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The time-course of cortico-limbic neural responses to air hunger

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Abstract

Several studies have mapped brain regions associated with acute dyspnea perception. However, the time-course of brain activity during sustained dyspnea is unknown. Our objective was to determine the time-course of neural activity when dyspnea is sustained. Eight healthy subjects underwent brain blood oxygen level dependent functional magnetic imaging (BOLD-fMRI) during mechanical ventilation with constant mild hypercapnia (~45 mmHg). Subjects rated dyspnea (air hunger) via visual analog scale (VAS). Tidal volume (V_T) was alternated every 90 seconds between high V_T (0.96 ± 0.23 L) that provided respiratory comfort ($12 \pm 6\%$ full scale) and low V_T (0.48 ± 0.08 L) which evoked air hunger ($56 \pm 11\%$ full scale). BOLD signal was extracted from *a priori* brain regions and combined with VAS data to determine air hunger related neural time-course. Air hunger onset was associated with BOLD signal increases that followed two distinct temporal profiles within sub-regions of the anterior insula, anterior cingulate and prefrontal cortices (cortico-limbic circuitry): (1) fast, BOLD signal peak <30 seconds and (2) slow, BOLD signal peak >40 seconds. BOLD signal during air hunger offset followed fast and slow temporal profiles symmetrical, but inverse (signal decreases) to the time-courses of air hunger onset. We conclude that differential cortico-limbic circuit elements have unique contributions to dyspnea sensation over time. We suggest that previously unidentified sub-regions are responsible for either

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AUTHOR CONTRIBUTIONS

A.P.B. – Study conception, protocol design, lead management and performance of all studies; analysis and manuscript preparation. K.C.E. – Hypothesis delineation, lead design and implementation of brain image analysis; manuscript preparation. J.D.R. – Brain image analysis design/implementation and manuscript preparation. S.H.M. – Protocol design, performing studies and manuscript preparation. R.B.B. – Study conception, team management, protocol design, performing studies, analysis design and manuscript preparation.

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the acute awareness or maintenance of dyspnea. These data enhance interpretation of previous studies and inform hypotheses for future dyspnea research.

Keywords

air hunger; dyspnea; fMRI; limbic; insula; time-course

1. Introduction

Despite its prevalence and importance as a cardinal symptom of cardiopulmonary disease (Hammond, 1964; Klein, 2002; Kroenke et al., 1990; Manning and Schwartzstein, 1995; Perna et al., 2004; Reuben and Mor, 1986; Rousseau, 1996), relatively little is known about the neural mechanisms of dyspnea, and dyspnea relief. No FDA approved agents list dyspnea as an indication. Since dyspnea is not only unpleasant but also evokes fear and anxiety (Banzett et al., 1996; Banzett et al., 2008; Evans and Banzett, 2014; O'Donnell et al., 2013) there is an increasing awareness of the need for compassionate management of this symptom (Brody et al., 1997; Campbell, 2004; Jones et al., 2004; LaDuke, 2001; Perkin and Resnik, 2002). Objective measurements of dyspnea will enhance drug development and help meet this obligation. The comparable field of pain research has faced similar challenges and has utilized neuroimaging techniques to delineate the neural circuitry underlying pain perception (Schweinhardt et al., 2006; Tracey and Mantyh, 2007; Wager et al., 2013). In contrast to hundreds of published studies in pain research, very few neuroimaging studies have examined dyspnea perception and its relief.

Objective signals of dyspnea perception have been localized within cortical and limbic/paralimbic (cortico-limbic) neural networks by positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) during laboratory induced dyspnea (Banzett et al., 2000; Evans et al., 2002; Peiffer et al., 2001). The activations identified during laboratory induced dyspnea are similar to findings during laboratory induced pain (Tracey and Mantyh, 2007). Although these studies provided objective measures of neural activity associated with dyspnea, the time-course of this neural activity and the relationship between dyspnea intensity and activation strength remain unknown. The delineation of neural substrates associated with dyspnea onset and sustained dyspnea (more akin to that experienced by patients) will facilitate progress in dyspnea research. Enhanced understanding of temporal dynamics promises to: 1) define the optimal time frame for imaging neural processes that mediate dyspnea, 2) aid objective quantification of efficacy for dyspnea therapeutics, and 3) guide novel therapeutic interventions for dyspnea.

We have previously used blood oxygen level dependent (BOLD) fMRI to study the form of dyspnea known as 'air hunger' or 'unsatisfied inspiration' – i.e., the feeling of not getting enough air (Banzett et al., 1990; Banzett et al., 1989; Wright and Branscomb, 1955). Air hunger is extremely unpleasant (Banzett et al., 2008) and is a key component of the dyspnea experienced by patients (Lansing et al., 2009; O'Donnell et al., 2007; Parshall et al., 2012; Smith et al., 2009). Air hunger can be evoked by lowering ventilation and relieved by raising it (Banzett et al., 2000; Manning et al., 1992) while holding the partial pressure of carbon

dioxide (P_{CO_2}) at a constant, modestly elevated level (Banzett et al., 1990; Banzett et al., 1989). This method minimizes CO_2 induced changes in global cerebral blood flow that would confound interpretation of the BOLD signal (Wise et al., 2004). Our previous BOLD-fMRI study used this technique to identify neural activity associated with rapidly alternating changes in air hunger stimulus (i.e., every 42 sec) (Evans et al., 2002). This frequency of stimulus alternation was optimized for nascent fMRI methodology of the late 1990s, and prevented conditions of air hunger and baseline from reaching “steady-state”, therefore precluded meaningful de-convolution of the neural dynamics of dyspnea. The present BOLD-fMRI study employed 100 sec conditions of sustained, steady-state air hunger (AH_{SS}) somewhat closer to the time-course of clinical dyspnea. We sought to determine the time-course of neural activity during: 1) the on-transition (AH_{ON}), from steady-state respiratory comfort/experimental baseline (BASE) to AH_{SS} , 2) AH_{SS} , and 3) the off-transition (AH_{OFF}), from AH_{SS} to BASE.

2. Methods

2.1 Subjects

Eight right-handed subjects with no history of neurological, cardiopulmonary, nor psychiatric illness were studied (23–31 years, 3 women). All subjects were naive to the underlying physiological mechanisms under investigation and the protocol objectives. The study was submitted to and approved by IRBs at Partners Health Care and the Harvard School of Public Health, and subjects gave informed consent.

2.2 Protocol

Physiological interventions and measurements are detailed in the on-line supplement. Briefly, subjects first underwent two training sessions outside of the scanner to become familiar with our dyspnea stimulus, the rating procedure and mechanical ventilation via mouthpiece. Subjects subsequently underwent BOLD-fMRI, during which they received volume-controlled ventilation (minute ventilation set to 0.13 l/kg/min and adjusted if necessary to achieve comfort in the baseline condition). End-tidal P_{CO_2} was monitored continuously (breath-by-breath) and the fraction of inspired CO_2 (F_{ICO_2}) was adjusted to maintain a constant end-tidal level (~5 mmHg above subject’s habitual baseline). As in previous studies (Banzett et al., 2000; Evans et al., 2002), air hunger was periodically evoked by reducing tidal volume (V_T). The F_{IO_2} was set at 30% during all conditions to assure >98% saturation of arterial oxygen pressure (SpO_2) and thereby prevent condition related changes in cerebral oxygenation that could impact interpretation of the BOLD signal). Subjects performed three 10-minute trials; each trial comprised ~100-second conditions that alternated between baseline and reduced V_T (i.e. 3 repeats of each condition per trial). Subjects rated air hunger every 15 seconds using a visual analog scale (AH-VAS). Full scale was defined to the subject as intolerable. During a post-study debriefing, subjects provided verbal description of their respiratory sensations (Banzett et al., 1996; Evans et al., 2002)).

2.3 Physiological and psychophysical data analysis

The physiological data, BOLD fMRI signal, and AH-VAS ratings were analyzed for each of four main conditions (Figure 1): 1) air hunger on-transient (AH_{ON}), 2) steady-state air hunger (AH_{SS}), 3) air hunger off-transient “relief” (AH_{OFF}) and 4) steady-state respiratory comfort/experimental baseline (BASE). Repeated measures ANOVA and between-condition t-tests were performed on the following variables: P_{CO_2} , V_T , and AH-VAS.

2.4 MRI data acquisition and analysis

High-resolution structural and BOLD-fMRI images were acquired using a Siemens Sonata 1.5 Tesla system with standard image acquisition parameters. Image pre-processing and analyses were performed within SPM5 analysis software. Analyses were conducted in two stages: Spatial Mapping Analyses and Time-course Analyses. Further details can be found in the on-line supplement.

2.5 Spatial mapping analyses

Group level voxel-wise tests were performed to identify regions of interest where changes in BOLD signal were significant for the following contrasts: 1) $AH_{ON} > BASE$, 2) $AH_{SS} > BASE$ and 3) $AH_{OFF} > BASE$. Statistical criteria for searches are discussed in the on-line supplement.

2.6 Time-course analyses

After identifying regions of interest at the group level (see above), the BOLD signal time-course data was extracted from the peaks of each identified region in the individual subjects. If the peak cluster within a region of interest did not reach significance in all subjects, then the region was not included in the time-course analysis.

The BOLD signal time-course data from each region of interest was then time-aligned with the corresponding V_T and AH-VAS data so it could be averaged across subjects for each experimental condition (i.e., BASE, AH_{ON} , AH_{SS} , AH_{OFF}). To determine the relationship between the BOLD time-course and AH-VAS ratings, a composite mean BOLD time-course was computed by averaging the BOLD signal from all of the individual regions of interest identified by the primary contrasts (i.e., $AH_{ON} > BASE$, $AH_{SS} > BASE$; see Figure 2., and the Supplement for details).

3. Results

3.1 Data quality control

Two of the eight subjects were excluded from analyses; one due to excessive task-related head movement (> 1 mm) and the other required excessive end-tidal P_{CO_2} (> 55 mmHg) to induce sufficient air hunger that precluded meaningful interpretation of the BOLD signal (Posse et al., 2001). Subsequent analysis was performed on data from the remaining six subjects (23–31 years old, three female).

3.2 Physiological and psychophysical

Physiological and psychophysical results are summarized in Table 1. Intrinsic to the study design, V_T during the AH_{SS} condition (group mean $V_T = 0.48 \pm 0.08$ L) was significantly less than V_T during the BASE condition (group mean $V_T = 0.96 \pm 0.23$ L). Also intrinsic to study design, subjects' AH-VAS ratings were significantly greater during the AH_{SS} condition (group mean = 57 ± 11 % full scale AH-VAS) than during the BASE condition (group mean = 12 ± 6 % full scale AH-VAS). The AH-VAS ratings were consistent across trials as repeated measures tests of within-subject effects demonstrated no significant differences in ratings for each condition across trials ($p = 0.362$).

The mean P_{CO_2} during fMRI data collection was 45.9 ± 3.9 mmHg. Despite our intent to maintain constant P_{CO_2} across the experimental conditions, small differences in P_{CO_2} were observed between conditions (maximum 0.7 mmHg); this was statistically significant in only one of the comparison pairs: (AH_{ON} vs. BASE, $p = 0.028$), but is probably unimportant physiologically and within the error of measurement. The variability in end-tidal P_{CO_2} did not vary across subjects ($p = 0.105$).

In order to verify that the stimulus provoked air hunger (Lansing et al., 2009), subjects were asked to provide a free-response description of their sensations. Subjects were asked “in your own words, what were you rating?” All subjects volunteered descriptions consistent with the sensation of air hunger: “desire to get more air,” “starving for air,” “highly uncomfortable urge to breathe”, “extreme desire for more air,” “the amount of air I was [not] getting.”

3.3 Spatial mapping: Identifying regions of interest

During both the AH_{ON} and AH_{SS} conditions the BOLD signal significantly increased above baseline in the anterior insula, dorsal anterior cingulate cortex (dACC) pre-supplementary motor area (pre-SMA) and middle frontal gyri (Tables 2, 3). Some local maxima were nearly identical in the two air hunger contrasts; other local maxima had slightly different regional localization (Figures 2, E1–E4). Local maxima within the premotor cortex and cerebellum showed a BOLD signal increase for the comparison of AH_{SS} > BASE, but not for the comparison of AH_{ON} > BASE (Tables 2, 3).

The air hunger contrasts also identified regions of increased BOLD signal outside the *a priori* regions (i.e., more than 15 mm from previously reported findings; Tables E1, E2). The inverse air hunger contrasts failed to yield any significant findings (e.g., AH_{ON} < BASE, AH_{SS} < BASE, AH_{OFF} < BASE). In addition, the contrast of “air hunger relief” (AH_{OFF} > BASE) failed to identify any regions of potential significance.

3.4 Time-course of neural activity related to air hunger and its relief

The group composite time-course data for the regions of interest identified by the contrasts of AH_{ON} > BASE and AH_{SS} > BASE are presented in Figure 2. The regional neural activity identified by each contrast was associated with a distinct profile of BOLD signal time-course. The on-transient time-course for the regions of interest identified by the AH_{ON} > BASE contrast had a fast profile in which the peak BOLD signal occurred approximately 18

seconds after a change in V_T , followed by a sharp decreased in BOLD signal seconds later (time-course plot on left, in red hue; Figure 2, Panel b). Whereas the on-transient time-course for the regions of interest identified by the $AH_{SS} > BASE$ contrast had a slow profile in which the peak BOLD signal occurred approximately 51 seconds after a change in V_T , followed by an elevated plateau in BOLD signal (time-course plot on left, in yellow hue; Figure 2, Panel b). Within the insula, dACC, SMA and middle frontal gyrus there were sub-regions that followed the fast neural profile (brain images with red overlay; Figure 2, Panel a) and other sub-regions that followed the slow/plateau profile (brain images with yellow overlay; Figure 2, Panel a). The BOLD signal time-course data for each individual local maximum identified by the air hunger contrasts are presented in the on-line supplement (Figures E1–E4).

The off-transient time-courses appeared as simply the inverse of the on-transient time-courses (time-course plots on right; Figure 2, Panel b). There were no regions in which a significant increase in BOLD signal was associated with the cessation of air hunger.

4. Discussion

4.1 Overall findings

The current study is the first to take advantage of the temporal resolution of fMRI to examine the time-course of neural activity relative to the time-course of dyspnea sensation. We found that the temporal profile of BOLD fMRI signals within cortico-limbic circuitry generally followed the time-course of subjective reports of dyspnea on-set and off-set, but that important differences can be seen among regions. Two distinct profiles of BOLD signal (neural) time-course were observed in the present study: 1) a fast rise in BOLD signal followed by an abrupt decrease within about 30 sec; 2) a slow rise in BOLD signal reaching a plateau after about 50 sec (time-course plots on left; Figure 2, Panel b). The insula, dACC, SMA and middle frontal gyrus had sub-regions with the fast profile and other sub-regions that followed the slow profile.

4.2 Implication of present findings in context of previous studies

Differential temporal dynamics within cortico-limbic circuits have also been observed in neuroimaging studies of pain (Downar et al., 2003; Ibinson and Vogt, 2013; Moulton et al., 2005). A direct comparison of findings from the present study to temporal dynamics reported in imaging studies of other sensations carry important caveats (e.g., pain and dyspnea may have similar and different afferent pathways, peripheral nerve fiber types, etc. (Banzett et al., 2007; Gracely et al., 2007; Morelot-Panzini et al., 2007)). Despite acknowledged limitations, the present findings appear to have temporal features in common with pain stimuli.

Prolonged neural responses (~ 60 sec) have been shown to follow the time-course of noxious stimuli (Downar et al., 2003). In a different study, Moulton et al., 2005 (Moulton et al., 2005) reported that innocuous and noxious pain stimuli share overlapping cortico-limbic localization for (e.g., insula, ACC, SMA) but have differences in time-course related to the magnitude of the stimuli: noxious stimuli exhibited a delayed peak (6–8 sec later than

innocuous stimuli) and longer overall response duration (>30 sec for noxious stimuli vs. <20 sec for innocuous stimuli). In the present study the group mean rating of air hunger was less for the AH_{ON} condition compared to the AH_{SS} condition (2.3 vs. 5.5, 0–10 scale, Table 1., Figure 2). Thus, periods of strong dyspnea (AH_{SS}) were associated with regional neural responses that exhibited a delayed peak and longer overall response compared to periods of mild dyspnea (AH_{ON}). Taken together, we suggest that a specific sub-circuit within the general cortico-limbic circuitry mediates the acute awareness of dyspnea onset and an anatomically similar yet functionally distinct sub-circuit mediates the maintenance of dyspnea activity.

As reviewed by Evans and Banzett (Evans and Banzett, 2014), several fMRI studies of dyspnea have been published over the last decade. Because signal/noise characteristics of BOLD fMRI are optimal for conditions of short duration, previous BOLD fMRI studies of dyspnea have used respiratory challenges of 24–45 seconds (Evans et al., 2002; Pattinson, 2009; von Leupoldt et al., 2008, 2009a; von Leupoldt et al., 2009b). Advances in fMRI analytic techniques (reviewed by Evans, 2010 (2010)) enabled the examination of the longer duration conditions employed in the current study. The present findings suggest that the findings of the earlier fMRI studies, especially those with stimuli less than 30 seconds, are probably dominated by transient neural processes related to the acute onset (or offset) of dyspnea. Conversely, PET images are collected over a period of 60 seconds or more, thus are ill-suited to resolve faster events (Evans, 2010).

The present findings challenge the interpretation of an earlier PET study of dyspnea relief (Peiffer et al., 2008). Despite nearly parallel methods of hypothesis testing (present study; AH_{OFF} > BASE, Peiffer et al., 2008 (Peiffer et al., 2008); load off-transient > no load), the present study failed to show any neural activation specific to dyspnea relief. Instead, we observed only a symmetrical decrease in neural activity in the cortico-limbic circuitry upon relief of air hunger. We suggest two possible interpretations of this discrepancy: 1) The relief activations observed by Peiffer et al., 2008 may be unique to the sudden cessation of external resistive loading, and not characteristic of dyspnea relief in general. 2) Alternatively, the time response of the PET imaging technique is not ideal for examination of neural dynamics. Given the relatively slow temporal resolution of oxygen¹⁵ PET, (~2 minutes; reviewed by Cherry and Phelps, 1996 (Cherry and Phelps, 1996)), neural signals related to the rapidly changing conditions were not faithfully represented.

Previous studies have used functional neuroimaging to examine the neural time-course of various respiratory stimuli and tasks, but these studies have focused on respiratory control mechanisms rather than dyspnea. Some of the ventilatory stimuli used in these studies may have evoked dyspnea and subsequently induced temporally related regional neural activity within cortico-limbic circuitry: hypercapnia (insula) (Harper et al., 2005), hypoxia (insula) (Macey et al., 2005) expiratory loading (dACC and insula) (Macey et al., 2004) breath-hold (dACC, insula, SMA) (McKay et al., 2008). However, because dyspnea ratings were not acquired during image acquisition in these studies, it is not possible to determine whether the neural responses were associated with respiratory discomfort, were part of a ventilatory control mechanism, or both. The present study acquired systematic measurements of

subjective air hunger ratings during a previously validated fMRI protocol designed to detect perceptual rather than physiological neural responses.

4.3 Replication of previous dyspnea neuroimaging findings

Although the primary aim of the present study was to describe the neural time-course of dyspnea perception, the maps of brain activation from the present study replicate and extend earlier functional neuroimaging studies that localized dyspnea perception within the anterior insula, dorsal anterior cingulate cortex (dACC) (Banzett et al., 2000; Evans et al., 2002; Peiffer et al., 2001), and prefrontal cortex (collectively referred to as cortico-limbic circuitry, reviewed by Evans, 2010 (Evans, 2010)). In particular, the local maxima identified by contrasts of air hunger were within close proximity to those identified in the only other published fMRI study of air hunger *per se* (Evans et al., 2002) (Tables 2, 3).

4.4 Novel dyspnea neuroimaging findings

Novel activations associated with air hunger in *post hoc* analyses are reported in Tables E1–E2. The localization of activity within the dorsal extent of bed nucleus of the stria terminalis (BNST) during sustained air hunger ($AH_{SS} > BASE$) was perhaps the most intriguing *post hoc* finding (Table E2). The BNST has integral connections to the amygdala, and has recently been implicated in conditions of sustained threat (Alvarez et al., 2011; Mobbs et al., 2010); this may have implications for future studies.

4.5 Methodological issues

As discussed above in Section 4.2, we consider the present findings in the context of BOLD temporal dynamics reported in imaging studies of other respiratory stimuli and tasks as well as other sensations (i.e., pain). We wish to emphasize that all BOLD-fMRI studies of physiological and/or psychophysical responses are all reliant on the accurate modelling of hemodynamic response (Friston et al., 2007; Friston et al., 1995). As such, the modelled BOLD response merely reflects an indirect measure of neuronal activity that is subject to variance in local cerebral metabolic rate of oxygen consumption, cerebral blood flow and cerebral blood volume (Logothetis, 2008). Thus we can only consider the regional BOLD signal time-course as a relative proxy for the time-course of local neural activity.

The interpretation of the present findings face additional challenges common to other published functional neuroimaging studies of dyspnea perception. These challenges include changes in respiratory related head motion related to experimental condition (Evans, 2010) and changes in P_{CO_2} related to experimental condition (Wise et al., 2004) that can impose artifacts in BOLD fMRI studies. Our study design, quality control, and analytic techniques served to minimize these sources of variance. Specifically, our induction of air hunger by reducing V_T and decreasing inspired P_{CO_2} evoked air hunger while avoiding large condition-related changes in end-tidal P_{CO_2} in this and previous studies (Banzett et al., 1996; Banzett et al., 2000; Evans et al., 2002). Our exclusion of data showing large head movement, and our use of P_{CO_2} and movement parameters as nuisance regressors in the SPM analytic model (see Supplement) further served to minimize artifacts associated with fluctuations in P_{CO_2} and respiratory related head movement. The use of mechanical ventilation may have prevented the subjects from reaching a true baseline of comfort despite the tidal volume

being manipulated to do so. The group mean change in AH-VAS score between BASE and AH conditions however was substantial (44% change of full scale). Given this significant difference in reported air hunger between the two states, combined with our analytic approach, we hold great confidence that the neural activity we report here is associated with the perception of air hunger.

Although significant, the magnitude of neural activity associated with air hunger within the insular cortices, opercula and cerebellum was relatively less than that observed in previous studies of dyspnea (Banzett et al., 1996; Brannan et al., 2001; Evans et al., 2002; Parsons et al., 2001; Peiffer et al., 2001). Because we imposed stringent criteria for inclusion, the failure of at least one subject to exhibit significant activation within the opercula and cerebellum, precluded our time-course analysis of these regions. While prominent activation of the amygdala had been observed in our earlier BOLD-fMRI study of air hunger (Evans et al., 2002), air hunger activity within *a priori* boundaries of the amygdala failed to reach significance (but *post hoc* exploratory analysis did show air hunger related activity within the peri-amygdaloid structure of the dorsal bed nucleus of the stria terminalis [BNST], Table E2, Supplement Discussion). The relatively small sample size and differences between experimental protocols may have contributed to differences in the magnitude of observed neural findings in the present study.

Beyond the aforementioned limitations, this study could have been improved in several respects. The sample size could have been larger, the MRI scanning could have been performed with a more powerful magnet (e.g., 3 Tesla vs. 1.5 Tesla) and greater number of head-coil elements (32 channels vs. 8 channels). However, the effect size and significance of the reported findings suggest that the sample size and technology used were adequate.

4.6 Conclusions

By delineating the time-course of air hunger associated neural activity within cortico-limbic circuitry, we have shown that regions previously shown to be associated with dyspnea contain sub-regions that have different time-courses of neural activity. The present findings provide new insights that improve understanding of previously published studies and also inform the design of future studies. We expect that enhanced understanding of neural time-course will serve to guide future neuroimaging studies of dyspnea in patients as well as studies focused on the development of novel therapeutics to relieve dyspnea.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Physiological waveforms and psychophysical ratings for a typical subject

Subjective ratings of air hunger (AH) via visual analog scale (VAS; 0–10) are presented in the second trace, interpolated AH-VAS ratings (down-sampled to scan domain; see E.1.3 for details) are shown in the first trace, expiratory tidal volume is shown in the third trace, tidal PCO_2 is shown in the fourth trace (peak values indicate end-tidal PCO_2 (P_{ETCO_2}); nadir values represent inspired PCO_2) and scan repetition times (TRs) are shown on the fifth (bottom) trace. Experimental conditions and transitions between conditions are indicated by patterns and shading (AH_{ON}; red checker board pattern, AH_{SS}; red shading, AH_{OFF}; blue grid pattern, BASE; blue shading).

Figure 2. Group Mean Time Courses

The regions of interest (ROIs) identified by the contrast of $AH_{ON} > BASE$ are shown in red; and ROIs identified by the $AH_{SS} > BASE$ contrast are shown in yellow. Panel (a) depicts the locations of significant maxima superimposed on coronal sections of a standardized structural image (numbers in white italic font indicate y-axis values within the Montreal Neurologic Institute coordinate system). The upper trace in Panel (b) depicts the time course of air hunger ratings (AH VAS) averaged over all subjects (top plot, diamonds). The two middle traces in Panel (b) depict the time course of BOLD signal for the group of regions identified by each contrast, averaged over all subjects ($\% \text{ BOLD} \pm \text{SEM}$; middle plots, circles). The lower trace depicts the tidal volume (V_T , liters; bottom plot, squares). The transition from high V_T to low V_T is shown by closed symbols in the left panels and the transition from low V_T to high V_T is shown by open symbols in the right panels.

Experimental conditions and transitions between conditions are labeled with abbreviations and indicated by patterns and shading (AH_{ON} ; checker board pattern, AH_{SS} ; dark gray shading, AH_{OFF} ; grid pattern, BASE; light gray shading). Within each time-course plot, data to the left of AH_{ON} and AH_{OFF} transient periods represent 30 seconds of the volume

condition preceding the transition in volume. To demonstrate the relative extent of % BOLD between volume conditions at each ROI, an experimental baseline BOLD value was computed (mean BOLD signal across the two 30-second periods preceding the volume transitions) and is indicated by the dashed horizontal line in each BOLD plot. Abbreviations: dACC; dorsal anterior cingulate cortex, L; liters, SMA; supplementary motor area, V_T ; tidal volume.

Table 1

lists condition mean values for tidal volume (V_T), partial pressure of carbon dioxide (P_{CO_2}), and air hunger as rated on the visual analog scale (VAS; 0–10).

	BASE	AH_{ON}	AH_{SS}	AH_{OFF}
V_T (L)	0.96 ± 0.23	0.66 ± 0.16	0.48 ± 0.08	0.86 ± 0.19
air hunger (VAS, 0–10)	1.2 ± 0.6	2.3 ± 1.0	5.7 ± 1.1	4.5 ± 1.6
PCO_2 (mmHg)	45.8 ± 4.2	46.5 ± 4.4	46.1 ± 4.2	45.1 ± 3.9

Abbreviations: AH_{ON}; air hunger on-transient, AH_{SS}; steady-state air hunger, AH_{OFF}; air hunger off-transient “relief,” BASE; steady-state respiratory comfort/experimental baseline.

lists MNI coordinates and Z-scores of local maxima for the $AH_{ON} > BASE$ contrast that met *a priori* significance criteria and fell within a 15 mm radius of findings reported in Evans et al., 2002 (Italic font). Regions are arranged in hierarchical order of Z-scores. Regions listed in the top panel were found to significant upon whole brain correction, whereas regions listed in the bottom panel were found to be significant only upon small volume correction for the anterior insula. An (×) in the “ROI” column indicates those regions with significant activation in each individual subject; selected for time-course analyses.

Table 2

Region	ROI	Side	x	y	z	Z-score	voxels
pre-SMA	×	R	3 -4	14 10	50 42	7.34	3925
middle frontal gyrus	×	R	41 46	53 48	15 12	7.11	3170
middle frontal gyrus		L	-35 -40	56 54	18 6	6.12	1142
middle frontal gyrus	×	R	53 50	11 12	36 36	5.62	477
dACC	×	L	-1 -4 2	25 24 18	36 24 32	5.53	921
anterior insula	×	L	-42 -46	13 18	-2 -8	4.75	229
	×	R	32 28	21 26	-10 -14	4.72	204
	×	R	37 32	23 28	6 6	4.54	1033

Abbreviations: dACC; dorsal anterior cingulate cortex, L; left, pre-SMA; pre-supplementary motor area, R; right.

lists MNI coordinates and Z-scores of local maxima for the $AH_{SS} > BASE$ contrast that met *a priori* significance criteria and fell within a 15 mm radius of findings reported in Evans et al., 2002 (Italic font). Regions are arranged in hierarchical order of Z-scores. Regions listed in the top panel were found to significant upon whole brain correction, whereas the region listed in the bottom panel was found to be significant only upon small volume correction for the anterior insula. An (x) in the “ROI” column indicates those regions with significant activation in each individual subject; selected for time-course analyses.

Table 3

Region	ROI	Side	x	y	z	Z-score	voxels
middle frontal gyrus	x	L	-31 -40	57 54	1 6	7.12	5174
		R	42 50	15 12	43 36	5.49	1551
dACC	x	L	-12 -4	38 32	26 20	4.81	297
		L	-2 4	4 8	63 48	5.41	394
insula/operculum	x	R	56 46	20 14	-6 -12	5.22	183
		R	23 24	2 6	55 50	4.98	182
cerebellum, semilunar (CrII)		L	-38 -38	-63 -72	-48 -42	4.79	141
cerebellum, quadrangular (VI)		L	-28 -28	-76 -70	-40 -28	4.66	173
anterior insula	x	L	-32 -46	22 18	-4 -8	3.96	58

Abbreviations: dACC; dorsal anterior cingulate cortex, L; left, pre-SMA; pre-supplementary motor area, R; right.