**Original**

**Breathlessness-related Brain Activation: Electroencephalogram Dipole Modeling Analysis**

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**Abstract**: Dyspnea is the feeling of shortness of breath and is a primary symptom of cardiopulmonary disease. Dyspneic symptoms include sensations such as labored respiration, chest tightness, air hunger, and uncomfortable or unpleasant urges to breathe. In this study, we investigated the brain areas associated with dyspnea using electroencephalogram dipole (EEG / DT) modeling. We hypothesize that good temporal resolution of EEG / DT recordings will enable determination of the neuroanatomical substrates of dyspnea in time course measures of inspiration. We measured EEG and respiration simultaneously during CO₂ rebreathing, which induced dyspnea in the subjects and allowed us to find inspiration-related potentials during dyspnea. The waveform of the potentials was composed of a negative peak at 100 ms and a positive peak at 250 ms. Our EEG / DT modeling estimated their source generators in the left superior frontal and left orbitofrontal cortex (OFC) at 100 ms after inspiration onset. In the next 100 ms, the anterior cingulate cortex was activated, followed by the superior frontal and OFC. At 200 ms to 300 ms, dipoles finally converged in the left insula and amygdala. The first component of inspiration-related potentials thus involved frontal areas that play a role in the intention to inspire and emotional guidance, while the late component incorporated areas related to emotional reaction. We suggest that dyspnea with increasing ventilation could involve intentions or efforts to continue inspiration activities, and consequently, the perception of dyspnea could be associated with unpleasant emotions.

**Key words**: dyspnea, respiration, electroencephalogram dipole modeling, insula, amygdala

**Introduction**

Dyspnea is the feeling of shortness of breath and is a primary symptom of cardiopulmonary disease¹, but it also occurs in anxiety and panic disorders². Dyspneic symptoms include sensations of labored respiration, chest tightness, air hunger, and uncomfortable or unpleasant urges to breathe. The emotional aspects of dyspnea are observed particularly with air hunger, which has been regarded as an essential vegetative sensation, such as hunger or thirst. Air hunger

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is increased by afferent inputs, such as CO$_2$ or hypoxia, which stimulate the respiratory chemoreceptors and increase air hunger$^3$, while mechanoreceptor-based tidal inflation of the lungs relieves air hunger$^4$. In addition to air hunger as a vegetative sensation, the effort required for breathing is also a possible cause of breathlessness. Breathlessness increases in proportion to the sense of effort, with a corresponding increase in respiratory resistive load$^5$.

Recently, brain imaging techniques such as functional magnetic resonance imaging (fMRI) and position emotion tomography (PET) revealed cerebral representations during dyspnea in humans$^{6-8}$. Using PET, Corfield et al$^6$ demonstrated involvement of the limbic areas in dyspnea perception, while Banzett et al$^7$ found that strong activation of the anterior insular cortex was associated with laboratory-induced dyspnea. An fMRI study also showed limbic and paralimbic loci of activation within the anterior insula, anterior cingulate, operculum, cerebellum, amygdala, thalamus, and basal ganglia during air hunger$^8$. The strengths of fMRI include its excellent spatial resolution and ability to identify anatomical localizations related to dyspnea; however, fMRI is limited by its lack of temporal resolution. Because dyspnea and air hunger may be associated with respiratory changes, we hypothesized that if CO$_2$ stimulation is applied through every inspiration, dyspnea would increase on a breath-by-breath basis, and to address this, we sought to determine how each brain area is involved in the processing of inspiration activities during rebreathing of CO$_2$. A candidate brain mapping technique with sufficient temporal resolution for such studies is electroencephalogram (EEG) dipole modeling analysis (EEG/DT), which can detect the source generators of event-related potentials such as movement-related potentials$^9$ and auditory-related potentials$^{10}$.

Recently, we investigated olfactory-inspiration potentials, which are averaged potentials triggered at the onset of inspiration during olfactory stimuli$^{11}$. As our data support the hypothesis that olfactory perception largely depends on inspiration, the olfactory-inspiration potentials at the onset of inspiration can be used as a trigger for EEG. Additionally, EEG/DT can estimate the source generators of this potential and detect olfactory processing related to inspiration onset with a good temporal resolution. The primary olfactory areas including the amygdala (AMG), entorhinal cortex, hippocampus, and orbitofrontal (OFC) were shown to be source generators of olfactory-inspiration potentials in accordance with inspiration onset$^{11}$.

Herein, by simultaneously recording EEG and respiration during CO$_2$ rebreathing, which induces dyspnea, we have identified dyspnea-related inspiration potentials associated with source localization in EEG/DT.

**Subjects and methods**

Five normal subjects (all males; mean age 22.6 years) participated in this study. All subjects gave informed consent and the study was approved by the Ethics Committee of Showa University School of Medicine.

**Experimental apparatus**

The subjects were seated at rest in a laboratory room. A facemask with a transducer for
measuring respiratory flow and volume was attached to each subject (CPX, Arco System, Chiba, Japan). The subjects rebreathed through a transducer from 6-L plastic bags containing a gas mixture of 5% carbon dioxide + 95% oxygen. Respiratory rate (RR), tidal volume (VT), minute ventilation (VE), and end tidal CO₂ concentration (ETCO₂) were continuously measured and the data was recorded on a computer. Every 10 seconds, we asked the subjects to evaluate their breathlessness by using a Borg scale

**Measurement of EEG and EEG/DT**

For the measurement of EEG and EEG/DT, 21 electrodes were positioned on the subjects according to the International 10–20 system, with the reference electrode on the right earlobe. An EEG and electro-oculogram were recorded and stored in a digital EEG analyzer (DAE-2100, Nihon Kohden, Tokyo, Japan). The EEG was sampled at 200 Hz through a 0.016- to 30-Hz bandpass filter. Impedances were kept below 10 KΩ. Signals of the onset of odor stimulation and respiratory flow (described later) were obtained simultaneously by the EEG and oculogram recordings and stored in the EEG analyzer. After electrodes were attached, the subject was moved to a shielded room and seated on a chair. Respiratory flow data obtained with the respiratory monitor were also stored in the EEG analyzer. Inspiration flows downward from the 0 level, and expiration flows upward. The onset of inspiration (0 level) was used as a trigger for collection of the averaging potentials.

To determine the generators of the averaged potentials, EEG/DT data was localized with a scalp-skull-brain head model, created with the Montreal Neurological Institute (MNI) standard coordinate system. EEG/DT involves calculating the location of source generators in the brain from the EEG data. The actual potential field distribution recorded from the 19 scalp electrodes (Vmeas) was compared with the calculated field distribution (Vcal) for an appropriately equivalent current dipole (for one-dipole estimations) or two appropriately equivalent dipoles (for two-dipole estimations). The inverse solution was used to determine the dipole location and orientation that best fit the recorded data 13. The locations and vector moments of one- or two-current dipoles were iteratively changed within the head model until the minimal squared difference between Vmeas and Vcal was obtained by the simplex method 14. Accuracy of generator location, estimated using an MNI standard head model, was confirmed by comparison with that estimated using individually created head models 9. In this study, we estimated the location of dipoles using both grand mean averaged potentials and individual averaged potentials with the MNI model. A detailed description of the dipole tracing method has been reported elsewhere 9–11.

**Statistical analysis**

We used a software package (SPSS, SPSS Japan) for all statistical analysis. One-way repeated measures analysis of variance (ANOVA) was used to test for the effect of CO₂ on respiratory variables. RR, VE, VT, ETCO2 and breathlessness during rest and CO₂ rebreathing were compared using the Bonferroni post-hoc multiple comparison.

The degree of source concentration can be calculated in terms of goodness of fit, with 100%
taken as ideal; however, in practice it is usually less than 100% owing to noise, electrode misalignment, or non-dipole components of the electric sources. In the present study, two-dipole estimates with a goodness of fit greater than 98% were considered to indicate a concentrated source.

**Results**

**Respiratory variables and breathlessness scores**

There was no difference in RR among measurements at baseline, during rebreathing of CO₂, and following rebreathing of CO₂ ($P = 0.22$), while $V_T$, $V_E$, and ETCO₂ increased significantly during CO₂ rebreathing. There were significant changes in breathlessness measured with the Borg scale. The post-hoc testing showed a significant increase in CO₂ during and after CO₂ rebreathing compared with the baseline period, while breathlessness changed significantly between during and after CO₂ rebreathing.

**EEG / DT results**

Grand averages of the potentials triggered at inspiration onset during CO₂ rebreathing across the five subjects are shown in Fig. 2 (top). The waveform of the potentials was composed of
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a negative peak at 100 ms and a positive peak at 250 ms. These potentials were referenced to inspiration-related potentials during dyspnea. The root mean square value (RMS) showed a slow negative slope from 0 to 200 ms that increased positively at 250 ms. Because the DT method detects moving dipoles over time after a triggering point (indicated as 0 in the Fig. 1), we analyzed the dipole locations at each of the three components (0 ~ 100 ms, 100 ~ 200 ms, and 200 ~ 300 ms).

The dipole results from five subjects located in each anatomical region are shown in Table 1. Anatomical areas of interest related to dyspnea or air hunger were determined based on previous PET and MRI studies. Dipoles with a GOF of 98% or higher were considered significant. Specific regions during each time period are indicated as numbers with a shaded column. From 0 ~ 100 ms, dipoles converged in the left OFC and the left superior frontal cortex (L S frontal). The left anterior cingulate (A cingulate) was activated from 100 ms to 200 ms. Finally, the left insula and the left AMG were activated from 200 ms to 300 ms. Typical dipole localizations in sagittal and horizontal sections during each time period are shown in Fig. 2 (bottom). Dipoles in sagittal sections corresponded to those in horizontal sections (some
dipoles are shown only in sagittal sections).

**Discussion**

In this study, we investigated the brain areas associated with dyspnea using EEG / DT modeling. We confirmed that dyspnea was successfully induced by CO$_2$ rebreathing. As ETCO$_2$ increased during CO$_2$ rebreathing, VT mainly contributed to the increase of V$_E$, whereas the RR was unchanged. Hypercapnia and exercise increase ventilation and thus increases in V$_E$ comprise various combinations of VT and RR. Izumizaki *et al.*$^{15}$ suggested that VT increases followed by RR increases during hypercapnia may be caused by dyspnea increases. As dyspnea causes negative emotions,$^{16}$ the perception of dyspnea is potentially associated with an increased respiratory rhythm during exposure to dyspnea-inducing stimuli in the laboratory. Negative emotions such as anxiety increase V$_E$ with a marked increase in RR during rest.$^{17}$ Thus, respiratory output in combination with VT and RR may produce a strong interaction between metabolic breathing and behavioral breathing. As observed in the responses during CO$_2$ breathing, only VT contributed to V$_E$ increases in the present study, suggesting that the dyspnea induced might not be strong enough to cause transitions to an RR increase. This observed respiratory response during mild dyspnea could reflect brain activities investigated by EEG / DT, and herein we found that the left superior frontal and left OFC were activated 100 ms after inspiration onset, while during the next 100 ms, the anterior cingulate cortex was activated.

The superior frontal areas are involved in motor planning and attention,$^{18}$ while the anterior cingulate plays a role in motor planning, and both are regarded as premotor areas.$^9$. These
areas of activations also were observed during movement-related potentials during voluntary motion\(^9\). On the one hand, the OFC plays a role in guiding emotional guidance and shaping survival strategies\(^7\). From our observations of the first component of the inspiration-related potentials during dyspnea, we suggest that the intention or plan to initiate inspiration is related to activities in the superior frontal and anterior cingulate cortices, whereas evaluating emotional reactions is related to activity in the OFC.

Activations in the superior frontal and anterior cingulate cortices suggest that intentions toward the next inspiration or effort to breathe may be involved. Between 200 and 300 ms, the dipoles finally converged in the left insula and amygdala. The insula is essential to the perception of dyspnea\(^7,8\), while electrophysiological and anatomical tracer investigations have linked the insula to afferents and motor centers relevant to breathing. Afferents from respiratory chemoreceptors and pulmonary stretch receptors project to the granular and dysgranular insula, neighboring the principal activation\(^20\). In addition, stimulation studies of the vagus and the insula have demonstrated reciprocal respiratory projections in man and other mammals\(^20\).

The insula has efferent and afferent connections with all of the neighboring limbic and paralimbic structures including the operculum, anterior cingulate, orbital frontal cortex, thalamus, amygdala, and basal ganglia\(^7\). Reiman\(^21\) proposed that the insula evaluates distressing stimuli carrying negative emotional valence, and dyspnea is a sensation that includes unpleasant affective responses, involving perception\(^7\). Interestingly, around 300 ms, the amygdala was also activated. Amygdala activity is thought to involve anxiety, fear, and unpleasantness\(^22\) and may be related to the aversive aspects of dyspnea. From these results, we propose that an emotional reaction can be recognized consciously as dyspnea at around 300 ms after inspiration onset.

As we noted earlier, increases of RR were not observed during CO\(_2\) rebreathing in this study. It has been reported that anxiety-increased RRrs occur in parallel to increased amygdala activity\(^23\), with source generators of anxiety-related potentials triggered by inspiration onset during anxiety in the amygdala within 100 ms after inspiration onset. In this study, AMG was found at 300 ms, which was already regarded as the time period for human recognition or perception. Therefore, in this study, anxiety-based increases in ventilation may be excluded, leaving purely dyspnea sensations during CO\(_2\) rebreathing.

In this study, we could detect activations simultaneous to inspiration activities because of the high temporal resolution of EEG/DT. Thus, we showed the areas associated with dyspnea from intention for inspiration and emotional guidance in the frontal cortex, followed by emotional reactions to the recognition of dyspnea. Recognition of air hunger may depend on attention and anxiety levels and may also depend on personality traits. Future studies could therefore investigate whether increases in attention or anxiety could increase dyspnea or whether dyspnea differs between individuals. Dyspnea is a major symptom for patients with pulmonary and cardiovascular diseases and also psychological diseases, such as patients with hyperventilation or panic disorders. Our ultimate goal is to find a way to reduce dyspnea using our new knowledge regarding its physiological and neurological aspects.
Acknowledgement

This work was supported by JSPS KAKENHI Grant Number 24500611.

Conflict of interest disclosure

The authors have declared no conflict of interest.

References


[Received December 25, 2014 : Accepted January 6, 2015]