D-cycloserine modulates breathlessness related brain activity over pulmonary rehabilitation

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Abstract

Rationale: For people with Chronic Obstructive Pulmonary Disease (COPD), improvements in breathlessness from pulmonary rehabilitation are neither long lasting nor guaranteed. Previously, we showed that pulmonary rehabilitation induced brain activity changes akin to those seen in exposure based cognitive behavioural therapies (CBT) in other conditions. D-cycloserine is a partial NMDA-receptor agonist which has been shown to enhance CBT.

Objectives: Here, we tested whether D-cycloserine would augment the effects of pulmonary rehabilitation on activity in brain areas that process breathlessness expectation.

Methods: 72 participants with mild-to-moderate COPD were recruited to a double-blind experimental medicine study running parallel to a pulmonary rehabilitation course. Participants were randomised to 250mg D-cycloserine or matched placebo, administered 15-30 minutes prior to the first four sessions of pulmonary rehabilitation. Brain functional magnetic resonance imaging, self-report questionnaires and clinical measures of respiratory function were collected at three time points: before, during (2-3 weeks) and after pulmonary rehabilitation (6-8 weeks).

Measurements: Primary and secondary outcome measures were difference in mean and voxel-wise brain activity across key brain regions of interest. An exploratory analysis determined the interaction with breathlessness-anxiety.

Main results: No difference was observed in either primary or secondary outcome measures. However, in the exploratory analysis, D-cycloserine attenuated the relationship between brain activity and breathlessness-anxiety within prefrontal cortex, superior frontal gyrus and precuneus.

Conclusions: The observed effects suggest that D-cycloserine augments pulmonary rehabilitation by dampening reactivity to breathlessness cues in brain areas associated
with breathlessness expectation and anxiety. This work highlights the opportunity to
test brain-active drugs in the context of augmenting behavioural interventions.

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behavioural therapy
Introduction

Chronic breathlessness is a central symptom of chronic obstructive pulmonary disease (COPD). Currently, pulmonary rehabilitation offers the most effective treatment strategy for chronic breathlessness in COPD, where over a 6-8 week programme, participants learn to manage their symptoms by engaging in supervised physical exercise and educational sessions. However, around 30% of patients derive no clinical benefit [1], and for those that do, health-related benefits plateau within the first 6-months following pulmonary rehabilitation, returning to pre-rehabilitation levels for the majority of patients after 12-18 months [2, 3]. Thus, strategies to enhance the therapeutic process capable of increasing or prolonging treatment effects are of considerable clinical relevance.

A body of evidence has shown that improvements in breathlessness over pulmonary rehabilitation result from a reappraisal of the sensory experience [4-7], which arrests the downward spiral of fear, avoidance and physical deconditioning [6, 8]. Clinical studies are supported by brain imaging evidence demonstrating changes in brain activity after pulmonary rehabilitation within areas including cingulate cortex, angular gyrus, insula and supramarginal gyrus which are linked to changes in ratings of breathlessness intensity and breathlessness anxiety [7]. The changes to activity within these networks, which are associated with attention and learned sensory and emotional expectations are similar to those observed during exposure-based cognitive behavioural therapies (CBT) for anxiety and panic disorder [9, 10].

In the field of psychiatry, drug interventions have been shown to enhance exposure-based CBT, successfully reducing anxiety and promoting cognitive reappraisal in some studies [11-14]. These findings suggest that targeting similar brain processes with drug interventions in chronic breathlessness may be an important focus for improving the effectiveness of pulmonary rehabilitation. One candidate drug is D-cycloserine, a
partial N-Methyl-D-Aspartate (NMDA) receptor agonist, which neuroscientific evidence suggests is associated with a reduced threat response in amygdala when paired with exposure based therapy [11]. The mechanism of effect is thought to occur at the glycine modulatory site of the NMDA receptor, where high affinity binding enhances synaptic plasticity, promoting emotional learning processes [13, 15] and boosting therapeutic effects as a result [12, 16-18].

Based on clinical and neuroscientific evidence in psychiatry and the parallels observed in pulmonary rehabilitation, we hypothesised that D-cycloserine would augment the neural responses to learned breathlessness associations connected with pulmonary rehabilitation. Neuroimaging techniques offer advantages in terms of detecting subtle changes in brain activity, which may precede clinical effects [19]; thus, an imaging study was could determine the value of proceeding to larger definitive clinical trials of brain-targeted agents to enhance pulmonary rehabilitation. Our primary hypotheses focused on brain activity changes within five key regions of interest, identified in previous studies of breathlessness [7, 20-22]. The regions of the anterior insular cortex, posterior insular cortex, anterior cingulate cortex, amygdala and hippocampus have all been linked to body and symptom perception as well as emotional salience [20, 23]. Secondary hypotheses examined the effect of D-cycloserine across a wider region of interest containing fifteen pre-defined brain areas. The fifteen brain areas encompassed regions associated with sensory and affective processing of breathlessness as well as body and symptom perception.

Since the inception of this trial, there has been an increasing number of null results from trials using D-cycloserine in combination with exposure-based CBT [24, 25]. While this trend may in part be explained by technical differences in study design, a number of well-powered and well-controlled studies have also revealed a more nuanced picture of D-cycloserine action. Several studies have shown that D-
cycloserine speeds therapeutic improvements rather than increasing them overall [14, 18], while Hofmann et al suggest that “D-cycloserine not only makes “good exposures” better but also may make “bad exposures” worse” [26]. To account for this updated literature, we conducted two additional analyses. Firstly, we examined the temporal action of D-cycloserine. Secondly, we tested whether brain activity changes relating to D-cycloserine were linked to improvements in breathlessness related anxiety during pulmonary rehabilitation.

**Methods and Materials**

An overview of the methodology is presented here. Full details, including sample size calculations, non-completion and sensitivity analysis can be found within supplementary materials. The study and statistical analysis plan were pre-registered on clinicaltrials.gov (ID: NCT01985750) prior to unblinding.

**Participants**

91 participants (30 female, median age 70 years; range 46-85 years) with COPD were recruited immediately prior to their enrolment in a National Health Service-prescribed course of pulmonary rehabilitation (full demographic information including non-continuation is shown in Supplementary material Table 1). From this population, 72 participants completed all study visits (18 female, median age 71 years (46-85 years)) (Table 1 and Figure 2). Written informed consent was obtained from all participants prior to the start of the study. Study approval was granted by South Central Oxford REC B (Ref: 118784, Ethics number: 12/SC/0713). Study inclusion criteria were: a diagnosis of COPD and admittance to pulmonary rehabilitation. Exclusion criteria were: inadequate understanding of verbal and written English, significant cardiac, psychiatric (including depression under tertiary care) or metabolic disease (including
insulin-controlled diabetes), stroke, contraindications to either D-cycloserine (including alcoholism) or magnetic resonance imaging (MRI), epilepsy, claustrophobia, regular therapy with opioid analgesics or home oxygen therapy.

Figure 1. Consort diagram illustrating stages of participant recruitment from initial screening through to completion at visit 3.
Table 1. Demographic information from the 72 participants who completed all three study visits.

Variance is expression either in terms of standard deviation (SD) or interquartile range (IQR) depending on the normality of the underlying data distribution. BMI = Body Mass Index, MRC = Medical Research Council. \( \text{SpO}_2 \% = \) Peripheral Oxygen saturation, expressed as a percentage. \( \text{FEV} = \) Forced Expiratory Volume. \( \text{FVC} = \) Forced Vital Capacity. Also listed with prevalence in brackets are recorded comorbidities ordered by frequency.

| Visit 1 | D-cycloserine | Placebo |
|---------|---------------|---------|
| (Pre-rehabilitation) | | |
| Age (median years / range) | 71.0 / (47-81) | 71.5 / (46-85) |
| Smoking pack-years (years / IQR) | 34.0 / (25.6) | 30.0 / (30.0) |
| Duration of breathlessness (years / IQR) | 8.0 (17.0) | 9.5 (10.0) |
| Total exacerbations (number / IQR) | 0.0 (1.0) | 1.0 (2.3) |
| BMI (kg.m-2 ± SD) | 27.3 ± 6.5 | 26.9 ± 5.7 |
| MRC breathlessness scale (IQR) | 3.0 (1) | 2.5 (1) |
| Resting \( \text{SpO}_2 \% \) (IQR) | 95 / (3.3) | 95 / (3.0) |
| Resting heart rate (beats.min-1 ± SD) | 80.8 ± 13.4 | 80.8 ± 14.9 |
| FEV1/FVC (IQR) | 0.53 / (0.17) | 0.56 / (0.13) |

Comorbidities (frequency)

| D-cycloserine | Placebo |
|---------------|---------|
| Asthma (14) | Asthma (11) |
| Hypertension (13) | Hypertension (11) |
| Gastro-oesophageal reflux (10) | Gastro-oesophageal reflux (12) |
| Swelling of both ankles (11) | Swelling of both ankles (8) |
| Surgery to the chest (6) | Surgery to the chest (7) |
| Depression (6) | Depression (2) |
| Diabetes (3) | Diabetes (6) |
| Heart attack (4) | Heart attack (5) |
| Bronchiectasis (3) | Bronchiectasis (4) |
| Osteoporosis (2) | Osteoporosis (4) |
| Arrhythmia (3) | Arrhythmia (4) |
| Inflammatory bowel disease (2) | Inflammatory bowel disease (3) |
| Peptic ulcer (3) | Peptic ulcer (2) |
| Heart failure (1) | Heart failure (1) |
| Tuberculosis (1) | Neuromuscular weakness (2) |
**Study drug**

Participants were randomised in a double-blinded procedure to receive either 250mg oral D-cycloserine or a matched placebo, administered by the study nurse 30 minutes prior to the onset of their first four pulmonary rehabilitation sessions. Study participants, investigators and those performing the analysis were blinded to the treatment allocation. Both D-cycloserine and placebo were over-encapsulated to appear identical. Full study drug description, randomisation protocol and minimisation criteria can be found within the supplementary materials.

**Study visit protocol**

Following telephone screening, participants were invited to attend their first research session (baseline) prior to starting pulmonary rehabilitation. A second study visit took place following the fourth pulmonary rehabilitation session but before the sixth session. Participants completed the remainder of their pulmonary rehabilitation course before attending a third study session (Figure 2) that occurred as close to the termination of pulmonary rehabilitation as possible and always within two weeks.

**Figure 2.** A schematic demonstrating order of visits, rehabilitation sessions and tablet administration throughout the study period. Participants took part in one study visit prior to their
first pulmonary rehabilitation session. Study drug/placebo were administered on four occasions over the first four rehabilitation sessions. A second study visit occurred after the final drug/placebo administration. Participants continued with their pulmonary rehabilitation course for a further four weeks before returning for a third study visit.

Self-report questionnaires:
Building on our previous work [7, 27] we selected a set of questionnaires designed to probe the experience of living with COPD, which were scored according to their respective manuals: Dyspnoea-12 (D12) Questionnaire [28], Centre for Epidemiologic Studies Depression Scale (CES-D) [29], Trait Anxiety Inventory (TRAIT) [30], Fatigue Severity Scale [31], St George’s Respiratory Questionnaire (SGRQ) [32], Medical Research Council (MRC) breathlessness scale [33], Mobility Inventory (MI) [34], Breathlessness catastrophising scale, adapted from the Catastrophic Thinking Scale in Asthma [35], Breathlessness vigilance, adapted from the Pain Awareness and Vigilance Scale [36].

Physiological measures:
Spirometry and two Modified Shuttle Walk Tests (MSWT) were collected using standard protocols [37, 38]. Participant height and weight were recorded at each visit. Oxygen saturations and heart rate were measured with pulse oximetry and were collected at rest and following the MSWT.

MRI measures
Image acquisition: Magnetic resonance imaging of the brain was carried out using a Siemens 3T MAGNETOM Trio. A T1-weighted (MPRAGE) structural scan (voxel size: 1 x 1 x 1 mm) was collected and used for registration purposes. A T2*-weighted, gradient echo planar image (EPI) scan sequence (voxel size: 3 x 3 x 3 mm), TR, 3000ms; TE 30ms was used to collect FMRI data.
**Word cue task:** To probe the neural responses of breathlessness-related expectations we examined the activity of brain regions responding to breathlessness-related word cues [7, 27, 39]. Brain activity was correlated with corresponding visual analogue ratings of breathlessness and breathlessness-anxiety collected during scanning. During the fMRI scanning, participants were presented with a word cue, e.g., “climbing stairs” in white text on a black background for 7 seconds. Participants were then asked, “how breathless would this make you feel” (wB) and “how anxious would this make you feel” (wA). To each question participants responded within a 7 second window using a button box and visual analogue scale (VAS). The response marker always initially appeared at the centre of the scale, with the anchors “Not at all” and “Very much” at either end.

**Control task:** A validated task of emotional faces was used as a control to separate generalized anxiety from breathlessness specific anxiety. Fearful or happy faces were presented on a black background was used to examine whether any differences in brain activity patterns between D-cycloserine and placebo groups was specific to breathlessness processing. Each face was shown for 500ms in blocks of 30 seconds. A fixation cross was interspersed for 30 seconds between the blocks of faces. Participants were instructed to respond via a button box to indicate facial gender. Reaction time and accuracy were recorded throughout the task.

**Outcomes**

The primary outcome comparison was brain activity difference between D-cycloserine and placebo in five pre-specified brain regions. Secondary outcomes were a voxel-wise difference in brain activity between D-cycloserine and placebo groups across one large region of interest which consisted of fifteen smaller brain regions. Exploratory
analyses examined the temporal action of D-cycloserine and updated the models used for primary and secondary outcomes to incorporate psychological variables.

**Analysis**

A summary of analyses is outlined here. Full details, including procedures for dealing with missing data and sensitivity analysis can be found within the supplementary materials. Our analysis for primary and secondary outcomes was pre-registered and made publicly available prior to unblinding (https://tinyurl.com/yxzky3p).

**Brain imaging analysis**

Image processing was carried out using the Oxford Centre for Functional Magnetic Resonance Imaging of Brain Software Library (FMRIB, Oxford, UK; FSL version 5.0.8; https://www.fmrib.ox.ac.uk/fsl/), MATLAB R2018b (Mathworks, Natick, MA), R-studio, R version 3.6.1 (2019-07-05), and associated custom scripts. Functional MRI processing was performed using FEAT (FMRI Expert Analysis Tool, within the FSL package).

Data were pre-processed according to standard protocols before being entered into single subject general linear models. These models captured brain activity during the periods in which the breathlessness-related word cues were presented allowing us to examine expectation-related processes.

**Group level analysis**

For each patient, the following metrics were extracted from each of the five regions of interest: anterior insula cortex, posterior insula cortex, anterior cingulate cortex, amygdala and hippocampus, at visits one, two and three:

1. Mean brain activity in response to breathlessness word-cue presentation
2. Mean brain activity for control task of emotional faces
To test for a drug effect across each metric, the values from visit two were entered into independent linear mixed effects models where they were adjusted for age, gender and scores at visit one. To correct for multiple comparisons across regions, permutation testing (with Family Wise Error Rate (FWE) 5%) was carried out. This process was repeated separately for data collected at visit three. Models were programmed using the lme4 function and permuco package within R version 3.6.1 (2019-07-05).

To test for a drug effect across the larger region of interest (panel B of Supplementary Figure 1), the following voxel-wise information was collected from within the region of interest at visits one, two and three:

1. Voxel-wise brain activity in response to breathlessness word-cues presentation
2. Voxel-wise brain activity in response the control task of emotional faces

Each of the values from visit two were entered into independent general linear model (GLM), controlling for age, gender and scores at visit one. Permutation testing was performed with threshold free cluster enhancement (TFCE) (a non-parametric test) [40] using FSL’s Randomise tool [41] at family wise error corrected p<0.05. The process was then repeated separately for data collected at visit three.

We repeated the primary and secondary analysis but with an additional term included in each model for the change in breathlessness related anxiety (wA). This allowed us to ask the question “Was there a difference in the relationship of brain activity and changes in breathlessness anxiety between the D-cycloserine and placebo groups?"

To answer the question “do changes to brain activity patterns occur more quickly in the D-cycloserine group” The difference between time points of each metric listed
above under secondary analysis was estimated using a form of linear regression known as the Robust Sandwich Estimator technique [42]. This implements a 2-way repeated measures ANOVA, 1 intrasubject factor of time (2 time points), 1 between subject factor (2 groups), and their interaction. Further details of this technique can be found in the supplementary materials.

Results

Of the 91 participants recruited (Figure 1), 72 participants completed all three study visits. Reasons for drop-out or exclusion included illness, scanner error and issues with data quality. One further participant was excluded due to an error in task-data collection. 71 participants were therefore assessed for study objectives. Sensitivity analysis was performed and is reported within supplementary materials.

There was no significant overall effect of D-cycloserine on mean brain activity within the five key regions of interest of anterior cingulate, anterior insula cortex, amygdala, hippocampus or posterior insula cortex (family wise error rate corrected, p>0.05) at visit two or visit three (Table 2).

Table 2. Significance of overall effect of D-cycloserine on mean brain activity within the five key regions of interest at visits two and three, having accounted for scores at visit one. Significance is reported as Family Wise Error (p<0.05) corrected p-values of the difference.

| Region of interest          | Visit two (p-value) | Visit three (p-value) |
|-----------------------------|---------------------|-----------------------|
| Anterior cingulate cortex   | 0.70                | 0.98                  |
| Anterior insula             | 0.98                | 0.98                  |
| Amygdala                    | 0.40                | 0.78                  |
| Hippocampus                 | 0.98                | 0.98                  |
| Posterior insula            | 0.98                | 0.70                  |
There was no significant overall effect of D-cycloserine across the broader mask of fifteen regions measured voxel-wise (family wise error rate correct, p>0.05) at visit two or visit three.

No significant differences in the questionnaire measures or physiology scores were found between the D-cycloserine and placebo groups at any point during the study. No differences were observed between the two groups either in breathlessness ratings (wB) or breathlessness related anxiety (wA) at visit two (both p=0.15 family wise error rate corrected) (Table 3) or visit three (both p=0.053 family wise error rate corrected) (Table 4). No significant effect of drug group was identified, using repeat measured ANOVA's, for the emotional faces control task (collected during FMRI scanning) at visit two (F(1,68) = 0.17, p=0.68) or visit three (F(1,68) = 0.001, p=0.97). Furthermore, no significant interaction between drug group and emotional valence (happy or fearful faces) was identified at visit two (F(1,68) = 0.36, p=0.55) (Table 3) or visit three (F(1,68) = 0.002, p=0.97) (Table 4).
Table 3. Scores on questionnaire, behavioural and physiological measures for drug and placebo group during (visit two). Measures are expressed as a “change score". Values are calculated as baseline visit (visit one) minus the later visit, thus positive values indicate an improvement or, in the case of the faces task, a faster reaction time (ms). Significance is reported as Family Wise Error (p<0.05) corrected p-values of the difference in scores between drug and placebo groups at visit two, accounting for scores at visit one. Values are either recorded as mean and standard deviation (1) or as median and interquartile range (2) depending on the normality of distribution. **BMI** = Body Mass Index, **MRC** = Medical Research Council clinical measure of breathlessness, **MSWT** = Modified Shuttle Walk Test, **HR** = Heart rate, **Sats** = Peripheral Oxygen saturation, expressed as a percentage

| Visit Two | D-cycloserine | Placebo | p-value |
|-----------|---------------|---------|---------|
| Catastrophising | 1.0 (5.3) ² | 2.0 (4.0) ² | 0.84 |
| Depression | 1.2 ± 6.4 ¹ | 3.2 ± 5.4 ¹ | 0.55 |
| D12 | 3.8 ± 5.4 ¹ | 2.2 ± 4.7 ¹ | 0.55 |
| Fatigue | 7.0 (12.3) ² | 4.5 (12.0) ² | 0.64 |
| BMI | -0.16 (0.6) ² | 0.0 (0.6) ² | 0.55 |
| MRC | 0 (0.5) ² | 0 (0.0) ² | 0.85 |
| MSWT Distance (m) | 0.0 (111.3) ² | 5.0 (70) ² | 0.85 |
| MSWT Borg Start | 0 (1) ² | 0 (1) ² | 0.85 |
| MSWT Borg Change | 0.5 (1.5) ² | 0.5 (2) ² | 0.85 |
| MSWT HR Start | -0.30 ± 13.9 ¹ | 1.4 ± 13.2 ¹ | 0.85 |
| MSWT HR Change | -45.4 ± 23.0 ¹ | -47.5 ± 25.4 ¹ | 0.90 |
| MSWT Stats Start | -0.30 ± 2.2 ¹ | -0.1 ± 2.2 ¹ | 0.85 |
| MSWT Stats Change | 0 (5) ³ | 0 (4.0) ³ | 0.91 |
| Vigilance | 3.8 ± 10.6 ¹ | 1.8 ± 13.6 ¹ | 0.85 |
| Spirometry | 0 (0.1) ² | 0 (0.1) ² | 0.55 |
| Avoidance – Alone | 0.1 (0.4) ² | 0.1 (0.2) ² | 0.91 |
| Avoidance -Accompanied | 0.0 (0.3) ² | 0.1 (0.2) ² | 0.55 |
| St George – Active | 6.7 (10.6) ² | 3.3 (12.8) ² | 0.55 |
| St George – Impact | 4.3 ± 7.7 ¹ | 1.5 ± 8.1 ¹ | 0.55 |
| St George – Symptom | 4.0 ± 11.5 ¹ | 4.3 ± 10.7 ¹ | 0.92 |
| Trait | 4.0 (5.5) ² | 1.0 (7.0) ² | 0.55 |
| Breathlessness Anxiety (wA) | 0.4 (29.0) ² | 5.0 (17.8) ² | 0.15 |
| Breathlessness Severity (wB) | -0.4 ± 21.3 ¹ | 9.2 ± 23.1 ¹ | 0.15 |
| Fearful Faces (ms) | 5.2 (116.5) | -13.5 (73.0) |
| Happy Faces (ms) | 12.1 (112.8) | -1.9 (106.9) |
| Main effect of drug group | F(1,68) = 0.17, p=0.68 |
| Interaction drug group:emotion | F(1,68) = 0.36, p=0.55 |
Table 4. Scores on questionnaire, behavioural and physiological measures for drug and placebo group following pulmonary rehabilitation (visit three). Measures are expressed as a “change score”. Values are calculated as baseline visit (visit one) minus the later visit, thus positive values indicate an improvement or, in the case of the faces task, a faster reaction time (ms). Significance is reported as Family Wise Error (p<0.05) corrected p-values of the difference in scores between drug and placebo groups at visit three, accounting for scores at visit one. Values are either recorded as mean and standard deviation (1) or as median and interquartile range (2) depending on the normality of distribution. BMI = Body Mass Index, MRC = Medical Research Council clinical measure of breathlessness, MSWT = Modified Shuttle Walk Test, HR = Heart rate, Sats = Peripheral Oxygen saturation, expressed as a percentage

| Visit Three          | D-cycloserine | Placebo | p-value |
|----------------------|---------------|---------|---------|
| Catastrophising      | 3.0 (7) 2     | 4.5 (8) 2 | 0.86    |
| Depression           | 3.1 ± 6.6 1   | 4.7 ± 5.3 1 | 0.86    |
| D12                  | 3.0 (8.3) 2   | 2.5 (8) 2 | 0.86    |
| Fatigue              | 9.7 ± 12.5 1  | 5.7 ± 12.0 1 | 0.86    |
| BMI                  | 0.0 (0.8) 2   | -0.1 (0.9) 2 | 0.86    |
| MRC                  | 0 (1) 2       | 0 (1) 2   | 0.86    |
| MSWT Distance (m)    | 40 (92.5) 2   | 30 (70) 2 | 0.86    |
| MSWT Borg Start      | 0 (0.5) 2     | 0 (1) 2   | 0.86    |
| MSWT Borg Change     | 0.1 ± 1.9 1   | -0.1 ± 1.6 1 | 0.86    |
| MSWT HR Start        | 2.7 ± 10.4 1  | 2.4 ± 13.5 1 | 0.98    |
| MSWT HR Change       | -51 (29.3) 2  | -53.5 (27) 2 | 0.86    |
| MSWT Stats Start     | 0.0 (2) 2     | 0.0 (3) 2  | 0.86    |
| MSWT Stats Change    | -1 (7) 2      | 0 (3) 2   | 0.86    |
| Vigilance            | 5.0 (12) 2    | 5.5 (12) 2 | 0.86    |
| Spirometry           | 0 (0.1) 2     | 0 (0.1) 2 | 0.86    |
| Avoidance – Alone    | 0.1 (0.3) 2   | 0.1 (0.3) 2 | 0.98    |
| Avoidance -Accompanied | 0.0 (0.3) 2   | 0.1 (0.3) 2 | 0.86    |
| St George – Active   | 6.0 (15.1) 2  | 6.2 (13.4) 2 | 0.86    |
| St George – Impact   | 5.1 (12.7) 2  | 5.0 (8.6) 2 | 0.86    |
| St George – Symptom  | 6.5 ± 12.5 1  | 6.4 ± 12.7 1 | 0.97    |
| Trait                | 2.7 ± 5.8 1   | 3.3 ± 7.6 1 | 0.86    |
| Breathlessness Anxiety (wA) | 3.0 (7.0) 2   | 7.3 (16.8) 2 | 0.053    |
| Breathlessness Severity (wB) | 3.1 (15.6) 2   | 8.4 (13.1) 2  | 0.053    |
| Fearful Faces (ms)   | -0.2 (70.7)   | -58.2 (88.4) |         |
| Happy Faces (ms)     | -10.5 (126.7) | -43.5 (106.4) |         |
| Main effect of drug group | F(1,68) = 0.001, p=0.97 |
| Interaction drug group:emotion | F(1,68) = 0.002, p=0.97 |

Following completion of the pulmonary rehabilitation course (visit three) we observed an interaction between changes in breathlessness anxiety, drug allocation (D-cycloserine/placebo) and brain activity (Figure 3). In the D-cycloserine group...
compared to the placebo group, for a given improvement in breathlessness anxiety there was an attenuated neural response to breathlessness cues ($z=2.3$, $p<0.05$) (Figure 4). This difference in brain activity was observed within the dorsolateral and medial prefrontal cortices, superior frontal gyrus, and precuneus. No significant relationship was observed between breathlessness related anxiety, drug allocation and brain activity at visit two.

**Figure 3.** Changes in Blood Oxygen Level Dependent (BOLD) activity that correlate with changes to ratings of breathlessness related anxiety (wA). Shown here for placebo group greater than D-cycloserine group during the presentation of breathlessness related word-cues (cluster corrected threshold of $z=2.3$, $p<0.05$). Abbreviations: dlPFC (dorsolateral prefrontal cortex), mPFC (medial prefrontal cortex/ frontal pole).
Figure 4. Pictorial representation of the relationship between drug allocation (D-cycloserine/placebo), changes to breathlessness anxiety and brain activity shown in Figure 3.

D-cycloserine was not found to affect the rate of change in brain activity for any of the regions of interest at visits two or three (Threshold Free Cluster corrEcted (TFCE) bootstrap-calculated, family wise error rate corrected p=0.68 and p=0.11 respectively).

Discussion

Key Findings

The results from the exploratory analysis give important insights into potential mechanisms of action and novel therapeutic avenues. In the D-cycloserine group, for a given change in ratings of breathlessness anxiety, there was correspondingly less brain activity in response to breathlessness cues than the placebo group. This effect was observed across a network of emotional salience regions which included superior frontal gyrus, precuneus, dorsolateral prefrontal cortex and medial prefrontal cortex. This suggests a down regulation of emotional responses to breathlessness word cues as a result of D-cycloserine in people who had derived positive change from pulmonary rehabilitation.

Pharmacological additions to pulmonary rehabilitation have already shown promise for their action on exercise capacity; oral nitrate supplementation has been shown to
improve exercise capacity over a course of pulmonary rehabilitation [43], while the long-lasting bronchodilator Tiotropium [44] also improved scores on the St Georges Respiratory Questionnaire which were sustained for three months following rehabilitation. While these studies focused on improving exercise capacity, the strong cognitive component to breathlessness suggests that the brain may be an important target that so far has been relatively ignored.

Work in social anxiety, post-traumatic stress disorder and panic disorder has shown that exposure-based CBT, which reduces anxiety and promotes reappraisal, may be enhanced by D-cycloserine [11-14]. Mechanisms of drug effect may occur via mediated activity within hippocampus, amygdala, dorsolateral prefrontal cortex and insula, regions which overlap with brain networks of internal bodily sensation (interoception) and reappraisal [11, 17, 18]. Our previous work, which examined changes in brain activity over a course of pulmonary rehabilitation, identified increased brain activity within posterior cingulate cortex, supramarginal gyrus and angular gyrus correlating with improvements to breathlessness anxiety [7]. It was hypothesised that these changes represented a shift in expectation-related brain activity towards that of healthy controls via somatosensory integration and attentional regulation. Based on these findings, we examined whether D-cycloserine would similarly augment the effect of pulmonary rehabilitation on neural responses to learned breathlessness associations.

Our primary and secondary outcomes (group mean differences in brain activity) were performed in predefined brain regions of interest. We tested for an overall group difference and observed no difference between D-cycloserine and placebo upon brain activity over the course of pulmonary rehabilitation.
Our exploratory analyses addressed more recent literature which has found D-cycloserine acts to reinforce both positive and negative experiences during exposure-based CBT [26]. This may render brain activity differences unobservable in a simple contrast of group means. Following the completion of pulmonary rehabilitation, for a given improvement in breathlessness anxiety, D-cycloserine suppressed brain activity in response to breathlessness-related word cues within superior frontal gyrus, precuneus, dorsolateral prefrontal cortex and medial prefrontal cortex compared to placebo. This interaction can be considered as a difference in slopes of the relationship between breathlessness anxiety and brain activity.

The networks targeted by D-cycloserine are associated with attentional regulation. In a previous study these networks were also shown to change over pulmonary rehabilitation [7]. However, while our previous work found co-activation within angular and supramarginal gyrus, regions associated with somatosensory integration, the current study’s co-activated networks are associated with emotional responsiveness. This finding demonstrated parallels between an earlier comparison of brain activity between patients with COPD and healthy controls, where breathlessness-related word cues elicited greater activity within medial prefrontal cortex than patients with COPD [27]. This difference was thought to reflect differences in emotional-cognitive aspects of breathlessness processing. Therefore, D-cycloserine, which we have shown here modulates the medial prefrontal cortex, may be driving activity towards that seen in healthy controls for whom the breathlessness-words hold less expectation-related significance. We speculate that our findings represent a down regulation of emotional responses to the breathlessness word cues as a result of D-cycloserine. This was particularly the case for people who had derived positive change (measured by breathlessness anxiety ratings) from pulmonary rehabilitation.

Our work has highlighted a potential brain-derived pathway of effect, for which other drugs may become more attractive candidates. As a stand-alone treatment for
treatment resistant depression, ketamine, which blocks pre-synaptic NMDA receptor signalling, increasing glutamate and thereby synaptic plasticity, has been linked with reductions in fear and anxiety [45], and rapid relief from symptoms [46]. While glucocorticoids such as cortisol, combined with exposure-based CBT, have shown promise in reduction of fear in phobias and post-traumatic stress disorder [12]. Collectively these candidate drugs boost synaptic plasticity, although via different neurochemical pathways, which may facilitate the re-setting of fearful associations within the brain. These could be used either during pulmonary rehabilitation, or as part of a precursor programme, helping to recruit harder to reach patients and support self-management. Successful self-management has been highlighted as a key objective by the department of health [47] and often follows on from CBT-based programmes such as the talking therapies recommended by the British Lung Foundation.

**Future considerations and limitations**

Our findings, which show that the action of D-cycloserine seems to relate to reductions in breathlessness anxiety, lend support to personalised approaches to treating breathlessness. We therefore suggest that future research should collect post-rehabilitation session outcome measures. Paired with post treatment outcome assessment, self-report measures of session success could affect the decision to administer drugs such as D-cycloserine. Questions remain regarding D-cycloserine’s optimum dosage, dose timing and number of administered sessions [24, 48]. We selected a dose of 250mg for this study based on its previous successful usage [11, 13] and practical considerations regarding drug availability. However, future trials may consider further investigating D-cycloserine (or alternative drug) dosage and dose timing in relation to this population of older adults who may demonstrate different responsivity to D-cycloserine given the well-established changes to NMDA receptor function as the brain ages [49]. Additionally, the action of D-cycloserine is known to be...
curtailed near the therapeutic ceiling [50], and pulmonary rehabilitation is a highly effective treatment [2], this may leave insufficient scope for improvement in some individuals. Future work may consider pairing D-cycloserine (or alternative) with a weaker behavioural intervention. Finally, given D-cycloserines’ supposed effect on learning, longer term follow-up would provide a clearer picture as to whether clinical scores were sustained for longer or whether relapse rates are affected.

**Conclusions**

We have shown evidence that D-cycloserine has the potential to augment the effects of pulmonary rehabilitation on the brain’s breathlessness perception networks. This may be extendable to other drugs working in the central nervous system. Such drugs could facilitate the creation of new, adaptive learned associations, boost mood and reduce anxiety. Future studies are needed to test other neuro-pharmacological additions to pulmonary rehabilitations and carry the translation of these findings into clinical benefit.

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References

1. Garrod, R., et al., Predictors of success and failure in pulmonary rehabilitation. European Respiratory Journal, 2006. 27(4): p. 788.
2. Haave, E. and M. Hyland, Different short-term and longitudinal results on perceived health status for asthma and COPD patients after pulmonary rehabilitation. Patients living alone have the largest improvements in perceived quality of life. Chron Respir Dis, 2008. 5(2): p. 69-73.
3. Walsh, J.R., et al., Longevity of pulmonary rehabilitation benefit for chronic obstructive pulmonary disease—health care utilisation in the subsequent 2 years. BMJ Open Respiratory Research, 2019. 6(1): p. e000500.
4. Carrieri-Kohman, V., et al., Additional evidence for the affective dimension of dyspnea in patients with COPD. Research in Nursing & Health, 2010. 33(1): p. 4-19.
5. Janssens, T., et al., Dyspnea Perception in COPD: Association Between Anxiety, Dyspnea-Related Fear, and Dyspnea in a Pulmonary Rehabilitation Program. Chest, 2011. 140(3): p. 618-625.
6. Reijnders, T., et al., The impact of disease-specific fears on outcome measures of pulmonary rehabilitation in patients with COPD. Respiratory Medicine, 2019. 146: p. 87-95.
7. Herigstad, M., et al., Treating breathlessness via the brain: changes in brain activity over a course of pulmonary rehabilitation. Eur Respir J, 2017. 50(3).
8. Corhay, J.-L., et al., Pulmonary rehabilitation and COPD: providing patients a good environment for optimizing therapy. International journal of chronic obstructive pulmonary disease, 2014. 9: p. 27-39.
9. Reinecke, A., et al., Early effects of exposure-based cognitive behaviour therapy on the neural correlates of anxiety. Translational psychiatry, 2018. 8(1): p. 225-225.
10. Reinecke, A., et al., Effective emotion regulation strategies improve fMRI and ECG markers of psychopathology in panic disorder: implications for psychological treatment action. Translational Psychiatry, 2015. 5(11): p. e673-e673.
11. Reinecke, A., et al., Neurocognitive processes in d-cycloserine augmented single-session exposure therapy for anxiety: A randomized placebo-controlled trial. Behaviour Research and Therapy, 2020. 129: p. 103607.
12. Singewald, N., et al., Pharmacology of cognitive enhancers for exposure-based therapy of fear, anxiety and trauma-related disorders. Pharmacol Ther, 2015. 149: p. 150-90.
13. Woud, M.L., et al., Investigating d-cycloserine as a potential pharmacological enhancer of an emotional bias learning procedure. J Psychopharmacol, 2018. 32(5): p. 569-577.
14. Hofmann, S.G., et al., D-Cycloserine as an augmentation strategy with cognitive-behavioral therapy for social anxiety disorder. Am J Psychiatry, 2013. 170(7): p. 751-8.
15. Davis, M., et al., Effects of D-Cycloserine on Extinction: Translation From Preclinical to Clinical Work. Biological Psychiatry, 2006. 60(4): p. 369-375.
16. Guastella, A.J., et al., *A randomized controlled trial of D-cycloserine enhancement of exposure therapy for social anxiety disorder.* Biol Psychiatry, 2008. **63**(6): p. 544-9.

17. Klass, A., et al., *D-Cycloserine facilitates extinction learning and enhances extinction-related brain activation.* Neurobiol Learn Mem, 2017. **144**: p. 235-247.

18. Nave, A.M., D.F. Tolin, and M.C. Stevens, *Exposure therapy, D-cycloserine, and functional magnetic resonance imaging in patients with snake phobia: a randomized pilot study.* J Clin Psychiatry, 2012. **73**(9): p. 1179-86.

19. Burggren, A. and J. Brown, *Imaging markers of structural and functional brain changes that precede cognitive symptoms in risk for Alzheimer’s disease.* Brain imaging and behavior, 2014. **8**(2): p. 251-261.

20. Faull, O.K., A. Hayen, and K.T.S. Pattinson, *Breathlessness and the body: Neuroimaging clues for the inferential leap.* Cortex; a journal devoted to the study of the nervous system and behavior, 2017. **95**: p. 211-221.

21. Craig, A.D., *How do you feel—now? The anterior insula and human awareness.* Nat Rev Neurosci, 2009. **10**(1): p. 59-70.

22. Rosenfield, D., et al., *Changes in Dosing and Dose Timing of D-Cycloserine Explain Its Apparent Declining Efficacy for Augmenting Exposure Therapy for Anxiety-related Disorders: An Individual Participant-data Meta-analysis.* J Anxiety Disord, 2019. **68**: p. 102149.

23. Mataix-Cols, D., et al., *D-Cycloserine Augmentation of Exposure-Based Cognitive Behavior Therapy for Anxiety, Obsessive-Compulsive, and Posttraumatic Stress Disorders: A Systematic Review and Meta-analysis of Individual Participant Data.* JAMA Psychiatry, 2017. **74**(5): p. 501-510.

24. Hofmann, S.G., *D-cycloserine for treating anxiety disorders: making good exposures better and bad exposures worse.* Depress Anxiety, 2014. **31**(3): p. 175-7.

25. Herigstad, M., et al., *Dyspnea-related cues engage the prefrontal cortex: evidence from functional brain imaging in COPD.* Chest, 2015. **148**(4): p. 953-961.

26. Yorke, J., et al., *Quantification of dyspnoea using descriptors: development and initial testing of the Dyspnoea-12.* 2010. **65**(1): p. 21-26.

27. Spielberger, C.D., *State-Trait Anxiety Inventory,* in The Corsini Encyclopedia of Psychology. 2010.

28. Radloff, L.S., *The CES-D Scale:A Self-Report Depression Scale for Research in the General Population.* 1977. **1**(3): p. 385-401.

29. Krupp, L.B., et al., *The fatigue severity scale: Application to patients with multiple sclerosis and systemic lupus erythematosus.* Archives of Neurology, 1989. **46**(10): p. 1121-1123.

30. Jones, P.W., et al., *A self-complete measure of health status for chronic airflow limitation.* The St. George’s Respiratory Questionnaire. Am Rev Respir Dis, 1992. **145**(6): p. 1321-7.
33. Bestall, J.C., et al., Usefulness of the Medical Research Council (MRC) dyspnoea scale as a measure of disability in patients with chronic obstructive pulmonary disease. Thorax, 1999. 54(7): p. 581-586.
34. Chambless, D.L., et al., The Mobility Inventory for Agoraphobia. Behav Res Ther, 1985. 23(1): p. 35-44.
35. De Peuter, S., et al., Illness-specific catastrophic thinking and overperception in asthma. Health Psychol, 2008. 27(1): p. 93-9.
36. McCracken, L.M., K.E. Vowles, and C. Eccleston, Acceptance-based treatment for persons with complex, long standing chronic pain: a preliminary analysis of treatment outcome in comparison to a waiting phase. Behav Res Ther, 2005. 43(10): p. 1335-46.
37. Bradley, J., et al., Validity of a modified shuttle test in adult cystic fibrosis. Thorax, 1999. 54(5): p. 437-9.
38. Levy, M.L., et al., Diagnostic spirometry in primary care: Proposed standards for general practice compliant with American Thoracic Society and European Respiratory Society recommendations: a General Practice Airways Group (GPIAG)1 document, in association with the Association for Respiratory Technology & Physiology (ARTP)2 and Education for Health3 www.gpiag.org 2 www.artp.org 3 www.educationforhealth.org.uk. Prim Care Respir J, 2009. 18(3): p. 130-47.
39. Herigstad, M., et al., Development of a dyspnoea word cue set for studies of emotional processing in COPD. Respiratory physiology & neurobiology, 2016. 223: p. 37-42.
40. Nichols, T.E. and A.P. Holmes, Nonparametric permutation tests for functional neuroimaging: A primer with examples. Human Brain Mapping, 2002. 15(1): p. 1-25.
41. Winkler, A.M., et al., Permutation inference for the general linear model. Neuroimage, 2014. 92: p. 381-97.
42. Guillaume, B., et al., Fast and accurate modelling of longitudinal and repeated measures neuroimaging data. Neuroimage, 2014. 94: p. 287-302.
43. Pavitt, M.J., et al., Oral nitrate supplementation to enhance pulmonary rehabilitation in COPD: ON-EPIC a multicentre, double-blind, placebo-controlled, randomised parallel group study. Thorax, 2020. 75(7): p. 547.
44. Casaburi, R., et al., Improvement in exercise tolerance with the combination of tiotropium and pulmonary rehabilitation in patients with COPD. Chest, 2005. 127(3): p. 809-17.
45. Glue, P., et al., Effects of ketamine in patients with treatment-refractory generalized anxiety and social anxiety disorders: Exploratory double-blind psychoactive-controlled replication study. J Psychopharmacol, 2020. 34(3): p. 267-272.
46. Berman, R.M., et al., Antidepressant effects of ketamine in depressed patients. Biol Psychiatry, 2000. 47(4): p. 351-4.
47. Health, D.o., An Outcomes Strategy for Chronic Obstructive Pulmonary Disease (COPD) and Asthma. 2011.
48. Hofmann, S.G., et al., Dose timing of D-cycloserine to augment cognitive behavioral therapy for social anxiety: Study design and rationale. Contemporary clinical trials, 2015. 43: p. 223-230.
49. Kumar, A. and T.C. Foster, *Alteration in NMDA Receptor Mediated Glutamatergic Neurotransmission in the Hippocampus During Senescence*. Neurochemical Research, 2019. **44**(1): p. 38-48.

50. Choi, D.C., et al., *Pharmacological Enhancement of Behavioral Therapy: Focus on Posttraumatic Stress Disorder*, in *Behavioral Neurobiology of Anxiety and Its Treatment*, M.B. Stein and T. Steckler, Editors. 2010, Springer Berlin Heidelberg: Berlin, Heidelberg. p. 279-299.
Supplementary Material

D-cycloserine modulates breathlessness related brain activity over pulmonary rehabilitation

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Online Data Supplement
Table 1. Demographic information from the 91 participants who were randomised. Variance is expression either in terms of standard deviation (SD) or interquartile range (IQR). BMI = Body Mass Index, MRC = Medical Research Council clinical measure of breathlessness. SpO₂% = Peripheral Oxygen saturation, expressed as a percentage.

| Visit 1 (N=91) | Total | D-cycloserine | Placebo |
|---------------|-------|---------------|---------|
| Age (median years/range) | 70 / (46-85) | 70 / (47-81) | 71.5 / (46-85) |
| Smoking pack-years (IQR) | 30 / (28.5) | 30 / (25.1) | 30 / (31.0) |
| BMI kg.m⁻² ± SD | 27.5 ± 5.4 | 26.9 ± 5.5 | 28.1 ± 5.3 |
| MRC (IQR) | 3 (1) | 3 (1) | 3 (1) |
| Resting SpO₂% (IQR) | 95 / (3.0) | 95 / (3.8) | 95 / (3.0) |
| Resting heart rate beats.min⁻¹ ± SD | 81.7 ± 13.9 | 82.3 ± 13.4 | 81.0 ± 14.6 |
| FEV₁/FVC (IQR) | 0.55 / (0.21) | 0.54 / (0.24) | 0.58 / (0.11) |

Study Drug

Study drugs were purchased from Ipswich Hospital Pharmacy Manufacturing Unit, Heath Road, Ipswich IP4 5PD, Tel: 01473 703603. Participants were administered with 250mg of D-cycloserine. While 50mg remains the most common dosage for D-cycloserine, there is no evidence to suggest that 50mg is more effective than higher dosages [1]. Furthermore, work conducted in healthy volunteers suggests that hippocampal learning only occurred at 250mg dose and not at 50mg [2]. The efficacy of 250mg dosage on brain plasticity is supported by more recent work [3] which found changes to amygdala reactivity after a single administration of D-cycloserine.

Randomisation Procedure

Once the participant gave written consent to the trial and completed the MRI scan, a member of the team submitted a randomisation form, entering eligibility criteria and
minimisation factors. Allocation to active or placebo capsules, which were both over-encapsulated to appear identical, was carried out by Sealed Envelope Randomisation Services (Sealed Envelope Ltd, Concorde House, Grenville Place, London NW7 3SA). The randomisation number was then provided to the Oxford Respiratory Trials Unit who dispensed the drug/placebo. Minimisation factors were as follows:

1. Centre
2. MRC grade
3. Diabetes
4. Antidepressant
5. Age at which the participant completed full time education
6. Previous rehabilitation

Randomisation codes were held by Sealed Envelope until study completion, after which at the first stage of unblinding an independent researcher provided study researchers with a coded binarised system for analysis. Researchers remained blinded to group identity until analysis was completed.

**Pulmonary Rehabilitation**

Pulmonary rehabilitation courses were run by either Oxford Health NHS Foundation Trust, West Berkshire NHS Foundation Trust, or Milton Keynes University Hospitals NHS Trust

**Sample Size**

At the time of study inception (and to a large extent still to date), the literature regarding D-cycloserine’s effects on functional brain activity is very limited. Therefore, in order to calculate the sample sizes required for this study we first took into account the described effects of d-cycloserine in clinical studies of augmentation for cognitive behavioural therapy for anxiety disorders, where effect sizes of up to 1.06 have been reported (although more commonly 0.4 to 0.7) [4-7]. The most relevant paper (on
treatment of snake phobia [8]) demonstrated that effects observed with neuroimaging were more sensitive than behavioural effects, therefore we believe that powering for a behavioural outcome measure (breathlessness-anxiety) provided a safe margin and was likely to be sufficiently conservative to detect our measures of interest. This was particularly the case as compared to the relatively blunt nature of behavioural data collection, functional neuroimaging carries considerably more specificity and statistical power. The study was not therefore specifically powered to investigate the clinical effects of D-cycloserine. In our previous study we observed an 11% (SD15%) improvement in breathlessness-related anxiety, measured with our FMRI word task (pre-treatment mean score 38%, post treatment mean score 27%, difference 11%, SD of difference 15%) [9]. Making a conservative assumption, we estimated that D-cycloserine augments this response with an effect size of 0.4. Assuming a similar coefficient of variation we anticipated an 18% (SD24%) improvement in breathlessness-anxiety (i.e. pre-treatment mean score 38%, post treatment mean score 20%, difference 18%, SD of difference 24%). Assuming α=0.05 and power 0.80, then we estimated a sample size of 36 in each group randomised 1:1. As this is a behavioural outcome, we expected this to have sufficient power to detect change in BOLD signalling.

Missing Data

The potential effect of missing brain imaging data was explored using a sensitivity analysis. Missing questionnaire and physiology data points were imputed using the Markov chain Monte Carlo method (multiple imputation technique) within the MICE package in R. A summary of missing data is provided below.

Behavioural Measures
**Questionnaire Measures**

**Dyspnoea-12 (D12) Questionnaire:** This is a 12-item questionnaire designed to measure the severity of breathlessness and has been validated for use in patients with respiratory disease [10].

**Centre for Epidemiologic Studies Depression Scale (CES-D):** Depressive symptoms are commonly observed in patients with respiratory disease. This brief questionnaire consists of 20 items investigates the symptoms of depression across a number of factors [11].

**State-Trait Anxiety Inventory (STAIT-T):** This questionnaire assesses participant’s general level of anxiety in particular scenarios via 20 questions asking “how anxious you generally feel” [12].

**Fatigue Severity Scale:** This 9-point questionnaire quantifies patient fatigue, which is well documented in its association with COPD [13].

**St George’s Respiratory Questionnaire (SGRQ):** There are 50 questions in this questionnaire, which has been developed and validated for use in COPD and asthma. The questions measure the impact of overall health, daily life and well-being [14].

**Medical Research Council (MRC) breathlessness scale:** The MRC scale quantifies perceived difficulty due to respiratory restrictions on a scale of 1 to 5 [15].

**Mobility Inventory (MI):** This questionnaire collects data regarding the extent to which a participant avoids certain situations, either alone or accompanied (21-items in each category) [16].

**Breathlessness Catastrophising Scale – adapted from the catastrophic thinking scale in asthma:** This 13-point questionnaire was modified for this study by substituting the word “asthma” for “breathlessness” in order to measure catastrophic thinking [17][18].

**Breathlessness Vigilance Scale – adapted from the pain awareness and vigilance scale:** This questionnaire was modified by substituting the word “breathlessness” for
the word “pain”. The 16-point scale measures how much a participant focuses their attention onto their breathlessness [19] [18].

Physiological Measures
A trained respiratory nurse collected spirometry measures of FEV₁ and FVC using Association for Respiratory Technology and Physiology standards [20]. Participants performed two modified incremental shuttle walk tests (MSWT) [21], and heart rate and oxygen saturations (SpO₂) were measured immediately before the MSWT and subsequently every minute until 10 minutes post-exercise (or until participants returned to their baseline state) using a fingertip pulse oximeter (Go2; Nonin Medical Inc). Before and after the MWST participants also rated their breathlessness on a modified Borg scale [22]. In a MWST participants must walk between and around two cones, placed 10m apart in time to a set of auditory beeps played from a laptop. Initially the speed of beep repetition is slow, but the participant must increase their walking speed each minute in order to reach the cone before the next beep. Participants continue to walk (or run) until they are too breathless to continue, at which point the total distance walked is recorded.

MRI Acquisition
Prior to each MRI session participants were screened for standard MRI contraindications including metal in or about their person, epilepsy and claustrophobia.

**Image acquisition:**
Hardware: A Tim System (Siemens Healthcare GmbH) 12-channel head coil.
T1 sequence parameters: TR, 2040ms; TE, 4.68ms; voxel size, 1 x 1 x 1 mm; FOV, 200mm; flip angle, 8°; inversion time, 900ms; bandwidth 130 Hz/Px).
T2*-weighted (functional) sequence parameters: TR, 3000ms; TE 30ms; voxel size 3 x 3 x 3 mm; FOV, 192mm; flip angle 87°; echo spacing 0.49ms.

Functional scan durations: word-task - 215 volumes, 10 minutes and 27 seconds duration and faces task - 168 volumes, 8 minutes and 24 seconds duration.

Field map scans of the B₀ field were obtained to aid the distortion correction of the functional scans: TR, 488ms; TE1, 5.19ms; TE2, 7.65ms; flip angle 60°; voxel size, 3.5 x 3.5 x 3.5 mm.

Word Task

This task was developed and published by Herigstad and colleagues in 2016 for use in the COPD population [23]. Word cues were developed in three key stages; firstly in collaboration with respiratory practitioners, academics and physiotherapists, a set of 30 word cues associated with breathlessness were created. Next, these cues were provided to patients with COPD alongside a VAS rating scale, allowing patients to rate how breathless and anxious the situations identified by the cues would make them feel. Following adjustments based on participant feedback, the word cues were then computerised and tested in a larger population of COPD patients [23]. Further validation was carried out in the fMRI environment and by for clinical sensitivity with comparisons between changes in key questionnaire measures and word-cue rating.

Before the first scan session, participants were given the opportunity to practice using the button box with a set of test words.

Symptom perception has been shown to be modulated by expectations [24-26]. Even in healthy volunteers, a cue paired with the expectation of a breathlessness-inducing situation can produce breathlessness and drive brain activity patterns in absence of afferent input [24]. When expectation incorrectly matches afferent inputs, maladaptive breathlessness can occur, and in populations with chronic symptoms of breathlessness the relationship between expectation and breathlessness-cues can become deeply reinforced. As a result, breathlessness-expectation is one target for
the CBT elements of pulmonary rehabilitations, and the most likely point of action for D-cycloserine.

Control tasks

1) A control condition, used as a baseline measure of activity in response to the presentation of a visual stimulus was presented 4 times over the course of the word-cue scan, consisting of a string of “XXXXXXXXXXXXXXXX” with fixed length of 15 characters, and each time was presented for 7 seconds. No rating period followed these control blocks [9, 18, 23].

2) A validated task of emotional faces was used as a control to separate generalized anxiety from breathlessness specific anxiety. Emotional facial expressions are widely recognised to activate the same brain pathways as the behavioural emotion conveyed by the expression itself. Fearful facial expressions, for example, have been shown to correspond to activity within the amygdala, a region known to modulate fear processing [26]. Faces were drawn from a set first developed by Ekman and Friesen [27] and furthered by Young et al [28]. Photographs of 10 faces (5 male, 5 female) with fearful or happy expressions of 100% intensity were used. Each face was shown for 500ms in blocks of 30 seconds. A fixation cross was interspersed for 30 seconds between the blocks of faces. Participants were instructed to respond via a button box to indicate facial gender. Reaction time and accuracy were recorded throughout the task. The task contrasting fear and happy facial expressions has been extensively used in previously studies and has been found to activate the amygdala in both healthy volunteers and in depressed patients [29, 30]. Neutral faces are not typically used as they can be interpreted as threatening or ambiguous in different settings [31].

Imaging Analysis

Functional MRI Preprocessing

Data denoising was carried out as follows: Before the first level analysis, each
functional scan was decomposed into maximally independent components using FMRIB’s MELODIC tool (Multivariate Exploratory Linear Optimised Decomposition into Independent Components). “Noise” components were identified by FIX (FMRIB’s auto-classification tool, [32, 33]) using the WhII.Standard.RData [34] trained classifier with aggressive clean up option. A Principal Component Analysis (PCA) was run on the FIX identified components to retrain 99% of the variance. Separately, the cardiac and respiratory related physiological signals (recorded via a pulse oximeter and respiratory bellows) were transformed into a series of regressors, (three cardiac and four respiratory harmonics) as well as an interaction term and a measure of respiratory volume per unit of time (RVT), using FSL’s physiological noise modelling tool (PNM). The signal associated with these waveforms (modelled using retrospective image correction (RETROICOR) [35, 36]) was then used to form voxelwise noise regressors.

The confounds identified by FSL’s FIX and PNM tools, along with sources of noise arising from motion, were then combined into a single model. This single noise model approach builds upon the technique outlined by [37]; and fully detailed by [38]. In these preceding works we employed a step-wise technique whereby physiological noise (identified by PNM) and FIX-identified noise were each removed from the data in separate steps prior to data entry into the lower level model. In the new cleanup pipeline, a single text file containing time-course information relating to FIX identified noise components along with white matter or CSF related noise was included as additional confound EV’s within the lower level model, while the PNM-identified noise was entered into the model as a standard voxel-wise confound list. In this updated denoising pipeline, confounds identified above are added to model at the stage of first-level analysis and thus the functional dataset can be corrected for sources of noise arising from motion, scanner and cerebro-spinal fluid artefacts, cardiac, and respiratory noise in a single step, rather than three.
**Functional MRI Analysis**

MRI processing was performed using FEAT (FMRI Expert Analysis Tool within the FSL package). The data were corrected for movement using MCFLIRT (Motion correction using FMRIB’s Linear Image Registration Tool [39]). Non-brain structures were removed using BET (Brain Extraction Tool [40]). Spatial smoothing was carried out using a full-width-half-maximum Gaussian kernel of 5mm, while high-pass temporal filtering (Gaussian-weighted least squares straight line fitting; 90 s) removed low frequency noise and slow-drift. Distortion correct of EPI data was carried out using a combination of FUGUE (FMRIB's Utility for Geometrically Unwarping EPI's [41, 42] and BBR (Boundary Based Registration; part of the FMR Expert Analysis Tool, FEAT version 6.0 [43]). The data were corrected for physiological noise using FSL's FIX-PNM pipeline. Functional scans were registered in a two-step process to the MNI152 (1x1x1 mm) standard space brain template. Firstly, each subject’s EPI was registered to their associated T1-weighted structural image using BBR (6 DOF) with nonlinear field map distortion correction [43]. In the second step the subject’s structural image was registered to 1mm standard space via an affine transformation followed by nonlinear registration (using FNIRT: FMRIB’s Non-linear Registration Tool [44]).

**Region of interest extraction**

The five bilateral regions of interest (ROI) were anterior insula cortex, posterior insula cortex, anterior cingulate cortex, amygdala and hippocampus. Seed voxels for each region of interest were identified as the peak voxel co-ordinates (Supplementary table 2) responding to breathlessness word-cues published by Herigstad et al 2017 [9] within the boundaries of each region of interest identified by standard atlas maps. The seed voxels were expanded to include the surrounding voxels within a 5 mm radius. Left and right masks of bilateral regions of interests (anterior insula, posterior insula, anterior cingulate, amygdala and hippocampus) were added together to form one
mask for each region of interest. Following registration each mask was re-thresholded at 40% probability to avoid interpolation errors before being binarised.

**Table 2. MNI coordinates for region of interest seeds**

| Region of interest       | Hemisphere | x   | y  | z  |
|--------------------------|------------|-----|----|----|
| Anterior insula cortex   | Left       | -31 | 7  | -14|
|                          | Right      | 38  | 13 | -10|
| Posterior insula cortex  | Left       | -31 | 7  | -15|
|                          | Right      | 37  | 10 | -12|
| Anterior cingulate cortex|            | -5  | 34 | -3 |
| Amygdala                 | Left       | -19 | -7 | -21|
|                          | Right      | 19  | -8 | -18|
| Hippocampus              | Left       | -22 | -12| -26|
|                          | Right      | 20  | -9 | -19|

**Network mask**

A second network mask region of interest was created from the 5 core regions of interest outlined above and an additional 11 regions defined by standard anatomical atlas maps (Harvard-Oxford Atlas and Destrieux' cortical atlas) (Supplementary Figure 1). A 40% probability threshold was applied to each region, before they were combined along with the original 5 regions into one network mask. This network mask was then registered to each individual before being re-thresholded at 40% probability to avoid interpolation errors and binarized. Combining the 16 regions into a single mask enabled us to appropriately correct for multiple comparisons.
Figure 1. Panel A highlights the 5 key regions of interest while Panel B shows the expanded region of interest map.

Word cue task

At the individual subject level, a general linear model (GLM) was created with explanatory variables (EVs) for the 7 seconds of word or non-word presentation, and two (7 second) de-meaned EVs modeling the reported breathlessness and anxiety response to the word cues. An additional explanatory noise variable was included to model the period during which the participant responded using the visual analog scale (VAS).
Control task

At the individual subject level, a GLM was created with explanatory variables for the 30 second stimulus presentation periods of happy and fearful faces, along with the associated (de-meaned) reaction times. Two additional explanatory variables were created to model participant (de-meaned) accuracy in identifying whether the presented faces were male or female.

Supplementary Figure 2 – An illustration of the generalised linear models (GLM) used for both lower and higher level analyses for the word and faces task. Abbreviations as follows – wB – breathlessness rating, wA – breathlessness anxiety rating, RT – reaction time, ACC – accuracy.
**Statistical Analysis**

Robust Sandwich Estimation: This method first estimates the parameters of interest using a simple Ordinary Least Square model before estimating the variances and covariances with the Sandwich Estimator (SwE). The SwE provides an estimate for standard errors that accounts for repeated measures correlations. When applied to the rate of change analysis the exact outcome is therefore the difference in slopes, i.e. difference between drug and placebo groups in the BOLD change per visit slope. A wild bootstrap was used to obtain voxelwise family error results within the pre-specified analysis mask.

**Sensitivity Analysis**

\[ \Delta = \Delta_{CC} + Y_1 P_1 - Y_2 P_2 \]

**Equation 1.** Formula for calculating the outcome of a range of missing not at random scenarios. Where delta is the treatment effect under the missing-not-at-random. Delta_{CC} is the treatment effect under a complete case scenario. Y_1 and Y_2 are the assumed mean responses for patients with missing data in treatment groups 1 and 2 respectively. P_1 and P_2 are the proportion of patients missing in groups 1 and 2 respectively.

Sensitivity analysis essentially asks the question “if all of the participants who were enrolled but did not complete the study actually had, would the result of the primary analysis have changed?” To answer this, a number of different brain activity levels are simulated to account for reasonable extreme scenarios.

Using Equation 1, three quantiles of brain activity within each of the five key regions of interest in response to breathlessness-related word cues were calculated for
participants in treatment group 1. This corresponded to 25%, 50% and 75% of activity observed within the group of participants who completed all three visits.

The standard error of delta is approximately equal to the standard error for delta_{cc}. Y_1 will be varied between Y_2 – (5%) and Y_2 + (5%). These values were multiplied by the proportion of missing participants from each group. The simulated complete datasets were entered into linear mixed effects models where they were adjusted for age and gender. To correct to multiple comparisons across regions, permutation testing (with Family Wise Error Rate (FWE) 5%) was carried out.

Results

Behavioural

Missing data

Table 3. Missing data reported as the total percentage of data collected for that measure.

|                  | Pre-rehabilitation | During rehabilitation | Following rehabilitation |
|------------------|--------------------|------------------------|-------------------------|
|                  | D-cycloserine | Placebo | D-cycloserine | Placebo | D-cycloserine | Placebo |
| **MSWT**         |                |          |                |          |                |          |
| Distance         | 11             | 9        | 14             | 3        | 14             | 9        |
| Borg change      | 11             | 9        | 14             | 3        | 14             | 9        |
| **HR change**    |                |          |                |          |                |          |
| Sats change      | 11             | 9        | 14             | 3        | 14             | 9        |
| **BMI**          | -              | -        | 8              | 3        | 30             | 21       |
| **MRC**          | -              | -        | -              | -        | 3              | 3        |

*MSWT* – modified shuttle walk test; *HR* – Heart rate; *Sats* – Oxygen saturation; *BMI* – Body mass index; *MRC* – Medical research council breathlessness scale
|                  | Visit One       | Visit Two       | Visit Three      |
|------------------|-----------------|-----------------|------------------|
|                  | D-cycloserine   | Placebo         | D-cycloserine    | Placebo         | D-cycloserine   | Placebo         |
| Catastrophising  | 6.0 (9.3) ²     | 8.0 (13.0) ²    | 5.0 (7.2) ²     | 5.5 (14.0) ²    | 3.0 (5.0) ²     | 5.0 (13.0) ²    |
| Depression       | 10.0 (9.0) ²    | 13.0 (8.0) ²    | 9.0 (9.3) ²     | 9.5 (12.0) ²    | 6.0 (10.3) ²    | 7.0 (12.0) ²    |
| D12              | 11.0 (11.3) ²   | 9.5 (10.0) ²    | 6.0 (6.5) ²     | 5.5 (10.0) ²    | 5.0 (7.0) ²     | 5.5 (10.0) ²    |
| Fatigue          | 43.0 (19.3) ²   | 33.5 (25.0) ²   | 35.0 (23.5) ²   | 32.5 (27.0) ²   | 29(18.0) ²      | 27.5 (18.0) ²   |
| BMI              | 27.3 (6.5) ²    | 26.9 (5.7) ²    | 27.3 ± 4.9 ¹    | 27.4 ± 4.4 ¹    | 27.7 ± 4.8 ²    | 27.0 ± 4.5 ²    |
| MRC              | 3 (1.0) ²       | 3 (1.0) ²       | 3 (1.0) ²       | 2 (1.0) ²       | 2 (1.0) ²       | 2 (1.0) ²       |
| MSWT Distance (m)| 280 (291) ²     | 325 (230) ²     | 290 (268)       | 355 (250) ²     | 340 (293) ²     | 340 (250) ²     |
| MSWT Borg Start  | 0.5 (1.0) ²     | 0.5 (1.0) ²     | 0.5 (0.5) ²     | 0.5 (1.0) ²     | 0.5 (0.6) ²     | 0.5 (1.0) ²     |
| MSWT Borg Change | 3.0 (1.5) ²     | 2.8 (2.5) ²     | 2.5 (1.5) ²     | 3.0 (1.0) ²     | 3.0 (2.0) ²     | 2.8 (1.5) ²     |
| MSWT HR Start    | 80.8 ± 13.4 ¹   | 80.8 ± 14.9 ¹   | 81.1 ± 14.8 ¹   | 79.4 ± 12.9 ¹   | 78.2 ± 13.1 ¹   | 78.4 ± 13.1 ¹   |
| MSWT HR Change   | 24.0 (27.3) ²   | 31.5 (16.0) ²   | 31.0 (19.5) ²   | 32.5 (28) ²     | 31.0 (23.5) ²   | 31.5 (25.0) ²   |
| MSWT Stats Start | 95.0 (3.3) ²    | 94.5 (3.0) ²    | 95.0 (4.0) ²    | 95.0 (3.0) ²    | 95.0 (2.5) ²    | 94.0 (5.0) ²    |
| MSWT Stats Change| 4 (6.3) ²       | 5 (5.0) ²       | 4 (4.3) ²       | 5 (7.0) ²       | 6 (8.3) ²       | 5 (5.0) ²       |
| Vigilance        | 37.0 ± 15.0 ¹   | 33.7 ± 17.7 ¹   | 33.2 ± 11.1 ¹   | 31.9 ± 13.8 ¹   | 33 ± 20.3 ¹     | 26.5 ± 27.1 ¹   |
| Spirometry       | 0.53 ± 0.17 ¹   | 0.56 ± 0.13 ¹   | 0.43 (0.36) ²   | 0.60 (0.12) ²   | 0.50 (0.34) ²   | 0.60 (0.22) ²   |
| Avoidance – Alone| 1.5 (0.63) ²    | 1.5 (0.8) ²     | 1.4 (0.43) ²    | 1.4 (0.6) ²     | 1.4 (0.46) ²    | 1.4 (0.65) ²    |
| Avoidance -Accompanied | 1.30 (0.53) ² | 1.40 (0.6) ² | 1.24 (0.36) ² | 1.20 (0.33) ² | 1.24 (0.44) ² | 1.24 (0.43) ² |
| St George – Active | 67.6 ± 19.0 ¹  | 57.1 ± 22.8 ¹  | 62.6 18.4 ¹    | 55.2 19.5 ¹    | 61.1 (22.3) ²   | 52.6 (19.5) ²   |
| St George – Impact | 32.7 ± 15.8 ¹  | 29.3 ± 16.4 ¹  | 24.8 (22.7) ²  | 23.2 (25.7) ²  | 22.4 (24.8) ²  | 20.9 (25.4) ²   |
| St George – Symptom | 60.7 ± 18.5 ¹  | 62.9 ± 18.5 ¹  | 56.7 ± 1.4 ¹   | 58.6 ± 21.2 ¹  | 54.2 ± 21.5 ¹   | 56.4 ± 20.2 ¹   |
| Trait            | 36.7 ± 9.9 ¹    | 38.5 ± 9.3 ¹    | 33.0 (12.3) ²   | 37.5 (16.0) ²   | 31.0 (14.8) ²   | 34.0 (16.0) ²   |
| Breathlessness Anxiety (wA) | 10.2 (28.9) ¹ | 27.8 (38.6) ¹ | 9.5 (34.7) ¹ | 9.5 (31.4) ¹ | 3.6 (32.7) ¹ | 6.4 (36.8) ¹ |
| Breathlessness Severity (wB) | 43.9 (16.1) ¹ | 51.5 (24.1) ¹ | 44.5 (19.5) ¹ | 42.2 (31.3) ¹ | 40.0 (21.1) ¹ | 41.8 (27.1) ¹ |
| Fearful Faces (ms) | 739.5 (179.5) ¹ | 757.6 (218.8) ¹ | 773.9 (196.0) ¹ | 796.1 (189.2) ¹ | 767 (165.5) ¹ | 837.7 (192.0) ¹ |
| Happy Faces (ms)  | 794.7 (174.6) ¹ | 774.8 (201.4) ¹ | 753.8 (151.8) ¹ | 778.6 (161.0) ¹ | 759.3 (178.0) ¹ | 808.3 (142.0) ¹ |

Table 4. Scores on questionnaire, physiology and behavioural measures for drug and placebo group before (visit one), during (visit two) and after (visit three) pulmonary rehabilitation. Variance is expressed either in terms of ¹standard deviation (SD) or ²interquartile range (IQR). BMI = Body Mass Index, MRC = Medical Research Council clinical measure of breathlessness, MSWT = Modified Shuttle Walk Test, HR = Heart rate, Sats = Peripheral Oxygen saturation, expressed as a percentage.
Table 4. Mean change scores on questionnaire and behavioural measures across both drug and placebo groups following four sessions of pulmonary rehabilitation (visit two). Measures are expressed as a “change score”, where visit two scores were subtracted from visit one. Variance is expressed either in terms of 1standard deviation (SD) or 2interquartile range (IQR). Significance is reported as exploratory uncorrected p-values and as Family Wise Error (p<0.05) corrected p-values.

| Cohort                        | Uncorrected p-values | Corrected p-values |
|-------------------------------|----------------------|--------------------|
| Catastrophising               | p=0.08               | p=0.097            |
| Depression                    | p=0.004*             | p=0.06             |
| D12                           | p<0.001*             | p<0.001*           |
| Fatigue                       | p=0.04*              | p=0.65             |
| BMI                           | p=0.71               | p=0.97             |
| MRC                           | p=0.71               | p=0.97*            |
| MSWT Distance                 | p=0.70               | p=0.97             |
| MSWT Borg Start               | p=0.63               | p=0.97             |
| MSWT Borg Change              | p=0.74               | p=0.97             |
| MSWT HR Start                 | p=0.75               | p=0.97             |
| MSWT HR Change                | p<0.001*             | p<0.001*           |
| MSWT Stats Start              | p=0.42               | p=0.97             |
| MSWT Stats Change             | p=0.97               | p=0.97             |
| Vigilance                     | p=0.78               | p=0.97             |
| Spirometry                    | p=0.75               | p=0.97             |
| Avoidance - Alone             | p=0.19               | p=0.97             |
| Avoidance - Accompanied       | p=0.47               | p=0.97             |
| St George - Active            | p=0.27               | p=0.97             |
| St George – Impact            | p=0.003*             | p=0.05             |
| St George – Symptom           | p=0.002*             | p=0.04*            |
| Trait                         | p=0.39               | p=0.97             |
Table 5. Mean change scores on questionnaire and behavioural measures across both drug and placebo groups following pulmonary rehabilitation (visit three). Measures are expressed as a “change score”, where visit three scores were subtracted from visit one. Variance is expressed either in terms of standard deviation (SD) or interquartile range (IQR). Significance is reported as exploratory uncorrected p-values and as Family Wise Error (p<0.05) corrected p-values.

| Measure                | Cohort Change | Uncorrected p-values | Corrected p-values |
|------------------------|---------------|----------------------|--------------------|
| Catastrophising        | 3.0 (8)       | p=0.001*             | p=0.02*            |
| Depression             | 3.9 ± 6.0     | p<0.001*             | p<0.001*           |
| D12                    | 3.0 (7.6)     | p<0.001*             | p=0.004*           |
| Fatigue                | 7.8 ± 12.4    | p<0.001*             | p<0.001*           |
| BMI                    | -0.04 (0.9)   | p=0.66               | p=0.99             |
| MRC                    | 0.0 (1)       | p=0.01*              | p=0.19             |
| MSWT Distance          | 30 (80.0)     | p=0.32               | p=0.99             |
| MSWT Borg Start        | 0.0 (0.88)    | p=0.38               | p=0.99             |
| MSWT Borg Change       | -0.01 ± 1.75  | p=0.95               | p=0.99             |
| MSWT HR Start          | 2.6 ± 11.9    | p=0.07               | p=0.84             |
| MSWT HR Change         | -51 (27.8)    | p<0.001*             | p<0.001*           |
| MSWT Stats Start       | 0.0 (3)       | p=0.65               | p=0.99             |
| MSWT Stats Change      | -1.0 (5.8)    | p=0.27               | p=0.99             |
| Vigilance              | 5.0 (11.8)    | p=0.11               | p=0.97             |
| Spirometry             | -0.01 (0.1)   | p=0.99               | p=0.99             |
| Avoidance - Alone      | -0.1 (0.3)    | p=0.10               | p=0.97             |
| Avoidance - Accompanied| 0.05 (0.3)    | p=0.12               | p=0.97             |
| St George – Active     | 6.1 (14.0)    | p=0.08               | p=0.84             |
| St George – Impact     | 5.1 (10.9)    | p=0.04*              | p=0.46             |
| St George – Symptom    | 6.5 ± 12.5    | p<0.001*             | p<0.001*           |
| Trait                  | 3.0 ± 6.6     | p<0.001*             | p<0.005*           |

Control Task
Table 6. Median change in reaction times (ms) in response to fearful or happy faces for both drug and placebo group following pulmonary rehabilitation (visit 3). Measures are expressed as a “change score”, where visit 3 scores were subtracted from visit 1. Variance is expressed as interquartile range (IQR). Significance set at exploratory uncorrected p-values and as Family Wise Error (p<0.05) corrected p-values.

|                | Cohort Average | Uncorrected p-values | Corrected p-values |
|----------------|----------------|----------------------|--------------------|
| Fearful        | -19.8 (104.2)  | p=0.28               | p=0.37             |
| Happy          | -30.4 (102.8)  | p=0.37               | p=0.37             |

Word Task

Table 7. Rating scores for breathlessness related anxiety (wA) and breathlessness (wB) for both drug and placebo group following pulmonary rehabilitation (visit 3). Measures are expressed as a “change score”, where visit 3 scores were subtracted from visit 1. Variance is expressed in terms of interquartile range (IQR). Significance set at exploratory uncorrected p-values and as Family Wise Error (p<0.05) corrected p-values.

|          | Cohort Average | Uncorrected p-values | Corrected p-values |
|----------|----------------|----------------------|--------------------|
| wA       | 5.3 (10.0) ²   | p=0.01               | p=0.02             |
| wB       | 6.3 (14.9) ²   | p=0.07               | p=0.07             |

Sensitivity Analysis and Study completeness

Ten participants who were randomised to the D-cycloserine group and eleven participants who were randomised to the placebo group did not complete all three visits. Sensitivity analysis revealed that the inclusion of the 21 missing participants would not have altered the primary outcome.
Results of sensitivity analysis for visit two. The significance of linear mixed effects models applied to the simulated complete datasets are reported as Family Wise Error Rate (FWE) 5% corrected p-values for each stimulus quantile.

| Quantile    | Region of interest |
|-------------|--------------------|
|             | Anterior cingulate cortex | Anterior insula | Amygdala | Hippocampus | Posterior insula |
| Lower 25%   | 0.58                | 0.96            | 0.12      | 0.83        | 0.96            |
| Lower 50%   | 0.42                | 0.99            | 0.13      | 0.99        | 0.99            |
| Lower 75%   | 0.35                | 0.82            | 0.31      | 0.82        | 0.76            |
| Upper 25%   | 0.81                | 0.97            | 0.31      | 0.99        | 0.99            |
| Upper 50%   | 0.56                | 0.98            | 0.26      | 0.91        | 0.91            |
| Upper 75%   | 0.61                | 0.80            | 0.65      | 0.65        | 0.65            |

Results of sensitivity analysis for visit three. The significance of linear mixed effects models applied to the simulated complete datasets are reported as Family Wise Error Rate (FWE) 5% corrected p-values for each stimulus quantile.

| Quantile    | Region of interest |
|-------------|--------------------|
|             | Anterior cingulate cortex | Anterior insula | Amygdala | Hippocampus | Posterior insula |
| Lower 25%   | 0.72                | 0.72            | 0.06      | 0.27        | 0.10            |
| Lower 50%   | 0.53                | 0.39            | 0.08      | 0.40        | 0.19            |
| Lower 75%   | 0.73                | 0.26            | 0.17      | 0.73        | 0.46            |
| Upper 25%   | 0.98                | 0.96            | 0.29      | 0.70        | 0.29            |
| Upper 50%   | 0.83                | 0.65            | 0.28      | 0.83        | 0.48            |
| Upper 75%   | 0.90                | 0.43            | 0.40      | 0.91        | 0.66            |

References

1. Rosenfield, D., et al., Changes in Dosing and Dose Timing of D-Cycloserine Explain Its Apparent Declining Efficacy for Augmenting Exposure Therapy for Anxiety-related Disorders: An Individual Participant-data Meta-analysis. J Anxiety Disord, 2019. 68: p. 102149.

2. Onur, O.A., et al., The N-Methyl-D-Aspartate Receptor Co-agonist D-Cycloserine Facilitates Declarative Learning and Hippocampal Activity in Humans. Biological Psychiatry, 2010. 67(12): p. 1205-1211.
3. Reinecke, A., et al., Neurocognitive processes in d-cycloserine augmented single-session exposure therapy for anxiety: A randomized placebo-controlled trial. Behaviour Research and Therapy, 2020. 129: p. 103607.

4. Ressler, K.J., et al., Cognitive enhancers as adjuncts to psychotherapy: use of D-cycloserine in phobic individuals to facilitate extinction of fear. Arch Gen Psychiatry, 2004. 61(11): p. 1136-44.

5. Rodebaugh, T.L., C.A. Levinson, and E.J. Lenze, A high-throughput clinical assay for testing drug facilitation of exposure therapy. Depression and anxiety, 2013. 30(7): p. 631-637.

6. Guastella, A.J., et al., A randomized controlled trial of D-cycloserine enhancement of exposure therapy for social anxiety disorder. Biol Psychiatry, 2008. 63(6): p. 544-9.

7. Hofmann, S.G., et al., Augmentation of exposure therapy with D-cycloserine for social anxiety disorder. Arch Gen Psychiatry, 2006. 63(3): p. 298-304.

8. Nave, A.M., D.F. Tolin, and M.C. Stevens, Exposure therapy, D-cycloserine, and functional magnetic resonance imaging in patients with snake phobia: a randomized pilot study. J Clin Psychiatry, 2012. 73(9): p. 1179-86.

9. Herigstad, M., et al., Treating breathlessness via the brain: changes in brain activity over a course of pulmonary rehabilitation. Eur Respir J, 2017. 50(3).

10. Yorke, J., et al., Quantification of dyspnoea using descriptors: development and initial testing of the Dyspnoea-12. 2010. 65(1): p. 21-26.

11. Radloff, L.S., The CES-D Scale: A Self-Report Depression Scale for Research in the General Population. 1977. 1(3): p. 385-401.

12. Spielberger, C.D., State-Trait Anxiety Inventory, in The Corsini Encyclopedia of Psychology. 2010.

13. Krupp, L.B., et al., The fatigue severity scale: Application to patients with multiple sclerosis and systemic lupus erythematosus. Archives of Neurology, 1989. 46(10): p. 1121-1123.

14. Jones, P.W., et al., A self-complete measure of health status for chronic airflow limitation. The St. George’s Respiratory Questionnaire. Am Rev Respir Dis, 1992. 145(6): p. 1321-7.

15. Bestall, J.C., et al., Usefulness of the Medical Research Council (MRC) dyspnoea scale as a measure of disability in patients with chronic obstructive pulmonary disease. Thorax, 1999. 54(7): p. 581-586.

16. Chambless, D.L., et al., The Mobility Inventory for Agoraphobia. Behav Res Ther, 1985. 23(1): p. 35-44.

17. De Peuter, S., et al., Illness-specific catastrophic thinking and overperception in asthma. Health Psychol, 2008. 27(1): p. 93-9.

18. Herigstad, M., et al., Dyspnea-related cues engage the prefrontal cortex: evidence from functional brain imaging in COPD. Chest, 2015. 148(4): p. 953-961.

19. McCracken, L.M., K.E. Vowles, and C. Eccleston, Acceptance-based treatment for persons with complex, long standing chronic pain: a preliminary analysis of treatment outcome in comparison to a waiting phase. Behav Res Ther, 2005. 43(10): p. 1335-46.

20. Levy, M.L., et al., Diagnostic spirometry in primary care: Proposed standards for general practice compliant with American Thoracic Society and European Respiratory Society recommendations: a General Practice
Airways Group (GPIAG) document, in association with the Association for Respiratory Technology & Physiology (ARTP) and Education for Health. Prim Care Respir J, 2009. 18(3): p. 130-47.

21. Bradley, J., et al., *Validity of a modified shuttle test in adult cystic fibrosis.* Thorax, 1999. 54(5): p. 437-9.

22. Mahler, D.A., et al., *Comparison of clinical dyspnea ratings and psychophysical measurements of respiratory sensation in obstructive airway disease.* Am Rev Respir Dis, 1987. 135(6): p. 1229-33.

23. Herigstad, M., et al., *Development of a dyspnoea word cue set for studies of emotional processing in COPD.* Respiratory physiology & neurobiology, 2016. 223: p. 37-42.

24. Vlemincx, E., C. Sprenger, and C. Büchel, *Expectation and dyspnea: The neurobiological basis of respiratory nocebo effects.* European Respiratory Journal, 2021: p. 2003008.

25. Janssens, T. and T. Ritz, *Perceived triggers of asthma: key to symptom perception and management.* Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology, 2013. 43(9): p. 1000-1008.

26. Pagnini, F., *The potential role of illness expectations in the progression of medical diseases.* BMC Psychology, 2019. 7(1): p. 70.

27. Ekman, P.F.W., *Pictures of Facial Affect.* Consult. Psychol. Press, 1975.

28. Young, A.W., et al., *Facial expression megamix: Tests of dimensional and category accounts of emotion recognition.* Cognition, 1997. 63(3): p. 271-313.

29. Godlewska, B.R., et al., *Short-term SSRI treatment normalises amygdala hyperactivity in depressed patients.* Psychological Medicine, 2012. 42(12): p. 2609-2617.

30. Rawlings, N.B., et al., *A single dose of mirtazapine modulates neural responses to emotional faces in healthy people.* Psychopharmacology, 2010. 212(4): p. 625-634.

31. Oliveira, L., et al., *What does brain response to neutral faces tell us about major depression? evidence from machine learning and fMRI.* PloS one, 2013. 8(4): p. e60121-e60121.

32. Griffanti, L., et al., *ICA-based artefact removal and accelerated fMRI acquisition for improved resting state network imaging.* Neuroimage, 2014. 95: p. 232-47.

33. Salimi-Khorshidi, G., et al., *Automatic denoising of functional MRI data: combining independent component analysis and hierarchical fusion of classifiers.* Neuroimage, 2014. 90: p. 449-68.

34. Filippini, N., et al., *Study protocol: the Whitehall II imaging sub-study.* BMC Psychiatry, 2014. 14(1): p. 159.

35. Harvey, A.K., et al., *Brainstem functional magnetic resonance imaging: disentangling signal from physiological noise.* J Magn Reson Imaging, 2008. 28(6): p. 1337-44.

36. Brooks, J.C., et al., *Physiological noise modelling for spinal functional magnetic resonance imaging studies.* Neuroimage, 2008. 39(2): p. 680-92.

37. Faull, O.K., et al., *Conditioned respiratory threat in the subdivisions of the human periaqueductal gray.* Elife, 2016. 5.
38. Hayen, A., et al., *Opioid suppression of conditioned anticipatory brain responses to breathlessness*. Neuroimage, 2017. 150: p. 383-394.

39. Jenkinson, M., et al., *Improved optimization for the robust and accurate linear registration and motion correction of brain images*. Neuroimage, 2002. 17(2): p. 825-41.

40. Smith, S.M., *Fast robust automated brain extraction*. Hum Brain Mapp, 2002. 17(3): p. 143-55.

41. Holland, D., J.M. Kuperman, and A.M. Dale, *Efficient correction of inhomogeneous static magnetic field-induced distortion in Echo Planar Imaging*. Neuroimage, 2010. 50(1): p. 175-83.

42. Jenkinson, M., *Fast, automated, N-dimensional phase-unwrapping algorithm*. Magn Reson Med, 2003. 49(1): p. 193-7.

43. Greve, D.N. and B. Fischl, *Accurate and robust brain image alignment using boundary-based registration*. Neuroimage, 2009. 48(1): p. 63-72.

44. Andersson, J., *Non-Linear registration, aka spatial normalisation*. FMRIB technical report, 2010. TR07JA2.