Dyspnea-related cues engage the prefrontal cortex - evidence from functional brain imaging in COPD

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ABSTRACT

Background: Dyspnea is the major source of disability in chronic obstructive pulmonary disease (COPD). In COPD, environmental cues (e.g. the prospect of having to climb stairs) become associated with dyspnea, and may trigger dyspnea even before physical activity commences. We hypothesised that brain activation relating to such cues would be different between COPD patients and healthy controls, reflecting greater engagement of emotional mechanisms in patients.

Methods: Using FMRI, we investigated brain responses to dyspnea-related word cues in 41 COPD patients and 40 healthy age-matched controls. We combined these findings with scores of self-report questionnaires thus linking the FMRI task with clinically relevant measures. This approach was adapted from studies in pain that enables identification of brain networks responsible for pain processing despite absence of a physical challenge.

Results: COPD patients demonstrate activation in the medial prefrontal cortex (mPFC), and anterior cingulate cortex (ACC) which correlated with the visual analogue scale (VAS) response to word cues. This activity independently correlated with patient-reported questionnaires of depression, fatigue and dyspnea vigilance. Activation in the anterior insula, lateral prefrontal cortex (lPFC) and precuneus correlated with the VAS dyspnea scale but not the questionnaires.

Conclusions: Our findings suggest that engagement of the brain's emotional circuitry is important for interpretation of dyspnea-related cues in COPD, and is influenced by depression, fatigue, and vigilance. A heightened response to salient cues is associated with increased symptom perception in chronic pain and asthma, and our findings suggest such mechanisms may be relevant in COPD.

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ABBREVIATION LIST

ACC – anterior cingulate cortex

BIS/BAS – behavioral inhibition system/behavioral activation system

COPD – chronic obstructive pulmonary disease

FEAT – FMRI expert analysis tool

FEV1 – forced expiratory volume in 1 second

FMRI – functional magnetic resonance imaging

GOLD - Global initiative for chronic obstructive lung disease

HR – heart rate

lPFC –lateral prefrontal cortex

MRC - Medical Research Council (UK)

MRI – magnetic resonance imaging

MSWT – modified shuttle walking test

mPFC – ventromedial prefrontal cortex

PFC – prefrontal cortex

SaO2 – oxygen saturation

SD – standard deviation

SGRQ – St George's Hospital Respiratory Questionnaire

VAS – visual analogue scale

INTRODUCTION

Dyspnea causes immense suffering for patients with chronic obstructive pulmonary disease (COPD). Objective measures of lung function, such as spirometry correlate poorly with dyspnea.[1] Despite dyspnea being subjective, it remains the best predictor of mortality,[2] and therefore there is a clear need to better understand its mechanisms, as this may lead to new treatments.

Contemporary models emphasize that the experience of dyspnea is strongly influenced by psychological processes, particularly depression[3-5] and dyspnea-related fear/anxiety.[5-9] Although the physical sensation of dyspnea commonly originates from sensory afferent sources, including the heart, lungs and muscles, conscious awareness of dyspnea, arises in the brain.[5-8]

Repeated association between environmental cues and dyspnea may increase the salience of such cues. For example, a ringing telephone could be associated with the need to move quickly to answer it, and thus may trigger brain anticipatory dyspnea circuitry even before physical activity commences. One way of measuring the activity in these brain circuits is with functional magnetic resonance imaging (FMRI).[10]

In this study, FMRI was used to investigate brain processes associated with the response to dyspnea-related cues in COPD. We adapted methodologies in which salient images or word-cues engage pain-processing networks in the brain despite the absence of a physical challenge[11, 12]. It is known that brain state prior to a stimulus influences its subsequent perception[13]. We hypothesised that differences in brain activation to dyspnea-related environmental cues between COPD patients and healthy controls may reflect differences in salience of these cues and changes in cognitive-affective state.

METHODS

Participants

We recruited 41 (15F, age 68.0 +/- 8.2 (SD)) patients with mild to moderate COPD (according to GOLD criteria) in the week before commencing a course of pulmonary rehabilitation, and 40 age-and-sex matched healthy controls (16F, age 69.1 +/- 8.1 (SD)). Demographics are listed in Table 1; medical details and recruitment procedures in the supplemental material. All participants gave written, informed consent. The study was approved by Oxfordshire Research Ethics Committee A (09/H0604/108).

FMRI

Imaging was performed with a Siemens 3 Tesla TIM-Trio scanner, using a 12-channel head coil. Participants underwent two FMRI scans (each 8m20s with a 30s break between) and one structural scan (Figure 1).

During scanning, participants were shown a randomised set of dyspnea-related word cues. These were designed to induce recall of everyday situations that may be associated with dyspnea in COPD patients, ranging from low to high valence (Figure 2). Participants viewed each word and rated according to how breathless and how anxious it would make them feel on a visual analogue scale (VAS, presented on screen, scale 0-100, anchors "not at all" and "very much") using a button box. To familiarise themselves with the protocol, prior to scanning, participants completed a set of 8 different test words.

Heart rate (HR), pulse oximetry (SaO2, multigas monitor, 9500, MR Equipment), respiration (chest respiratory bellows) and end-tidal partial pressures of oxygen and carbon dioxide (Datex, Normocap; nasal cannula, Salter Labs) were continuously measured throughout the scan. All physiological data were sampled at 50Hz and recorded along with scan volume triggers via PowerLab8, using Chart5 (ADinstruments).

Psychological measurements

Prior to scanning, a comprehensive assessment of respiratory perception/impact was obtained using the following self-report questionnaires: St George's Hospital Respiratory Questionnaire (SGRQ),[14] MRC dyspnea scale,[15] Dyspnoea-12 questionnaire,[16] Catastrophic Thinking Scale in Asthma,[17] and Pain Awareness and Vigilance Scale[18] (modified by substituting 'breathlessness' for the words 'asthma' and 'pain' respectively). Depression, anxiety, fatigue and demotivation are important co-existing psychological processes in COPD and were measured with the Center for Epidemiologic Studies Depression Scale,[19] State-Trait Anxiety Inventory,[20] Fatigue Severity Scale[21] and Behavioral Inhibition System/Behavioral Activation System (BIS/BAS) scale.[22]

Spirometry and exercise testing

Participants undertook spirometry, performed by a trained respiratory nurse using Association for Respiratory Technology and Physiology standards[23] and a Modified Shuttle Walking Test (MSWT), performed twice. Before, during and after the MSWT, HR and SaO2 were measured every minute using a fingertip pulse oximeter (Go2, Nonin Medial). Participants rated their dyspnea on a modified Borg scale immediately before and after the MSWT (Table E1).

Data analysis

All FMRI data processing was carried out within FEAT (FMRI Expert Analysis Tool, version5.98, FMRIB Software Library www.fmrib.ox.ac.uk/fsl), using a whole-brain approach with standard parameters.

First-level analyses used a general linear model with multiple explanatory variables which were: presentation of word cues, trial-by-trial dyspnea and anxiety ratings of word cues, random letter strings and periods when subjects were rating using the VAS. Physiological noise correction was performed using RETROICOR.[24, 25]

A multiple regression analysis (IBM SPSS Statistics) was performed upon all questionnaire scores to identify the major psychological factors contributing to the Dyspnea-12 scores (dependent variable). The following scores were identified and included as additional regressors in the higher-level analysis: state anxiety, fatigue, depression and Awareness and Vigilance Scale.

A higher (group) level analysis was performed. As fatigue, depression and vigilance are known to be major factors in dyspnea these were considered regressors of interest. As state anxiety may have been confounded by experimental factors this was considered a regressor of no interest. As we had no prior expectation of any link between these factors and breathlessness in controls, these regressors were not further interrogated in the control group.

To correct for multiple comparisons, Z statistic images were thresholded using clusters determined by $Z>2.3$ and a (corrected) cluster significance threshold of $p=0.05$ across the whole brain.[26]

We performed conjunction analyses (conjunction null) to determine common areas of mean brain activation in each group and between the questionnaire regressors in patients only. Unpaired t-tests determined between-group differences. F-tests were performed to test for shared variance between the different.

Questionnaires were scored according to their respective manuals. For spirometry, the measurement associated with the highest FEV1 was used. For MSWT, the measurement associated with the furthest distance walked was used. Data was compared using Student's t-test. Correlations between FEV1, MSWT and Dyspnoea-12 scores were assessed with Matlab (Mathworks), and p values <0.017 (Bonferroni corrected) considered significant.

A detailed description of study methods can be found in the supplemental material.

RESULTS

Participants

Table 1 presents participants' demographics, spirometry and MSWT values. Patients' FEV1 did not correlate with Dyspnoea-12 score or MSWT distance, but a negative correlation was observed between Dyspnoea-12 score and MSWT distance (Figure 3). In patients, dyspnea and anxiety VAS scores correlated with Dyspnoea-12 scores (dyspnea VAS r=0.51, p=0.0007, anxiety r=0.56, p<0.0001).

Questionnaires and VAS ratings

Patients scored significantly higher than controls on the following questionnaires: Dyspnoea-12, SGRQ, Catastrophic Thinking Scale, Awareness and Vigilance Scale, fatigue, depression, state and trait anxiety, but not BIS/BAS (Table 2). During FMRI, patients rated word cues higher for both dyspnea (mean patients:53.6+/-13.5 (SD); controls:8.4+/-10.4 (SD); p<0.001) and dyspnea-anxiety (mean patients:43.1+/-18.6 (SD); controls:5.8+/-10.8 (SD); p<0.001) compared to controls (Figure 4).

FMRI

A full list of additional activations, their co-ordinates and z scores are presented in Tables E2 and E3.

Correlation of fMRI signal with dyspnea VAS ratings, patients: Activations were observed in the medial prefrontal cortex (mPFC), anterior insula, lateral prefrontal cortex (lPFC), anterior cingulate cortex (ACC) and precuneus (Figure 4)

Correlation of fMRI signal with dyspnea VAS ratings, controls. Activations were observed in the lPFC, anterior insula, putamen, caudate, angular gyrus, supramarginal gyrus and superior frontal gyrus (Figure 4).

Correlation of fMRI signal with VAS ratings, comparison of patients and controls: Common activation was found in the left anterior insula (Figure 4). A direct group comparison revealed stronger activation in the left mPFC and the ACC in patients; the reverse contrast showed stronger activation in the supramarginal gyrus, angular gyrus, precuneus and middle frontal gyrus regions in controls (Figure 4).

Influence of depression, fatigue and vigilance upon correlation of fMRI signal with dyspnea VAS ratings, patients only:

Significant negative correlations were observed between both depression and fatigue and activatons in the mPFC, lPFC and the ACC. We observed clusters of sub-threshold positive activation at Z=2.0 for vigilance scores (Figure 5, Table E3) in the mPFC and ACC. F-tests revealed no shared variance between the contrasts. Conjunction analysis between all questionnaires and the mean activation revealed significant overlap in the mPFC and ACC (Figure 5).

Correlation of fMRI signal with anxiety VAS ratings: No significant mean effects were observed in either patients or controls. No group differences were identified.

DISCUSSION

In patients, we observed activation of the mPFC, lPFC, ACC, anterior insula and precuneus that correlated with the subjective dyspnea (VAS) response to the word cues. Furthermore, some of the variability in the brain response to these word cues is explained by measures of depression, fatigue and vigilance.

The findings in the mPFC and ACC are of particular interest as FMRI activation in these areas was stronger than in healthy controls (Figure 4). The mPFC has been linked with fear-related memory processes and emotional learning, and is a key structure that is engaged in chronic but not acute pain.[27, 28] For example, in patients with chronic back pain, acute thermal pain engages the insula, whereas spontaneous chronic pain is associated with activity in the mPFC.[27] The mPFC is considered a key component of a "chronic pain suffering" model that includes the lPFC and ACC.[29] The mPFC has not been identified in any previous FMRI studies of dyspnea, however none have examined the chronic state. Activation in the ACC has been identified in two FMRI studies of experimental dyspnea,[30, 31] although in those studies activations were not correlated with a particular psychological measure.

Depression is a well-established, major influence in dyspnea. Taking parallels from the study of pain, where depression enhances pain unpleasantness via the mPFC[32], the present data suggests in dyspnea similar mechanisms may be in play. Despite strong clinical associations with COPD, the brain mechanisms of fatigue on sensory processes remain poorly understood. Our data begin to elucidate this by showing that COPD patients' level of fatigue correlates with prefrontal activation in response to dyspnea-related cues, which might indicate that it influences emotional processing. Hypervigilance is similarly well described in chronic dyspnea[6, 9] and an important component of fear-avoidance.[33] Hypervigilance may amplify dyspnea perception by altering the way the mPFC and ACC respond to dyspnea-specific situations.

Activation was also observed in the lPFC which is known for its role in cognitive decisions

regarding reacting to potentially harmful stimuli[29]. The data presented here suggest that fatigue and depression influence the brain processing of dyspnea-cues by acting in this structure. This may represent the patients' altered evaluation of the word-cues in the context of real life experiences. The lPFC has been identified in experimentally induced acute dyspnea,[30] but this is the first time it has been linked to specific behavioural measures.

Dyspnea ratings were correlated with activation in the left anterior insula, a structure associated with interoception, the conscious awareness of bodily sensations.[34, 35] The insula has been identified in all FMRI studies of acutely induced dyspnea.[7] Although the overall insular activation to the word-cue task was stronger in patients than controls (Figure E2), the correlation between dyspnea-VAS ratings and FMRI activations was the same (Figure 4). Taken together these findings raise the question as to whether activity in the insula is dyspnea-specific or relates to more universal interoceptive processes and whether emotional engagement in dyspnea is "downstream" or separate to interoception.

The findings in healthy controls (Figure 4) identify broadly the same brain areas as in other healthy volunteer FMRI studies of dyspnea and respiratory sensation.[7, 36, 37] In the mPFC and the ACC, COPD patients demonstrate FMRI activation to these cues that is not observed in healthy controls. We propose that the findings relate to the different psychological processing of these environmental cues, with greater salience and stronger negative meaning in COPD. In the everyday life of COPD patients, these cues may be learned associations of normally innocuous scenarios with dyspnea, or simply anticipation of physical activities, which may serve to prime the brain in readiness. It is known that cues can exacerbate symptom perception in pain[38, 39], and asthma[40] by enhancing brain activity in relevant areas prior to stimulus onset[41]. The data presented here suggests that similar mechanisms of heightened responses to dyspnea-related environmental cues could serve to increase the threat value or amplify the sensations of dyspnea, making it more unpleasant or more frightening.

We further speculate that engagement of these frontal areas may either lead to reorganization of the brain's emotional circuitry (as has been proposed in chronic pain[29]) or alternatively that these circuits are simply recruited more readily, however, further research is necessary.

The interpretation of our imaging findings is supported by clinical evidence suggesting that emotional learning processes contribute to dyspnea. To characterise the mechanisms of emotional learning, and determine the effect of chronicity, longitudinal FMRI studies are necessary. These could examine either natural change in dyspnea over time or brain changes in response to an intervention. For example, dyspnea-related fear measured prior to pulmonary rehabilitation correlates with improvements in dyspnea[9] strongly suggesting the importance of emotional learning.

Limitations

This study used word cues to engage brain networks responsible for dyspnea processing. The approach may be more suited to interrogating the emotional-cognitive aspects that modulate dyspnea rather than those brain activations related to direct sensory input.[42, 43] It is worth noting that the word cue responses correlate with Dyspnoea-12 scores so are likely to represent a meaningful aspect of clinical dyspnea. Healthy controls may interpret word cues differently, reflecting a differing response to real life situations. Targeting such variation was the aim of the study. However, to compare absolute dyspnea between patients and controls, a different approach might be taken, for example adopting the paradigm of O'Donnell et al[44] or comparing word cues with similar dyspnea valence.

The activations relating to the vigilance contrast did not survive cluster thresholding at the standard threshold of $z>2.3$. Although we have less confidence in this particular finding, it would be misleading to ignore it completely. More work is needed to determine the role of vigilance in COPD.

As smoking history was strongly associated with group (i.e. patients or controls) we did not include it as a regressor in the FMRI analysis. Smoking has known effects upon the brain, but it remains unclear whether it has specific effects upon the FMRI signal. We therefore included a control task (random letter strings) which demonstrated no difference between the groups. We suggest that future work might compare brain responses in groups matched for smoking history.

Conclusions

Our findings suggest that emotional processes such as depression, fatigue and vigilance play an important role in shaping the brain mechanisms associated with interpretation of dyspnea-related cues in COPD. Heightened responses to salient cues are associated with increased symptom perception in other disorders, and our findings suggest similar mechanisms may also be relevant in COPD. Engagement of these emotion-regulating areas may contribute to the poor correlation between lung function and dyspnea severity.

Future work would look in more detail at these structures and how interventions may affect dyspnea processing. Understanding the neural processing of dyspnea in a clinical population is crucial for advances to be made in its treatment, for example the development of neuroimaging biomarkers that allow patient stratification leading to individualised treatments.

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FIGURE LEGENDS

Figure 1. FMRI task and protocol. Participants were presented with, and asked to rate, dyspnea-related word cues. Dyspnea-related word cues and ratings were each displayed for 7 seconds. Every block was separated by a fixation cross (12 seconds) and every third block was followed by random letter strings (7 seconds).

Figure 2: Mean visual analogue scales responses (VAS) in response to the question "how breathless would this make you feel" in 41 COPD patients and 40 healthy age and sex matched controls. Anchors were "not at all" and "very much". Error bars represent standard error of the mean.

Figure 3: FEV1, exercise performance and dyspnea in COPD patients. Percent predicted FEV1 plotted against Dyspnoea-12 score and distance walked on modified shuttle walking test (MSWT) for patient group. Dyspnoea-12 score plotted against distance walked on MSWT for patient group. Simple linear model representing a least-squares fit of the data (line) with confidence bounds (thin lines).

Figure 4: Activation (contrasts and conjunction) correlating with VAS ratings to dyspnea-word cues. Maps are whole-brain analysis, cluster level corrected for multiple comparisons at $p<0.05$. Maps represent conjunction analysis (activations common to both groups) and comparisons between groups, (patients>controls in red-yellow, controls>patients in blue-lightblue), and mean activations in patients and controls. Bar graph is average+/-SD dyspnea ratings for each group. mPFC (medial prefrontal cortex), ACC (anterior cingulate cortex), Ins (insula), Pcun (precuneus cortex), SMG (supramarginal gyrus), SFG (superior frontal gyrus), ACC (anterior cingulate cortex), AngG (angular gyrus), Cerebellum (CrusI and VI).

Figure 5: Activations correlating with VAS ratings to dyspnea-word cues for patients only. a) depicts mean positive activations in patients in the two slices common to b) c) d). b) c) and d) represents activations that correlate negatively with depression, negatively with fatigue and positively with vigilance respectively. Conjunction analysis represents brain areas where there is significant overlap between these activations. Maps for mean, depression, and fatigue are whole-brain analysis, cluster level corrected for multiple comparisons at $p<0.05$, with a cluster threshold of $z<2.3$, and activations are presented using standard red-yellow scaling. Activation in the vigilance contrast is presented with a cluster threshold of $z<2.0$ and activations are presented in pink scale to highlight this different statistical threshold. Maps represent mean correlation with vigilance scores in patients only. ACC (anterior cingulate cortex).

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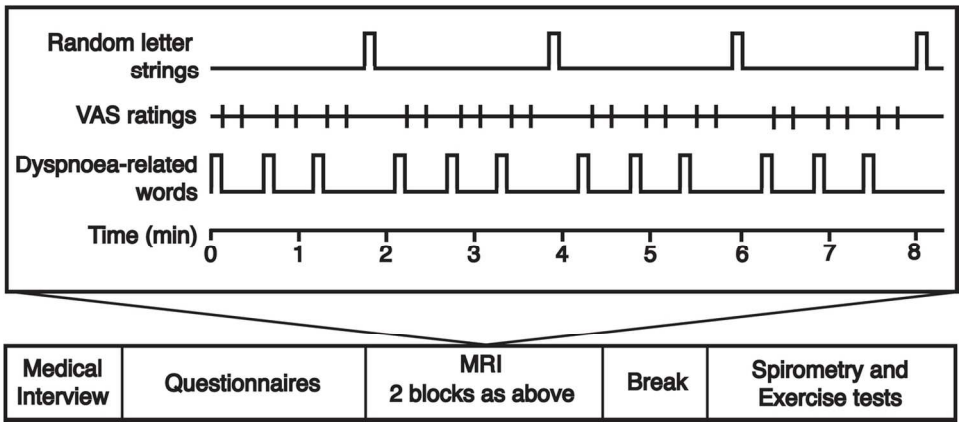

Figure 1. FMRI task and protocol. Participants were presented with, and asked to rate, dyspnea-related word cues. Dyspnea-related word cues and ratings were each displayed for 7 seconds. Every block was separated by a fixation cross (12 seconds) and every third block was followed by random letter strings (7 seconds).
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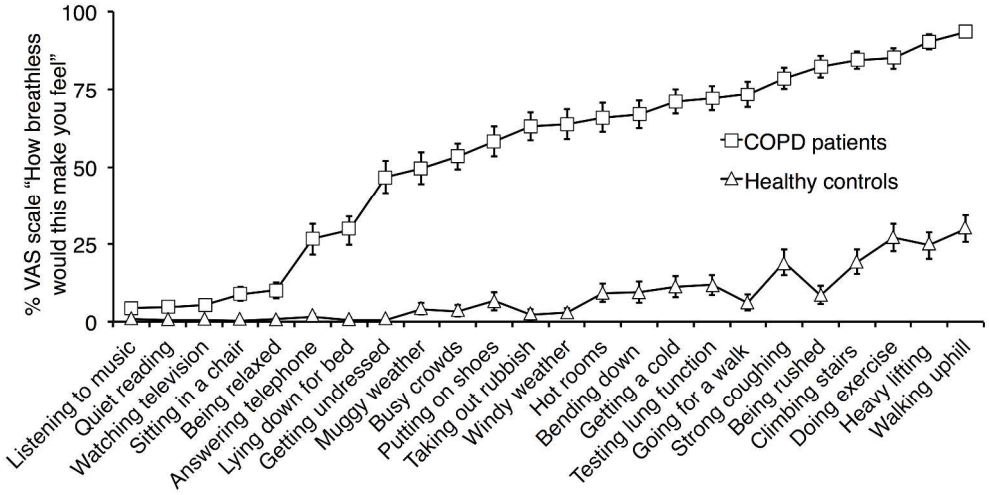

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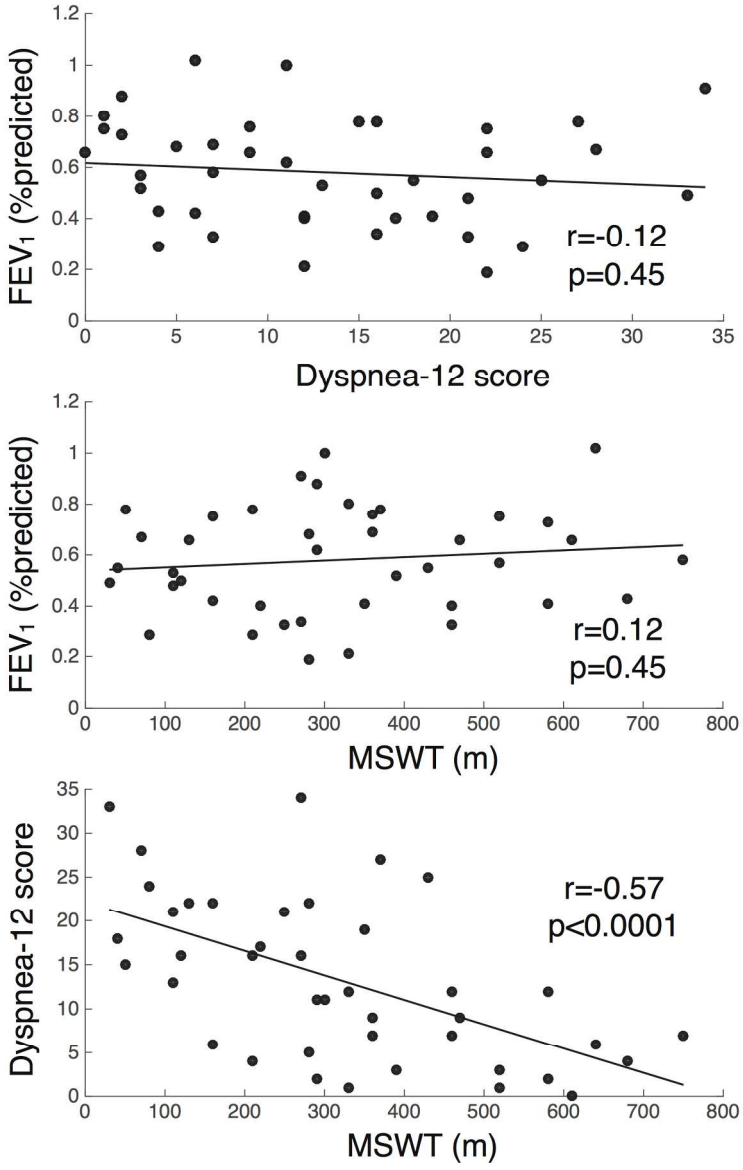

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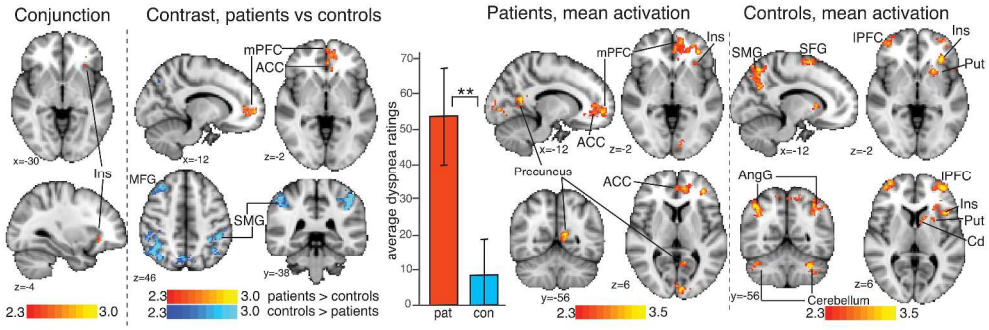

Figure 4: Activation (contrasts and conjunction) correlating with VAS ratings to dyspnea-word cues. Maps are whole-brain analysis, cluster level corrected for multiple comparisons at p<0.05. Maps represent conjunction analysis (activations common to both groups) and comparisons between groups, (patients>controls in red- yellow, controls>patients in blue-lightblue), and mean activations in patients and controls. Bar graph is average+/-SD dyspnea ratings for each group. mPFC (medial prefrontal cortex), ACC (anterior cingulate cortex), Ins (insula), Pcun (precuneus cortex), SMG (supramarginal gyrus), SFG (superior frontal gyrus), ACC (anterior cingulate cortex), AngG (angular gyrus), Cerebellum (CrusI and VI). 929x313mm (600 x 600 DPI)

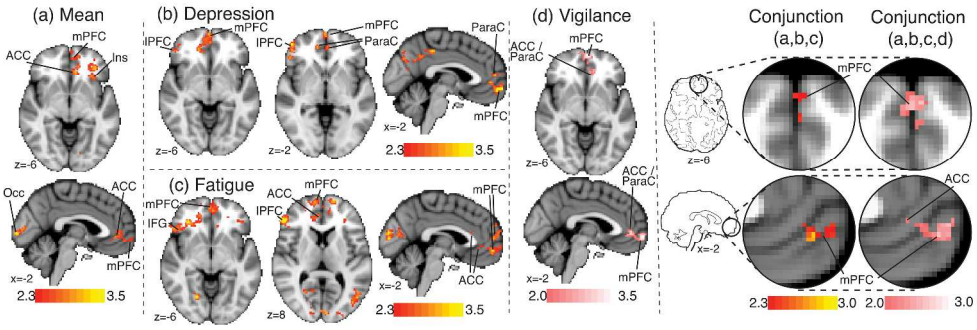

Figure 5: Activations correlating with VAS ratings to dyspnea-word cues for patients only. a) depicts mean activations in patients in the two slices common to b) c) d). b) c) and d) represents activations that are explained by patient variability in depression, fatigue and vigilance respectively. Conjunction analysis represent brain areas where there is significant overlap between these activations. Maps for mean, depression, and fatigue are whole-brain analysis, cluster level corrected for multiple comparisons

at $p<0.05$, with a cluster threshold of $z<2.3$, and activations are presented using standard red-yellow scaling. Activation in the vigilance contrast is presented with a cluster threshold of $z<2.0$ and activations are presented in pink scale to highlight this different statistical threshold. Maps represent mean correlation with vigilance scores in patients only. ACC (anterior cingulate cortex).

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Table 1: Participant details and physiological data

	Patients (mean (SD))	**Controls (mean (SD))**
Age (years)	68.0 (8.2)	69.1 (8.1)
Sex (females/males)	15/26	16/24
IMD score	12.1 (6.8)	11.7 (9.2)
BMI (kg/m^2)	28.4 (6.7)	25.2 (3.2)*
MRC score (1-5)	3 [IQR 1]	1 [IQR 0]**†
GOLD stage (0-4)	2 [IQR 1]	
Resting Borg score (1-10)	0.8 (1.1)	0.06 (0.2)**
Resting SaO2 (%)	94.4 (2.6)	96.4 (1.3)**
Resting HR (bpm)	82.8 (13.7)	72.2 (11.0)**
MSWT (distance walked, m)	320 (185)	804 (274)**
Pack years	40.3 (33.3)	3.1 (6.6)**
FEV1 (% predicted)	58 (21)	99 (24)**

*Demographic data for patients and controls (mean results and standard deviation, except for MRC score which is median and interquartile range (IQR)). Abbreviations: MRC score (Medical Research Council breathlessness score), IMD score (English Indices of Deprivation score, 2010 data), BMI (body mass index), SaO2 (blood oxygen saturation), MSWT (modified shuttle walking test), *p<0.01, ** p<0.001. †Mann-Whitney-Wilcoxon test.*

Table 2: Questionnaire scores

Questionnaire	Patients (mean (SD))	Controls (mean (SD))
Dyspnoea-12	13.2 (9.2)	0.0 (0.0)***
Physical	8.1 (5.2)	0.0 (0.0)***
Affective	5.1 (4.3)	0.0 (0.0)***
SGRQ	52.0 (17.0)	6.9 (5.1)***
Symptom	61.8 (18.0)	2.7 (2.1)***
Activity	69.7 (22.4)	3.1 (3.1)***
Impact	39.0 (18.0)	0.6 (1.4)***
Catastrophic Thinking Scale †	14.5 (12.0)	0.0 (0.2)***
Helplessness	5.6 (5.7)	0.0 (0.2)***
Magnification	4.1 (3.1)	0.0 (0.0)***
Rumination	4.9 (4.4)	0.0 (0.0)***
Awareness and Vigilance Scale ‡	41.7 (14.6)	12.9 (11.4)***
Fatigue Severity Scale	42.9 (11.0)	22.3 (12.0)***
BIS/BAS	53.6 (8.6)	54.7 (8.5)
BAS: Drive	10.0 (2.8)	10.5 (2.7)
BAS: Fun-seeking	9.2 (2.5)	9.4 (2.4)
BAS: Reward responsiveness	9.5 (2.9)	9.5 (3.1)
BIS: Inhibition	16.4 (3.8)	16.5 (3.7)
CES-D	14.8 (9.3)	7.2 (6.6)***
State anxiety	35.1 (9.9)	25.6 (7.5)***
Trait anxiety	37.6 (11.0)	29.1 (6.8)***

*Behavioural measurements in patients (n=41) and controls (n=40, mean results and standard deviation). Component scores are included where appropriate. SGRQ (St George's Respiratory Questionnaire), BIS/BAS (Behavioral Approach System/ Behavioral Inhibition System), CES-D (Center for Epidemiologic Studies Depression Scale) Modified from use in asthma †. Modified from use in pain ‡. **p<0.01, *** p<0.001.*

SUPPLEMENTARY MATERIAL

TITLE: Dyspnea-related cues engage the prefrontal cortex - evidence from functional brain imaging in COPD.

Running head: Brain processing of dyspnea in COPD

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Summary COI statement: All authors declare no conflict of interest.

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EXTENDED METHODS SECTION

Participants

We recruited 44 patients with mild to moderate COPD in the week before commencing a course of pulmonary rehabilitation, and 40 age-and-sex matched healthy controls (see Table E5 for medical questionnaire summary). Three patients who were studied were subsequently excluded from the analysis as neither the medical history obtained, nor spirometry, indicated COPD. Two of these patients had restrictive lung disease, and in the other patient the cause of dyspnea was undefined but probably cardiac.

The analysis presented therefore includes 41 patients (15F, age 68.0+/-8.2(SD)) and 40 controls (16F, age 69.1+/-8.1(SD)). See Table 1 for GOLD staging and its relation to MRC dyspnea scores. At the time of the study, a primary care diagnosis of COPD was an entry criterion for pulmonary rehabilitation. However, based on our testing in this study, four of these patients were categorised as GOLD stage 0 (i.e. FEV1>80%), these patients were included in the analysis as they had symptomatic dyspnea, had a strong history of exposure to cigarette smoke, had been referred for pulmonary rehabilitation and had no clear alternate cause for their dyspnea.

Exclusion criteria were MRI contraindications (e.g. ferrous implants), dependency on oxygen therapy, inability to complete the research tasks, diabetes, history of stroke, opioid use and epilepsy.

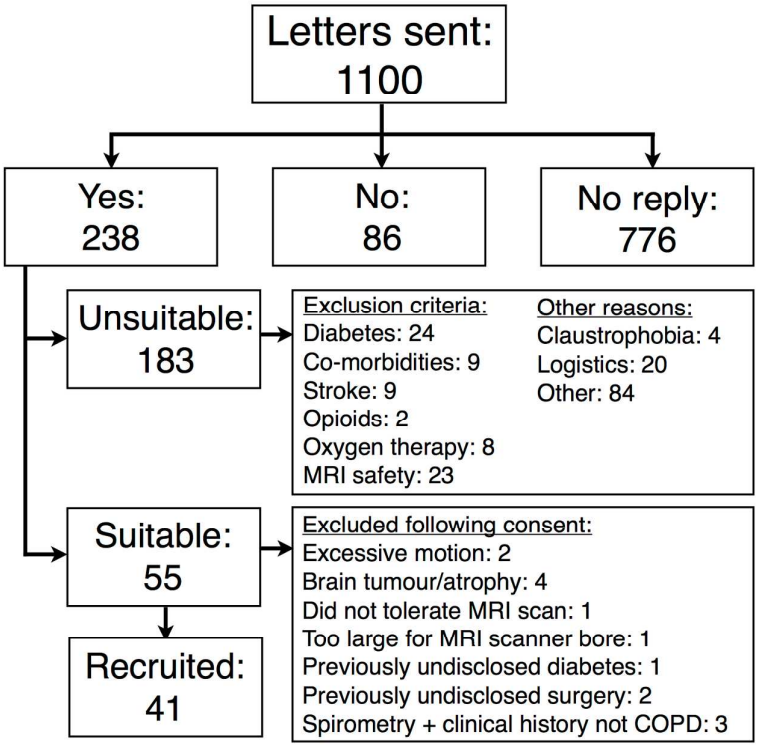

Figure E1: *Details of recruitment numbers for COPD patients and reasons for exclusion, FMRI study. MRI safety includes all MRI contraindications, such as ferrous implants, pacemakers and large tattoos. COPD patients were recruited as part of their invitation for pulmonary rehabilitation treatment via the Oxfordshire Primary Care Trust, Oxford University Hospitals NHS Trust and Oxford Health NHS Foundation Trust. Controls were recruited through poster advertisements on public noticeboards in Oxfordshire.*

FMRI

Imaging was performed at the University of Oxford Centre for Clinical Magnetic Resonance Research with a Siemens 3Tesla TIM Trio scanner, using a 12-channel head coil. Participants undertook two FMRI scans, each using a BOLD echo-planar image (EPI) acquisition (time repetition (TR)=3000ms, time echo (TE)=30ms, field of view 192x192mm, voxel size 3x3x3mm, 45 slices, 168 volumes). Each FMRI scan lasted 8 minutes and 20 seconds, with the break between scans allowing participants a chance to cough. A structural T1-weighted, whole-brain scan (MPRAGE sequence, TR=2040ms, TE=4.68ms, flip angle of 8°, voxel size 1.0x1.0x1.0mm) was obtained and used for image registration. Scans were always performed in the same order.

During the FMRI scans, participants were presented with a set of randomised dyspnea-related cues (24 in total) on a screen (white text, black background; displayed for 7 seconds). Dyspnea-related cues ranged from near-neutral (e.g. 'Sitting in a chair') to strenuous/distressing scenarios (e.g. 'Walking uphill') and were developed in a pilot trial in a separate group of COPD patients. Participants were asked to rate each cue, first according to how breathless they would feel ('How breathless would this make you feel?') and second how anxious they would feel ('How anxious would this make you feel?'), in each given scenario on a visual analogue scale (VAS) scale (range:0-100; anchors: ‘Not at all’ and ‘Very much’, 7 seconds). Ratings were always in the same order. A control task to assess potential differences in baseline BOLD responsiveness between groups was employed in the form of random letter strings which were presented after every third dyspnea-related word cue. These were not followed by any ratings and participants were instructed to ignore them. Participants were trained to do the task immediately prior to the scan, using a different set of cues. Training was repeated until participants could complete the task

reliably on their own. Participants were instructed to keep their eyes open for the full duration of the BOLD sequences.

Data analysis

All FMRI data processing was carried out within FEAT (FMRI Expert Analysis Tool) Version 5.98, part of FSL (Oxford Centre for Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library, version 5.0 www.fmrib.ox.ac.uk/fsl).

Prestatistic processing of the data included MCFLIRT motion correction,[1] removal of nonbrain structures,[2] spatial smoothing (full-width-half-maximum Gaussian kernel of 5mm) and high-pass temporal filtering (Gaussian-weighted least-squares straight line fitting, high-pass filter cut-off of 90s). First-level data were modeled (voxel-by-voxel) using FMRIB's Improved Linear Model (FILM) with local autocorrelation correction.[3] FSL Motion Outliers was used to detect timepoints in the dataset subject to large, rapid motion artefacts (such as coughs). A confound matrix was generated and used in the general linear model (GLM) to remove the effects of these timepoints. Physiological measurements of the respiratory cycle and pulse oximetry during the scan were used to account for respiratory- and cardiovascular-related noise effects, using RETROICOR,[4,5] with 4 cardiac, 4 respiratory, 2 interaction terms and respiratory-volume-time (RVT) smoothing of 15.

Images were then registered to the MNI152 standard space using an affine registration (FMRIB Linear Image Registration Tool, FLIRT) between the EPI and T1 structural scan and a nonlinear registration (FNIRT) between T1 structural scans and the MNI standard brain.

First-level analyses used a general linear model (GLM) with multiple explanatory variables (EVs) which were: presentation of word cues, trial-by-trial dyspnea and anxiety ratings of word cues, random letter strings and periods when subjects were rating using the VAS in both patients and controls. The GLM assumed a 6-second haemodynamic delay. Contrast images were calculated as appropriate and used for higher-level analyses.[6]

In ten cases, individuals rated their anxiety consistently at zero, setting contrasts involving the anxiety EV to zero in the first-level analysis. Higher-level FEAT analysis does not automatically omit contrasts that involve empty EVs but rather inserts a parameter estimate image with the value zero, which is not an accurate representation of an empty EV. Therefore, contrasts for such empty EVs were manually removed from higher-level analyses of anxiety ratings.

For all lower-level analyses, the two scans for each participant were combined using a fixed-effects model. This was done by forcing the random effects variance to zero in FLAME (FMRIB's Local Analysis of Mixed Effects).[6-8] Inputs were lower-level FEATs (two per participant). Each contrast of parameter estimates (COPE) image from this second-level analysis could then be entered into a third, higher-level analysis to examine group differences.

A higher (group) level mixed-effects analysis was performed, to compare brain activation across groups using FLAME 1+2.[7] The COPEs analysed were the presentation of dyspnea-related cues and random letter strings from the first lower-level analysis, and breathlessness and anxiety ratings of the dyspnea-related cues from the second lower-level analysis.

As fatigue, depression and vigilance are known to be major factors in dyspnea these were considered regressors of interest. As state anxiety may have been confounded by experimental factors this was considered a regressor of no interest. We had no prior expectation of any link between these factors and breathlessness in controls, and these regressors were therefore not further interrogated in the control group.

To correct for multiple comparisons, Z statistic images were thresholded using clusters determined by $Z > 2.3$ and a (corrected) cluster significance threshold of $p=0.05$ across the whole brain.[9] To detect important sub-threshold activations, the analysis was then repeated in which Z statistic images were thresholded using clusters determined by $Z > 2.0$ and a (corrected) cluster significance threshold of $p=0.05$ across the whole brain.

We performed conjunction analyses (conjunction null) to determine common areas of mean brain activation in each group and between the questionnaire regressors in patients only. F-tests examined shared variance between these questionnaire-based regressors.

To account for potentially confounding differences in psychological functioning, behavioural measures were also added to the GLM as additional regressors: State Anxiety, Fatigue Severity Scale, CES-D and Awareness and Vigilance Scale. These were identified using multiple regression analysis (IBM SPSS Statistics) with dyspnea scores obtained through the Dyspnoea-12 questionnaire as the dependent variable and other questionnaire scores as independent variables (see next section of online supplement). The Dyspnoea-12 was chosen as it is a validated, dyspnea-specific measure of respiratory symptoms in COPD. Variables with a variable inflation factor above five were excluded from the regression analysis.

We did not include pack-years as a nuisance regressor because this variable is strongly linked to group, i.e. the COPD patients had substantial smoking history and the healthy controls did not. As smoking (and COPD) has unknown effects on BOLD responsiveness, we included a control task (random letter strings) to specifically test whether the BOLD response behaved similarly in both groups (described above).

Two main group level analyses were conducted.

- Main analysis: EVs for patients and controls were modelled separately, accounting for any differences in the linearity of response between patients and controls. As fatigue, depression and vigilance are known to be major factors in dyspnea these were considered regressors of interest. As state anxiety may have been confounded by experimental factors this was considered a regressor of no interest. We had no prior expectation of any link between these factors and breathlessness in controls, these regressors were not further interrogated.
- A supplementary basic analysis (presented only in the supplementary material) was also performed in which the EVs included the presentation of dyspnea-related word cues (across the whole range from low to high valence), random letter strings and the periods when subjects were rating dyspnea (VAS scale presentation) but without VAS scores or questionnaire scores.

Conjunction analyses (conjunction null) were performed to determine common areas of brain activation across groups, and unpaired t-tests were used to determine group differences.

To assess group differences in generalised BOLD responsiveness, a region-of-interest (ROI) analysis explored between-group differences in the BOLD response to visual stimulation for random letter strings. A ROI (visual cortex, bilateral V1) mask was created using a standard atlas (Juelich Histological Atlas, thresholded at 50%) and used to identify the average signal change within this mask for all participants [10] in response to the random letter strings (visual stimulus). Student's T-test was then used to assess group differences in visual stimulus-induced BOLD response.

Identification of additional regressors for group analysis

Patients show significantly higher psychomorbidity than the general population and such psychological factors are interlinked with the dyspnea symptom. In order to address effects of psychological factors, we included selected questionnaire scores as additional regressors in the higher level analysis. To choose the most relevant behavioural measures, a multiple regression analysis was used to identify psychological factors predicting dyspnea levels (measured by the Dyspnoea-12 questionnaire, as it is a measure specific for both dyspnea and COPD). These scores were then added as additional regressors in the group analysis.

The Dyspnoea-12 questionnaire contains a total score and two sub-scores measuring physical aspects and affective aspects of dyspnea. Each score was used as a dependent variable in a separate multiple linear regression (IBM SPSS Statistics). Candidate models were fitted separately and then combined. Independent variables were behavioural measures.

Potential inter-correlations were controlled for as follows: To ensure that our set of predictors was not weakened by unacceptably high levels of intercorrelation, we first employed a cut-off level of $r>0.8$ for correlation between measures. That led to the exclusion of the trait anxiety questionnaire. We then performed a multiple regression analysis in SPSS and checked co-linearity diagnostics. Variance inflation factors (VIFs), tolerance values and condition indices were checked. VIFs over 5 were considered unacceptable, as were condition indices over 15 (paired with high variance proportions (0.5 or larger)) and tolerance of less than 0.2.

Interrogating VIFs and tolerance values examines the independent variable in relation to all other independent variables, and interaction effects are therefore taken into account. This provides a more robust exclusion parameter than simple correlations. Independents that conformed to these cut offs were dropped. The remaining independents were included in the final multiple regression analysis. None of the independents that made this final set of predictors employed VIFs, condition indices or tolerance values above the above-cited cut offs.

The prediction model for the total score of the Dyspnoea-12 questionnaire accounted for 66.0% of the variance of the score ($R2=0.674$, adjusted $R2=0.660$ and was statistically significant ($F(3,74)=50.9, p<0.01$). The model contained 3 of 6 predictors, with the primary predictor being higher values on the Awareness and Vigilance Scale (beta: 0.369) followed by higher values on the CES-D (beta: 0.386) and Fatigue Severity Scale (beta: 0.237). The prediction models for the component scores showed the following: physical component $R2=0.674$ (adjusted $R2=0.656$), $p<0.001$ ($F(4,73)=37.8$), containing 4 of 6 predictors (higher values on the Awareness and Vigilance Scale (beta: 0.442), State Anxiety Inventory

(beta: 0.171, CES-D (beta: 0.212) and Fatigue Severity Scale (beta: 0.188)); affective component R2=0.639 (adjusted R2=0.624), $p<0.001$ ($F(3,74)=43.6$) containing 3 of 6 predictors (higher values on the CES-D (beta: 0.456), Awareness and Vigilance Scale (beta: 0.268) and Fatigue Severity Scale (beta: 0.240)).

Analysis of respiratory pattern in the patient group.

A separate assessment of the respiratory data was also conducted to investigate whether COPD patients altered their breathing in response to dyspnea-related cues. Respiratory bellows systems measure the tension produced by the change in chest circumference with each breath. While chest expansion cannot account for lung expansion into the abdomen, it nevertheless provides a representation of tidal volume. Respiratory rate and chest expansion were thus calculated from the individual respiratory bellow traces and interpolated over the scan sessions (one data point per second) using custom-made Matlab scripts (Mathworks Inc., US). Respiratory rate multiplied by chest expansion did not change significantly neither between the presentation of dyspnea-related word cues and random letter strings ($p=0.98$), nor the presentation of dyspnea-related word cues and fixation crosses ($p=0.96$) in COPD patients.

Comparison of generalised BOLD responsiveness between patients and controls

Visual stimuli may be used to address differences in BOLD responsiveness between groups. BOLD FMRI relies on a relative change in paramagnetic deoxy-hemoglobin concentration. Certain conditions (e.g. hypertension, stroke) may potentially alter the coupling of cerebral blood flow and blood volume to metabolic rate, thus changing/abolishing the BOLD signal. This baseline difference in BOLD responsiveness in the patient group might be taken as a 'real' group difference in neuronal activation. At the time of writing there were only three

other published neuroimaging studies in COPD patients (none of which address respiratory sensations), and little was known about BOLD responsiveness in this patient population. As such, it was possible that neurovascular coupling could be different in COPD patients due to disease process or drugs, even when correcting for age and sex.

We would expect baseline differences in BOLD responsiveness to manifest as group differences across the whole brain rather than a subset of regions linked by function (anxiety responses). Therefore, to investigate whether changes in BOLD signal observed arose from 'real' differences in neuronal activation, we interrogated the response to random letter strings specifically in the visual cortex in both groups.

The average activation level of the voxels within the visual cortex (V1) did not differ significantly between groups (mean signal change 0.52% (SD 0.28%) in patients, 0.54% (SD 0.26%) controls p=0.69). This suggests that there was no significant between-group difference in BOLD responsiveness, or that BOLD responsiveness in COPD patients is altered to a lesser degree than is detectable by our significance criteria, and that BOLD signal changes in the present study thus reflect actual differences in neuronal activation.

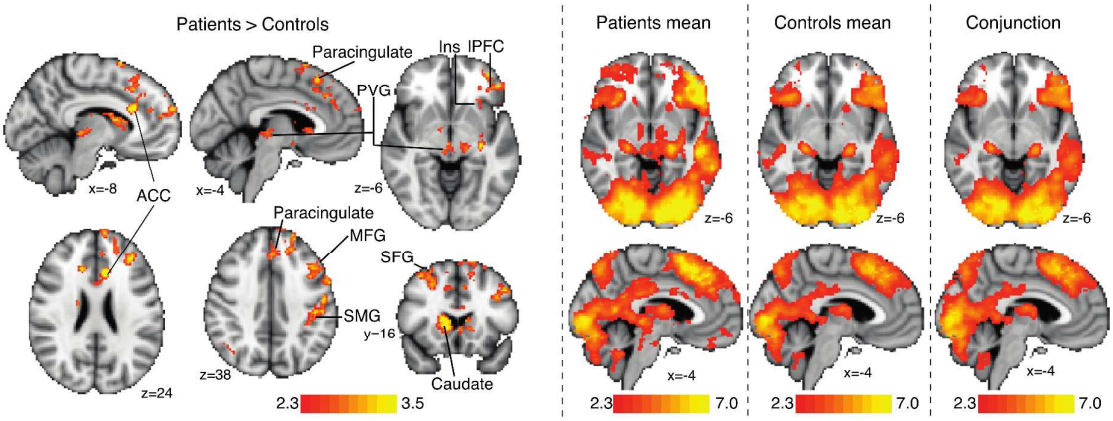

Figure E2: Activation during the presentation of words for patients and controls, not scaled by VAS ratings. Maps are whole-brain analysis, cluster level corrected for multiple comparisons at $p<0.05$. Maps represent comparison between groups (patients>controls), patient mean, controls mean, and conjunction as labelled. No activation was greater in controls than in patients. Abbreviations: ACC (anterior cingulate cortex), PVG (periventricular grey), Ins (insula), lPFC (lateral prefrontal cortex), MFG (middle frontal gyrus), SFG (superior frontal gyrus), SMG (supramarginal gyrus).

Supplementary analysis: results

Both groups exhibited bilateral activation in response to word cues in an extended network comprising illustrated above (Fig E2) and in Table E4. Compared to healthy controls, the patient group showed stronger activation in the left lateral PFC, supramarginal gyrus and insular cortex, and bilateral anterior cingulate cortex (ACC), caudate, thalamus and periventricular/periaqueductal grey (PVG/PAG). In no brain regions did healthy controls show stronger activation than patients.

Supplementary analysis: discussion

Patients demonstrated greater activation in the left dorsal and ventrolateral PFC, insular cortex, ACC, PVG/PAG and thalamus, which are areas known to be involved in anxiety,

threat and fear processing. Abnormal insular activity is observed in patients with anxiety disorders,[11] and insular activation is associated with negative emotions in experimentally-induced dyspnea.[12] The ACC[11,13] and PFC[13] are implicated in the appraisal of threat associated with pain and noxious respiratory challenges. The PVG and PAG are associated with anxiety and fight-or-flight escape behaviours.[14] The pattern also broadly reflects brain areas activated in FMRI studies of experimentally-induced dyspnea.[12] These findings are plausible in as much as patients demonstrated higher levels of generalised anxiety (STAI questionnaires) and higher levels of specific dyspnea-related anxiety (VAS recordings).

Table E1: MODIFIED BORG SCALE

0	Nothing at all
0.5	Very, very slight (just noticeable)
1	Very slight
2	Slight
3	Moderate
4	Somewhat severe
5	Severe
6	
7	Very severe
8	
9	Very, very severe (almost maximal)
10	Maximal

Table E2: FMRI activation, group interactions, Correlation of FMRI signal with VAS rating of dyspnea word cues

Contrast	Region	Peak voxel location X,Y,Z (z max)	
		Left	**Right**
Patients > Controls			
	medial prefrontal cortex	-12, 58, 0 (3.13)	
	medial prefrontal cortex	-16, 48, 2 (3.08)	
	anterior cingulate cortex	-6, 32, -6 (3.08)	
	lateral prefrontal cortex	-36, 42, -2 (3.77)	
	precuneous	-12, 58, 14 (3.72)	
Controls > Patients			
	medial frontal gyrus		36, 32, 44 (3.95)
	lateral occipital cortex		14, -70, 58 (3.42)
	angular gyrus		40, -52, 46 (3.81)
	supramarginal gyrus		54, -42, 52 (3.67)
	superior parietal lobule	-44, -40, 48 (4.14)	
	cerebellum, crus 1	-34, -60 ,-40 (3.8)	
Patients mean			
	lateral prefrontal cortex	-36, 40, -4 (4.22)	
	medial prefrontal cortex	-12, 56, -4 (3.52)	
	paracingulate	-10, 46, 6 (3.48)	
	anterior cingulate	-6, -34, 5 (2.79)	
	insula	-30, 26, 0 (2.46)	
	precuneous	-12, -58, 14 (3.72)	
	occipital pole	-4, -94, 4 (4.13)	
Controls mean			
	lateral prefrontal cortex	-38, 54, 4 (4.37)	36, 58, 2 (4.31)
	paracingulate gyrus	-6, 30, 40 (3.6)	
	frontal orbital cortex	-34, 32, 2 (4.61)	
	insula	-32, 18, -8 (2.85)	
	frontal operculum	-40, 18, 6 (3.61)	
	caudate	-14, 14, 4 (3.79)	
	putamen	-26, 10, 4 (3.18)	
	superior parietal lobule	-38, -50, 58 (4.89)	
	supramarginal gyrus		48, -40, 52 (4.33)
	superior frontal gyrus	-22, 10, 54 (4.43)	
	cerebellum crus 1	-32, -56, -34 (4.11)	34, -66, -34 (3.73)

Peak voxel locations and peak z scores (z max) during FMRI, MNI standard space.

Table E3: FMRI activation, Correlation of FMRI signal with VAS rating of dyspnea word-cues, questionnaire contrasts, patients only

Contrast	Region	Peak voxel location X,Y,Z (z max)	
		Left	**Right**
Depression			
	medial prefrontal cortex	0, 58, -10 (4.75)	
	anterior cingulate cortex		2, 36, -8 (2.77)
	paracingulate cortex	0, 52, 10 (3.39)	
	lateral prefrontal cortex		46, 52, 0 (4.09)
	supramarginal gyrus		42, -36, 38 (4.09)
	precuneous cortex	-8, -48, 38 (3.54)	
	inferior frontal gyrus		50, 10, 18 (3.84)
	supramarginal gyrus	-38, -48, 42 (3.73)	
	lateral occipital cortex		44, -72, 30 (3.46)
Fatigue			
	medial prefrontal cortex		8, 60, 12 (4.43)
	anterior cingulate cortex	0, 26, 20 (2.85)	6, 42, 14 (3.11)
	lateral prefrontal cortex		46, 40, 22 (3.73)
	inferior frontal gyrus		50, 30, -2 (4.27)
	lateral occipital cortex	-42, -76, 0 (3,58)	
	occiptial pole		18, -90, 16 (4.3)
Conjunction: mean, depression, fatigue			
	medial prefrontal cortex	-2, 54, -8 (2.87)	
	anterior cingulate cortex / paracingulate cortex	-2, 50, -4 (2.41)	
Vigilance (cluster threshold z>2.0)			
	medial prefrontal cortex	*-4, 62, -4 (3.89)*	
	anterior cingulate cortex / paracingulate cortex	*-4, 46, 0 (3.29)*	
Conjunction: mean, depression, fatigue, vigilance (cluster threshold z>2.0)			
	medial prefrontal cortex	*-2, 62, -4 (2.5)*	
	anterior cingulate cortex	*0, 44, 2 (2.2)*	

Peak voxel locations and peak z scores (z max) during FMRI, MNI standard space.

Table E4: Results of basic analysis. FMRI activation to word cues without weighting by VAS ratings or questionnaires

Contrast	Region	Peak voxel location X,Y,Z (z max)	
		Left	**Right**
Patients > Controls			
	lateral prefrontal cortex	-38, 48, -12 (4.96)	
	medial prefrontal cortex	-22, 46, 32 (3.39)	
	anterior cingulate	-8, 24, 24 (4.27)	
	caudate	-8, 8, 12 (3.62)	12, 16, 10 (4.54)
	insula / operculum	-32, 24, 6 (3.23)	
	periventricular gray		6, -26, -6 (2.89)
	thalamus	-14, -22, -2 (3.56)	8, -22, -2 (2.89)
	Angular gyrus		
	Middle frontal gyrus		30, 22, 54 (3.69)
	Superior parietal lobe	-34, -44, 62 (4.96)	
Patients and controls conjunction			
	Visual cortex		12, -88, 2 (8.07)
	frontal orbital cortex	-32, 26, 0 (7.89)	34, 26, 2 (6.41)
	cerebellar vermis		2, -54, -36 (4.9)
	middle temporal gyrus		58, -38, -10 (3.35)
	lateral geniculate body		24, 24, -4
	cerebellum crus x		22, -40, -44
	temporal fusiform cortex	-40, -14, -22 (2.78)	
	superior parietal lobe	-30, -58, 48 (6.3)	
	superior frontal gyrus	-6, 20, 52 (6.9)	
	caudate	-12, 8, 12 (5.66)	
	lateral occipital cortex		34, -66, 30 (4.62)

Peak voxel locations and peak z scores (z max) during FMRI, MNI standard space.

Table E5: MEDICAL QUESTIONNAIRE SUMMARY

A researcher-administrated structured interview was conducted by a trained respiratory nurse. The aim was to assess the participant's full medical, social and occupational history, as well as family history of respiratory disease. A summary of the main findings from this interview can be found below:

Table E4: Medical Interview Data

	COPD patients	Healthy controls
Age of COPD diagnosis (years)	59.3 (14.1) [a]	n/a
Time since last COPD exacerbation (months)	6.7(12.6) [a]	n/a
Number of exacerbations in previous year	2.1(2.1) [a]	n/a
Past medical history		
Asthma	18	3*
Bronchiectasis	3	0
Hypertension	18	6
Myocardial infarction, atrial fibrillation, angina	23	8
Osteoporosis	3	0
Irritable bowel disease, ulcers or reflux and	31	11
Depression requiring treatment	16	5
Other major psychiatric illness	0	0
Tuberculosis of the lungs	2	2
Family history of respiratory disease (parent)	16	4
Family history of respiratory disease (sibling)	4	2
Currently employed	10	12
Smoking history		
Smoker	10	0
Ex-smoker	29	19
Never smoker	2	21
Exposed to cigarette smoke at home	10	0
Exposed to cigarette smoke during childhood	37	26
Units of alcohol per week	9.2 (13.5) [a]	9.1(8.9) [a]

Values are averages (and standard deviations) †, or sums.* childhood asthma, not on any current treatment ** not formally diagnosed

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