m²) with no prior experience with respiratory or neurophysiology experiments participated in the study. Two subjects were occasional smokers. All the others were nonsmokers and had normal forced expiratory flow-volume curves. None of them took any psychotropic substances on a long-term basis, either for therapeutic or recreational use (e.g., cannabis derivatives). In addition, they were asked to refrain from consuming caffeine in any form (7), tea, alcohol, benzodiazepines, cannabis, and generally any medication during the 24 h preceding the experiments, and finally, they were advised to avoid sleep deprivation. The study received legal and ethical clearance from the appropriate external authority (Comité de Protection des Personnes Ile-de-France 6 Pitié-Salpêtrière, Paris, France). Prior to the experiment, the subjects were given detailed information about the methods used, but not about the actual purpose of the study, in order not to interfere with the results. This information was given at the end of the experimental session. The subjects gave their written consent, and none withdrew their consent at the end of the experiment.

Experimental conditions. The experiments took place in a semi-dark quiet room. The subjects were installed in a reclining armchair, which provided head, back, and arm support. They were asked to avoid body and eye movements. They were distracted from the experimental context by watching a movie during the entire experiment, on a screen placed in front of them. To minimize emotional interferences, the movie watched was chosen by the subjects themselves among a selection of neutral fictions and documentaries. They had to be already familiar with the chosen title. The experimental setup is depicted in Fig. 1. Of note, and also to minimize interferences, the subjects were given no instruction regarding breathing.

Electrophysiological measurements. Electroencephalographic activity was recorded via surface electrodes (Acticap, Brain Products, Gilching, Germany) placed at Fz, FCz, Cz, Fp1, Fp2, F3, F4, C3, C4, T7, T8, A1, and A2, according to the international 10/20 system. The FCz electrode served as reference for signal recording. Impedance was kept below 2 kΩ at all times. The signal was fed to a dedicated amplifier (BrainAmp, Brain Products), filtered, sampled at 2.5 kHz, and stored on hard disk for offline analysis (Recorder, Brain Products).

The EMG activity of the right external oblique muscle was recorded using surface electrodes. The EMG signal was preamplified with a 2,000-fold gain, 0.05–500 Hz band-pass filtered (Electroenceph. Clin. Neurophysiol.), and then fed to the EEG amplifier (BrainAmp, Brain Products).

Respiratory measurements. The subjects breathed through a mouthpiece connected to a two-way nonrebreathing valve, the expiratory limb of which can be either free (“unloaded expiration”) or connected in series with various expiratory loads. EEG, electroencephalogram; PETCO₂, end-tidal carbon dioxide partial pressure in the expired gas; EMG, electromyogram (external oblique muscle).

Fig. 1. Schematic representation of the experimental setup. A: subject breathes through a mouthpiece connected to a two-way nonrebreathing valve, the expiratory limb of which can be either free (“unloaded expiration”) or connected in series with various expiratory loads. EEG, electroencephalogram; PETCO₂, end-tidal carbon dioxide partial pressure in the expired gas; EMG, electromyogram (external oblique muscle).
Assessment of respiratory discomfort. The degree of “respiratory discomfort” was self-assessed by the subjects on a visual analogue scale (VAS). The subjects were asked to set the cursor on a 10-cm horizontal line, between the descriptions “no respiratory discomfort” on the left and “intolerable respiratory discomfort” on the right.

Protocol. Five conditions were studied in random order, with a 10-min break between each condition: 1) normal, unloaded breathing (control); 2) 50 cmH2O·s−1·m−2 expiratory resistive load (ERL); 3) 20 cmH2O expiratory threshold load (high ETL); 4) 10 cmH2O expiratory threshold load (low ETL); and 5) self-paced voluntary forced expirations (“forced expiration”), as a “positive control” condition (known to normally elicit preexpiratory potentials) (25). A minimum of 100 cycles were recorded for each condition.

EEG data processing. EEG data were processed according to previously described principles (35). Briefly, the EEG signal was re-referenced with an average reference and filtered (0.05–5 Hz). The signal was split into 5.5-s segments, extending from 3.5 s before to 2 s after the onset of expiration defined from the airflow pressure signal. In practice, the pressure signal was used to time-lock the EEG via an electrical pulse automatically generated at the beginning of expiration (the flow signal, used for this purpose in previous studies, could not be used here because during expiratory threshold loading, the onset of flow is delayed until the subject reaches the target expiratory pressure). The correct position of each marker on the pressure signal was controlled visually (for example, coughing generates markers that must be deleted because they do not correspond to the beginning of a normal expiration), and segments showing obvious artifacts were removed from the analysis data set (average number of rejected segments: 17%), and a minimum of 80 segments were averaged under each condition. A pre-expiratory potential was defined as a slow negative shift starting between 2 and 0.5 s before the onset of expiration. The Fz, Cz, and FCz channels were focused on to look for the presence or absence of pre-expiratory potentials, in line with the results of earlier experiments (34, 35). The other derivations were summarily inspected to verify that the observed signal did actually fit previous descriptions (present in central derivations, absent in other derivations). EEGs were assessed visually by two examiners blinded to the loading conditions. In cases in which the two observers disagreed about the presence or absence of pre-expiratory potentials, a third blinded examiner was consulted whose opinion prevailed.

A similar analysis was performed using inspiratory time-locked EEG epochs of the same duration to detect preinspiratory potentials, the onset of inspiration being defined as the zero crossing of the ascending pressure signal.

Statistical analysis. Statistical analyses were performed with SPSS 19.0 and GraphPad 5.0 softwares. Friedman’s nonparametric ANOVA and Dunn’s post hoc test test were used for continuous variables and Cochran’s Q test was used for dichotomous variables, followed by a pairwise comparison. Benjamini-Hochberg correction was used to account for multiplicity (18).

Differences in the distribution of breathing pattern and respiratory discomfort variables according to the presence of a pre-expiratory potential were assessed by covariance analysis using “subject” as a dummy variable to avoid introducing an intrasubject reproducibility bias. To increase statistical power, this analysis was performed after pooling the resistance, high ETL, and low ETL conditions. For all analyses, a P value of 0.05 was considered to be statistically significant. For the analysis of variance, effect size was estimated using ε² (the proportion of the total variance that is attributed to an effect). The inter-rater agreement for the presence or absence of pre-expiratory potentials was tested using Cohen’s κ coefficient (before the intervention of a third observer when needed).

RESULTS

All subjects underwent the complete protocol. One subject did not exhibit pre-expiratory cortical activity during voluntary expiratory maneuvers, which was considered to be anomalous. This subject was, therefore, excluded from the analysis. The tracings of three other subjects were discarded because they exhibited gross oscillations of the baseline EEG traces synchronous with breathing during the loading conditions. These oscillations persisted after the averaging process and made it impossible to identify pre-expiratory potentials. The consolidated results, therefore, pertain to 16 subjects with complete data sets.

Breathing pattern and respiratory discomfort. All of the forms of expiratory loading tended to increase tidal volume. Respiratory rate was significantly slower with high ETL and ERL (Fig. 2). Overall, a trend toward increased ventilation and reduced PETCO2 were observed with threshold loading. Expiratory time was significantly prolonged in all forms of expiratory loading (Fig. 2). Inspiratory time was prolonged during high ETL (Fig. 2).

The abdominal EMG signal was consistently silent during tidal breathing in all subjects. In contrast, it exhibited expiratory-related activity under all other study conditions in all subjects.

Visual analog scale ratings of respiratory discomfort significantly increased with respect to baseline during ERL (P < 0.001) and high ETL (P < 0.001). A marked increase of VAS scores was also observed during low ETL, but was not statistically significant (Fig. 3), possibly because two subjects reported abnormally high discomfort during the control condition (Fig. 3).

Pre-expiratory potentials. Overall, Cohen’s κ coefficient to assess interobserver agreement concerning the presence or absence of a pre-expiratory potential was 0.87 (very good agreement), before any intervention of a third observer.

All subjects with a complete data set included in the analysis (n = 16) displayed pre-expiratory potentials under the “forced expiration” condition. One of these subjects displayed a pre-expiratory potential during unloaded breathing. Pre-expiratory potentials were present in 8 out of 16 subjects during low ETL, in 15 out of 16 subjects during high ETL and in 13 out of 16 subjects in ERL (Fig. 4, Fig. 5). (Figs. 4 and 5). According to Cochran’s Q test for dichotomous variables, pre-expiratory potentials were significantly more frequent in the loaded conditions and during forced expirations than during the control condition (overall Cochran, P < 0.001; control vs. low ETL, P = 0.008; control vs high ETL, P < 0.001; control vs ERL, P < 0.001; and control vs. forced expiration, P < 0.001). Pre-expiratory potentials were significantly less present in low ETL than in forced expiration (P = 0.016). The latencies and amplitudes of the pre-expiratory potentials are given in Table 1. There was no significant differences in latencies and amplitudes across conditions.

Covariance analysis showed that breathing pattern descriptors were not statistically related to the presence or absence of a pre-expiratory potential. In contrast, a statistical association was demonstrated for respiratory discomfort that was more intense in the presence of pre-expiratory potentials (P < 0.001, ε² = 0.25, namely, effect size of modest magnitude). There was no significant subject effect.

Preinspiratory potentials. Two subjects displayed preinspiratory potentials during quiet breathing (one of those also displayed a pre-expiratory potential in the control condition). Preinspiratory potentials tended to be more frequent with
expiratory loading (six subjects during low ETL, five subjects during high ETL, four subjects during ERL, and among those, three subjects during the three conditions). Nevertheless, there was no significant difference between control and the expiratory loading conditions, and no significant relationship between preinspiratory potentials and psychophysiological reporting. Of note, no evidence of inspiratory reafferent potentials was visible on the EEG tracings processed for preinspiratory potentials, even when preinspiratory potentials were present (probably as a consequence of the unloaded nature of inspiration).

**DISCUSSION**

This study confirms that the physiological response to experimental expiratory loading in humans involves cortical mechanisms. The presence of pre-expiratory premotor potentials preceding loaded expirations indicates that the supplementary motor area (or, more generally, the supplementary motor complex) is most likely implicated. It is, therefore, likely that sustained expiratory loads trigger a cortical drive to expiratory muscles.

**Methodological considerations.** In contrast with earlier approaches (34, 35), we identified pre-expiratory potentials ex-
clusively on the basis of visual inspection rather than statistical comparison of the slope of putative potentials with respect to baseline. This is common practice in cortical potential studies, and our blinded interobserver agreement was very good with a Cohen's $\kappa$ value of 0.87. Therefore, we are confident that our observations correspond to a physiological reality. One subject displayed a pre-expiratory potential during unloaded quiet breathing (and two displayed preinspiratory potentials). Similar findings have been observed in inspiratory loading studies [(1 out of 10 subjects in Ref. 35), (one out of nine subjects in Ref. 44), (two out of seven subjects in Ref. 34)]. In this study, as in the previous studies, and despite the various precautions taken (see METHODS), we cannot exclude the possibility that some subjects focused on their respiratory activity, for example, as a consequence of apparatus-related respiratory ungating and that this, in turn, resulted in premotor and motor activation in a manner similar to that described for other types of imagined movement (21). Of note, the lack of pre-expiratory potentials at low expiratory loading levels in some of our subjects may be due to insufficient sensitivity of this particular electroencephalographic approach to identify respiration-related cortical activity.

Motor-related potentials have been associated with perceived effort, with a relationship between their amplitude and effort perception (8), but the neurophysiological mechanisms for effort perception and discomfort may differ (27). We did not ask our subjects to rate effort perception independently of respiratory discomfort. This prevents us from establishing, for example, a threshold for discomfort in relationship with cortical activity. It may also explain why we did not find a relationship between discomfort and the latency and amplitude of the pre-expiratory potentials, in the hypothesis that such a relationship exist for effort but not discomfort (plateau effect). Also, the relationship between perception and motor potentials might be different from the relationship between perception and premotor potentials, an issue that has seemingly not been studied. We focused on premotor potentials because they were unambiguously identifiable, which was not the case of motor potentials [possibly in relationship with the automatized nature of breathing under loading (see discussion below), as opposed to the fully volitional nature of arm movements in the study by de Morree et al. (8)].

Physiological considerations: comparison with literature data. Our data corroborate the previous description of pre-expiratory potentials during voluntary expiratory maneuvers...
(25). They fuel the notion that respiratory muscles respond to cortical control and have a cortical representation similar to that of limb muscles (9, 13, 14, 25, 31, 41, 46). This study also shows that the cortical circuitry activated during voluntary expiratory efforts is probably also activated during expiratory load compensation, which is reminiscent of inspiratory loading experiments in which inspiratory threshold loads and inspiratory resistances give rise to preinspiratory cortical potentials (34, 35). In the case of inspiration, several arguments suggest that the cortical activity related to loading does not correspond to voluntary “breath-by-breath” inspirations, but to cortical automatization. First, Tremoureux et al. (44) reported that this activity is not transient, but persists for as long as the load is maintained. Secondly, Raux et al. (36) studied the brain-blood oxygen-dependent signal of subjects submitted to single-breath inspiratory threshold loading and then to sustained inspiratory threshold loading. They observed changes in cortical activation similar to the changes associated with the automatization of motor skills (22, 32), namely, a reduction of the activation of the cortico-subcortical areas involved in the acute response and a strengthening of the SMA activation. By analogy, we postulate that the cortical activity elicited by expiratory loading in the present study also proceeds from cortical automatization. This is consistent with the fact that our subjects did not receive any instructions on how to behave when confronted with expiratory loads. To the best of our knowledge, this is the first time that cortical activity related to expiration has been observed despite the absence of an explicit voluntary command, in contrast with previous studies (24–26, 33).

Our data suggest a marked similarity between cortical control of expiration and cortical control of inspiration in response to mechanical loading. Like their limb muscle counterparts, preinspiratory and pre-expiratory potentials likely originate in the anterior cingulate cortex, the premotor cortex, and the supplementary motor area (4), in line with the functional imaging data reported by Ramsay et al. (33), showing that the cortical areas involved in active expiration largely overlap the cortical areas involved in voluntary inspiration.

Putative relevance to speech breathing. Speech implies major perturbations of breathing control. Regarding inspiration, speech involves the preparation and production of phonatory breaths adapted to the intended subsequent utterances. This is associated with preinspiratory cortical potentials similar to those observed during inspiratory loading (45). Speech also involves the active control of expiratory muscles (11, 19) that produce expiratory flow without lung recoil and against the active vocal cords (natural expiratory load). By evidencing a cortical control of loaded expiratory muscles likely to involve cortical networks similar to those involved in inspiratory load compensation (34, 35) and prephonatory inspirations (45), the present data provide a rationale for future studies of the involvement of these networks in the speech-related cortical control of expiration, keeping in mind that the cortical networks engaged in phonation and voluntary expiration do overlap (24).

Dyspnea and disease-related expiratory loads. Expiratory resistive loading is associated with dyspnea, which is all of the more intense in the presence of high expiratory load (6). The subjects of the present study reported breathing discomfort in response to resistive and threshold expiratory loads (Fig. 3). An association was observed between respiratory discomfort and the presence of pre-expiratory potentials, this study being seemingly the first to establish a link between movement-related potentials and uncomfortably perceived respiratory efforts, even though we previously hinted at an association between dyspnea and the presence of preinspiratory potentials in normal subjects exposed to inspiratory loading under mechanical ventilation (34). These findings could pave the way to the development of neurophysiological surrogate markers of dyspnea, in which premotor potentials could be interpreted as the “cortical reaction” to dyspnea-generating afferences projecting onto the SMA (23), in the frame of the corollary discharge theory of dyspnea (30). However, the characteristics of the pre-expiratory potentials did not differ across conditions (Table 1), and there was, therefore, no relationship between the perception of respiratory discomfort and the magnitude of the potentials [which is similar to the findings reported by de Morree et al. (8) in limb muscles: these authors did not find a difference in the amplitude of the premotor potential between high and low load conditions during elbow flexor muscle contractions]. Notwithstanding the above considerations (see Methodological considerations), this could be the result of the important interindividual variability of the pre-expiratory potentials amplitudes and latencies (Table 1). Further studies (e.g., involving a simpler experimental design in a greater number of subjects, with distinction of effort and discomfort) will be needed to unmask such a relationship.

In conclusion, our findings provide a rationale to include the study of the cortical contribution to breathing control in the description of the physiological response to disease-related expiratory loads, particularly in respiratory diseases characterized by expiratory loads and active expiration, such as asthma or COPD (29).

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REFERENCES


