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The effect of D-cycloserine on brain processing of breathlessness over pulmonary rehabilitation - an experimental medicine study

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Short title: D-cycloserine's effect on breathlessness brain processes

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Abstract

Research Question: Pulmonary rehabilitation is the best treatment for chronic breathlessness in COPD but there remains an unmet need to improve efficacy. Pulmonary rehabilitation has strong parallels with exposure-based cognitive behavioural therapies (CBT), both clinically and in terms of brain activity patterns. The partial NMDA-receptor agonist, D-cycloserine has shown promising results in enhancing efficacy of CBT, thus we hypothesised that it would similarly augment the effects of pulmonary rehabilitation in the brain. Positive findings would support further development in phase 3 clinical trials.

Methods: 72 participants with mild-to-moderate COPD were recruited to a double-blind pre-registered (ID: NCT01985750) experimental medicine study running parallel to a pulmonary rehabilitation course. Participants were randomised to 250mg D-cycloserine or placebo, administered immediately prior to the first four sessions of pulmonary rehabilitation. Primary outcome measures were differences between D-cycloserine and placebo in brain activity in the anterior insula, posterior insula, anterior cingulate cortices, amygdala and hippocampus following completion of pulmonary rehabilitation. Secondary outcomes included the same measures at an intermediate time point and voxel-wise difference across wider brain regions. An exploratory analysis determined the interaction with breathlessness-anxiety.

Results: No difference between D-cycloserine and placebo groups was observed across the primary or secondary outcome measures. D-cycloserine was shown instead to interact with changes in breathlessness anxiety to dampen reactivity to breathlessness cues. Questionnaire and measures of respiratory function showed no group difference. This is the first study testing brain-active drugs in pulmonary rehabilitation. Rigorous trial methodology and validated surrogate end-points maximised statistical power.

Conclusion: Although increasing evidence supports therapeutic modulation of NMDA pathways to treat symptoms, we conclude that a phase 3 clinical trial of D-

cycloserine would not be worthwhile.

Key Words: Neuroimaging, breathlessness-expectation, brain-active drugs, cognitive 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. However, around 30% of patients derive no clinical benefit [1]. 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]. Thus, there remains an unmet need to develop strategies to increase or prolong the beneficial effects of pulmonary rehabilitation.

A body of evidence has shown that improvements in breathlessness over pulmonary rehabilitation result from a reappraisal of the sensory experience [3, 4], arresting the downward spiral of fear, avoidance and physical deconditioning. The safe, graded exposure to breathlessness within pulmonary rehabilitation parallels techniques of exposure-based cognitive behavioural therapy (CBT), in which pathological fears are first activated and then disconfirmed by new adaptive information. Although these parallels are drawn observationally, practitioners in CBT are explicitly trained in graded exposure theories, while pulmonary rehabilitation physiotherapists are not, clinical studies of both pulmonary rehabilitation and cognitive behavioural therapy have both shown changes to brain activity within areas associated with attention and learned sensory and emotional expectations such as cingulate cortex, angular gyrus, insula and supramarginal gyrus, [4, 5].

In the field of psychiatry, there has been great interest in the partial NMDA agonist D-cycloserine, as a pharmacological adjunct to enhance efficacy of exposure-based CBT [6-8]. D-cycloserine is thought to act at the glycine modulatory site of the NMDA receptor. Its high affinity binding enhances synaptic plasticity, promoting emotional learning processes [8] boosting therapeutic effects of CBT as a result [7, 9,

10]. Experimental medicine studies have demonstrated reductions in emotional response within the amygdala when paired with CBT [6], increasing activity within hippocampus in a manner linked to learning [11]. In CBT for alcoholism, D-cycloserine decreased cue induced brain activity across the ventral and dorsal striatum which was associated with reductions in alcohol craving [12]. Clinical trials of D-cycloserine paired with CBT have demonstrated reductions in symptoms of acrophobia [13], social phobia [14], panic disorder [15] and obsessive compulsive disorder (OCD) over placebo [16, 17], often with medium to large effect sizes.

Given the strong parallels between pulmonary rehabilitation and exposure-based CBT, and the strong effects of pulmonary rehabilitation on affective components of breathlessness [3, 4] we hypothesised that D-cycloserine may have therapeutic benefits in enhancing pulmonary rehabilitation.

To test this hypothesis, we performed an experimental medicine study using functional magnetic resonance imaging (FMRI) markers of drug efficacy in the brain. We chose this approach over a phase 3 clinical trial because the differences between COPD populations and those with primary psychiatric conditions in which D-cycloserine has been tested so far might necessitate a bespoke trial design. Therefore, we wanted to test efficacy in the brain first. A positive result from an FMRI study would facilitate clinical trial design and help early decisions of go/no-go on further clinical development and subsequent larger phase 3 clinical trials.

Since the inception of this study, there has been an increasing number of null results from studies using D-cycloserine in combination with exposure-based CBT [16, 18]. 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. Hofmann et al suggest that “D-cycloserine

not only makes “good exposures” better but also may make “bad exposures” worse” [17]. To account for this updated literature, we conducted an additional analysis. which tested whether brain activity changes relating to D-cycloserine was linked to improvements in breathlessness related anxiety during pulmonary rehabilitation.

Methods and Materials

An overview of the methodology is presented here. Full details, including 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. This is the first example of pre-registration of both study design and analysis plan in a respiratory neuroimaging study.

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) [13, 14, 19]**.** The most relevant paper (on treatment of snake phobia [10]**)** demonstrated that effects observed with neuroimaging were more sensitive than behavioural effects, therefore 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%) [4]. 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.

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 e-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.

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. 250mg dosage has been shown to be efficacious [6, 11]. D-cycloserine exerts its best effects when given immediately before exposure based therapy and only for a limited number of times [20] with daily administration associated with tachyphylaxis [13]. Therefore, the dose timing of D-cycloserine was deliberately selected to be the first four sessions, where the greatest potential for emotional

learning occurs as patients become habituated to pulmonary rehabilitation. 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.

The following minimization criteria were used for randomisation a) centre at which pulmonary rehabilitation was performed, MRC breathlessness grade, presence of diabetes, whether the participant was taking antidepressants, the age at which the participant completed full time education and previous pulmonary rehabilitation. Full details of randomisation 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.

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. The full course ran for 6 weeks with two sessions per week including an hour of exercises and an hour of education, as part of a standard pulmonary rehabilitation programme. For programme details see Supplementary materials.

***Self-report questionnaires*:** Building on our previous work [4, 21] we selected a set of questionnaires with proven sensitivity to changes across pulmonary rehabilitation [4], which were designed to probe the experience of living with COPD. These were

scored according to their respective manuals: Dyspnoea-12 (D12) Questionnaire [22], Centre for Epidemiologic Studies Depression Scale (CES-D) [23], Trait Anxiety Inventory (TRAIT) [24], Fatigue Severity Scale [25], St George's Respiratory Questionnaire (SGRQ) [26], Medical Research Council (MRC) breathlessness scale [27], Mobility Inventory (MI) [28], Breathlessness catastrophising scale, adapted from the Catastrophic Thinking Scale in Asthma [29], Breathlessness vigilance, adapted from the Pain Awareness and Vigilance Scale [30].

Physiological measures: Spirometry and two Modified Shuttle Walk Tests (MSWT) were collected using standard protocols [31, 32]. 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 [4, 21, 33]. This paradigm has previously been shown to be sensitive to improvements in breathlessness over a course of pulmonary rehabilitation. Brain activity was correlated with corresponding visual analogue ratings of breathlessness and breathlessness-anxiety collected during scanning. [4]. During 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. Scan duration was 7 minutes and 33 seconds.

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. Scan duration was 5 minutes and 42 sections.

Outcomes: Our primary outcomes focused on brain activity changes within five key regions of interest, identified in previous studies of breathlessness [4, 34]. 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 [35, 36]. Focusing on a small number of regions of interest is more statistically powerful and therefore more likely to detect a hypothesised difference. 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 regions. Exploratory analyses 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 primary and secondary analysis was pre-registered and made publicly available prior to unblinding https://mfr.osf.io/render?url=https://osf.io/wqyf4/?action=download%26mode=render.

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://ww.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

Baseline group level brain activity in response to breathlessness related word cues

can be seen in Finnegan et al 2021 Supplementary Figure 7 [37] in which activity was observed within inferior frontal gyrus, posterior cingulate, anterior cingulate and insula. 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 e-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) [38] using FSL's Randomise tool [39] at family wise error corrected $p<0.05$. The process was then repeated separately for data collected at visit three.

We then 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?"

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.

Primary outcomes: 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).

Secondary outcomes: 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) (Supplementary e-Table 9) or visit three (both $p=0.053$ family wise error rate corrected) (Supplementary e-Table 10). Group level effects of pulmonary rehabilitation on brain activity in response to breathlessness related word-cues can be observed in Supplementary e-Figure 3. 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 ($F(1,68) = 0.36$, $p=0.55$) (Supplementary e-Table 9) or visit three ($F(1,68) = 0.002$, $p=0.97$) (Supplementary e-Table 10). Raw scores are reported in Supplementary e-Table 4.

Overall changes in self-report questionnaires over the course of pulmonary rehabilitation were as expected and are presented in Supplementary e-Table 5.

Exploratory outcomes

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 4). 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 5). 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.

Discussion

Key Findings

We found that 250mg of D-cycloserine administered prior to the first four sessions of pulmonary rehabilitation showed no mean effect on breathlessness related brain activity in the 5 regions of interest tested: amygdala, anterior insula, posterior insula, anterior cingulate cortex or hippocampus when assessed either during (after 4-5 sessions) or after the course. Likewise, there was no mean effect on the secondary endpoints of voxel-wise activity across a wider selection of brain regions. These findings suggest that D-cycloserine does not have the potential to move to phase 3 clinical trials in pulmonary rehabilitation and that alternative drug candidates should be considered. However, the results from the exploratory analysis give important insights into potential mechanisms of action for brain-targeted drugs like D-cycloserine. 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 expectation related responses to breathlessness word cues as a result of D-cycloserine in people who had derived positive change from pulmonary rehabilitation.

This is the first study to test a neuro-pharmacological adjunct to pulmonary rehabilitation. A key strength of this study, in addition to its sample size, which is large for a neuroimaging study of breathlessness, is its use of robust clinical trial methodology which was monitored in accordance with GCP standards. We included a formal sample size calculation powered for validated end points relevant to the patient population, pre-registered the study design and published a statistical analysis plan ahead of unblinding. Additionally, our hypotheses focused on five a-priori defined brain regions with demonstrated evidence of strong modulation by D-cycloserine [9, 40] or links to body, symptom perception as well as emotional salience [35, 36]. Together these steps, which are rarely carried out for neuroimaging studies, ensured rigorous methodology, robust findings and provide the best chance to detect an effect if present.

Why was no drug effect observed across the group?

D-cycloserine may not have sufficient glutamatergic activity: While D-cycloserine has not shown sufficient promise to be progressed to full-scale clinical trials, other drugs may now be more attractive candidates. Paired with cognitive therapies as treatment for treatment resistant depression, ketamine and its derivative Esketamine, which blocks pre-synaptic NMDA receptor signalling, increasing glutamate and thereby synaptic plasticity, has been linked with reductions in fear and anxiety , and rapid relief from symptoms [41].

Individual differences: Recent literature has highlighted the importance of individual differences in response to D-cycloserine. New evidence suggests that D-cycloserine has the potential to reinforce either positive or negative experiences during exposure based CBT [17]. Direction of action appears to depend on stress levels, which at the neurochemical level influence neurotransmitter concentrations surrounding the NMDA-receptors [42]. These opposing effects may render brain activity differences unobservable in a simple contrast of means. These effects may be further compounded by the multidimensional nature of breathlessness and corresponding heterogenous symptoms of people living with COPD [37, 43, 44]. Our exploratory analysis investigated this further. 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 [4]. 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 [21]. 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 for people who had derived positive change (measured by breathlessness anxiety ratings) from pulmonary rehabilitation.

Ceiling effect: The action of D-cycloserine is known to be curtailed near the therapeutic ceiling [45], and pulmonary rehabilitation is a highly effective treatment [2], this may leave insufficient scope for improvement in some individuals.

Little evidence of D-cycloserine in older adults: Older adults are not well represented within the evidence base regarding D-cycloserine's action. Given the well-established changes to NMDA receptor function as the brain ages, D-cycloserine may act differently in this population [46].

Alternative brain pathways for pharmacological targets. In this study we specifically investigated drug effect on breathlessness related brain activity. However, other targets may also positively impact breathlessness or pulmonary rehabilitation outcomes via other mechanisms. For example, mindfulness-based CBT

in pulmonary rehabilitation can improve quality of life without affecting breathlessness [47]. Glucocorticoids such as cortisol, combined with exposure-based CBT, have shown promise in reduction of fear in phobias and post-traumatic stress disorder [7] via their action on glucocorticoid receptors along the hypothalamic-pituitary-adrenal (HPA) axis. Findings regarding selective serotonin uptake inhibitors (SSRIs) meanwhile are mixed. Paired with CBT, Paroxetine was found to reduce panic attacks by 50% compared to placebo [48]. However, a review of wider SSRI literature found that chronic and sub-chronic administration was associated with reduced CBT response, leading to questions as to whether SSRI's may even interfere with CBT effectiveness [49]. 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.

Dosing and dose timing. Questions do still remain regarding D-cycloserine's optimum dosage, dose timing and number of administered sessions [16]. Based on the available literature at the time [6, 8] and practical considerations regarding drug availability, we selected a dose of 250mg for this study, administered at the first four rehabilitation sessions. However, given that our maximum effect size was 0.18 (Figure 3), even a 50% increase would be below the commonly reported effect sizes of 0.4-0.7, and up to 1.06 [10, 13, 14, 19]. This strongly suggests that dose and dose timing did not drive the negative result.

Conclusions

We have shown evidence that D-cycloserine does not have a mean effect on breathlessness related brain activity, behavioural or physiological measures over the course of pulmonary rehabilitation. Instead, the drug appears to work in a more

nuanced manner and interacts with changes in breathlessness anxiety to influence the brain's breathlessness perception networks, lending support to personalised approaches to treating breathlessness. This study contributes important information regarding the overall (un)suitability of D-cycloserine as a candidate for phase 3 clinical trials in pulmonary rehabilitation.

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Disclosures

Dr. Harmer has valueless shares in p1vital and serves on their advisory panel. She has received consultancy payments from p1vital, Zogenix, J&J, Pfizer, Servier, Eli-Lilly, Astra Zeneca, Lundbeck. Dr. Pattinson and Dr Ezra are named as co-inventors on a provisional U.K. patent application titled “Use of cerebral nitric oxide donors in the assessment of the extent of brain dysfunction following injury. Dr. Rahman, has received consulting fees from Rocket Medical U.K. Prof. Nichols has received consulting fees from Perspectum Diagnostics. The remaining authors have no biomedical financial interests or potential conflicts of interest.

Author contributions

S.L.F – Acquisition of data, analysis, interpretation, drafting, editing and approving manuscript, guarantor of the paper

O.K.H - Interpretation, editing and approving manuscript

S.B - Study design, editing and approving manuscript

A.D - Acquisition of data, analysis, approving manuscript

M.E – Analysis, editing and approving manuscript

B.G – Analysis, approving manuscript

C.J.H – Study design, editing and approving manuscript

M.H - Study design, acquisition of data, editing and approving manuscript

T.N – Analysis, approving manuscript

N.M.R - Study design, editing and approving manuscript

O.R – Analysis, approving manuscript

A.R - Study design, editing and approving manuscript

K.T.S.P - Study design, interpretation, editing and approving manuscript, guarantor of the paper

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. Carrieri-Kohlman, 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.
4. 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).
5. 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.
6. 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.
7. 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.
8. 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.
9. Klass, A., et al., *d-Cycloserine facilitates extinction learning and enhances extinction-related brain activation.* Neurobiol Learn Mem, 2017. **144**: p. 235-247.
10. 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.
11. 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.
12. Kiefer, F., et al., *Effects of d-cycloserine on extinction of mesolimbic cue reactivity in alcoholism: a randomized placebo-controlled trial.* Psychopharmacology, 2015. **232**(13): p. 2353-2362.
13. 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.
14. 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.
15. Otto, M.W., et al., *Efficacy of d-cycloserine for enhancing response to cognitive-behavior therapy for panic disorder.* Biol Psychiatry, 2010. **67**(4): p. 365-70.
16. 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.
17. 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.
18. 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.
19. 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.
20. Norberg, M.M., J.H. Krystal, and D.F. Tolin, *A meta-analysis of D-cycloserine and the facilitation of fear extinction and exposure therapy.* Biol Psychiatry, 2008. **63**(12): p. 1118-26.
21. 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.
22. Yorke, J., et al., *Quantification of dyspnoea using descriptors: development and initial testing of the Dyspnoea-12.* 2010. **65**(1): p. 21-26.
23. Radloff, L.S., *The CES-D Scale:A Self-Report Depression Scale for Research in the General Population.* 1977. **1**(3): p. 385-401.
24. Spielberger, C.D., *State-Trait Anxiety Inventory*, in *The Corsini Encyclopedia of Psychology*. 2010.
25. 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.
26. 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.
27. 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.
28. Chambless, D.L., et al., *The Mobility Inventory for Agoraphobia.* Behav Res Ther, 1985. **23**(1): p. 35-44.
29. De Peuter, S., et al., *Illness-specific catastrophic thinking and overperception in asthma.* Health Psychol, 2008. **27**(1): p. 93-9.
30. 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.
31. Bradley, J., et al., *Validity of a modified shuttle test in adult cystic fibrosis.* Thorax, 1999. **54**(5): p. 437-9.
32. 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 1*

www.gpiag.org 2 www.artp.org 3 www.educationforhealth.org.uk. Prim Care Respir J, 2009. **18**(3): p. 130-47.
33. 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.
34. Esser, R.W., et al., *Brain Activation during Perception and Anticipation of Dyspnea in Chronic Obstructive Pulmonary Disease.* Frontiers in physiology, 2017. **8**: p. 617-617.
35. Craig, A.D., *How do you feel--now? The anterior insula and human awareness.* Nat Rev Neurosci, 2009. **10**(1): p. 59-70.
36. 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.
37. Finnegan, S.L., et al., *Breathlessness in COPD: linking symptom clusters with brain activity.* European Respiratory Journal, 2021. **58**(5): p. 2004099.
38. 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.
39. Winkler, A.M., et al., *Permutation inference for the general linear model.* Neuroimage, 2014. **92**: p. 381-97.
40. Thompson, L.T., J.R. Moskal, and J.F. Disterhoft, *Hippocampus-dependent learning facilitated by a monoclonal antibody or D-cycloserine.* Nature, 1992. **359**(6396): p. 638-641.
41. Ziffra, M., *Panic disorder: A review of treatment options.* Ann Clin Psychiatry, 2021. **33**: p. 124-33.
42. Schade, S. and W. Paulus, *D-Cycloserine in Neuropsychiatric Diseases: A Systematic Review.* The international journal of neuropsychopharmacology, 2016. **19**(4): p. pyv102.
43. Finnegan, S.L., et al., *A common model for the breathlessness experience across cardiorespiratory disease.* ERJ Open Res, 2021. **7**(2).
44. Morélot-Panzini, C., et al., *Real-life assessment of the multidimensional nature of dyspnoea in COPD outpatients.* Eur Respir J, 2016. **47**(6): p. 1668-79.
45. 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.
46. 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.
47. Farver-Vestergaard, I., et al., *Mindfulness-based cognitive therapy in COPD: a cluster randomised controlled trial.* Eur Respir J, 2018. **51**(2).
48. Oehrberg, S., et al., *Paroxetine in the Treatment of Panic Disorder a Randomised, Double-Blind, Placebo-Controlled Study.* British Journal of Psychiatry, 1995. **167**(3): p. 374-379.
49. Reinecke, A. and C.J. Harmer, *A Cognitive-Neuropsychological Account of Treatment Action in Anxiety: Can We Augment Clinical Efficacy?* Psychopathology Review, 2016. **a3**(1): p. 77-109.

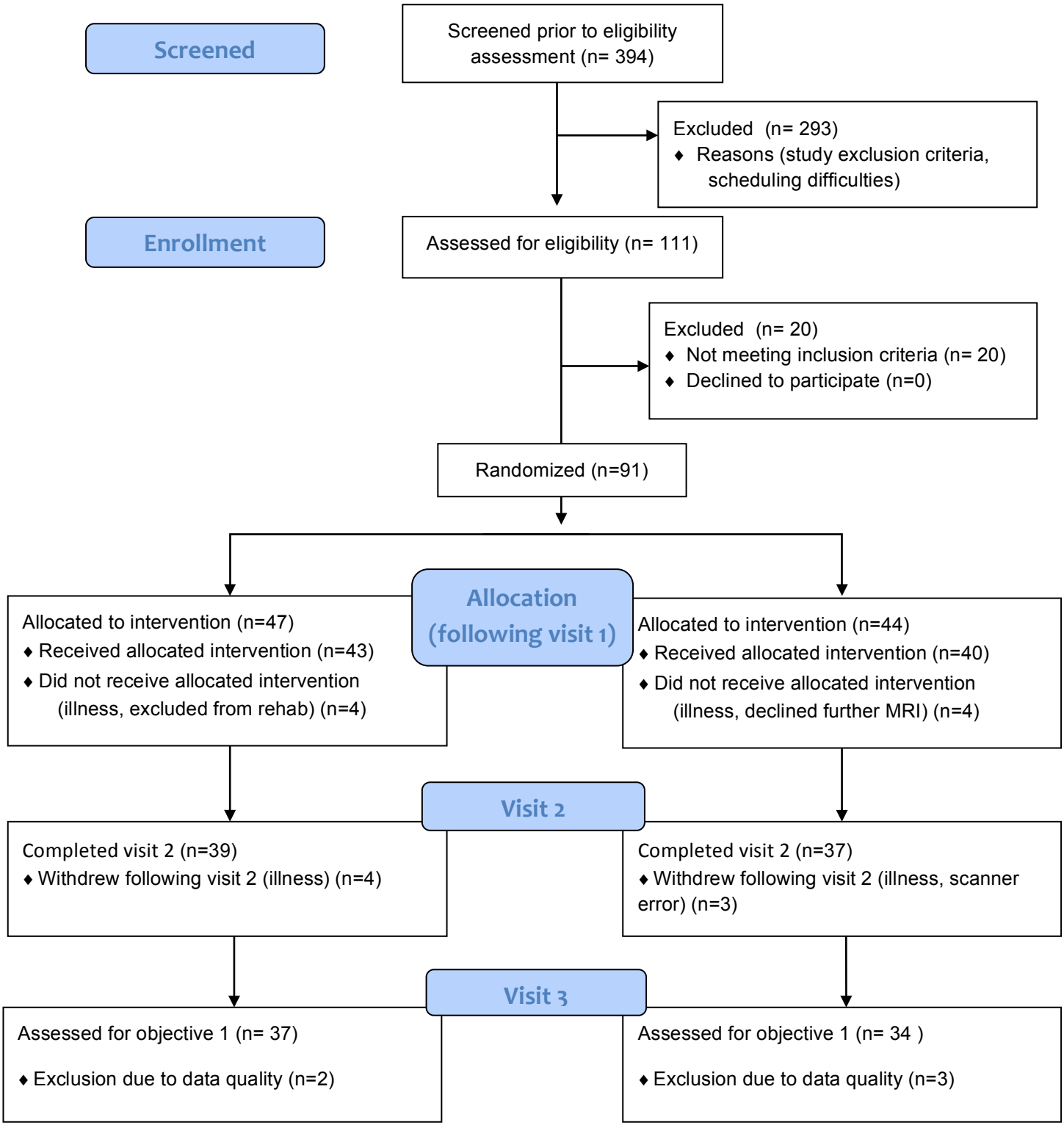
Table 1. *Demographic information from the 72 participants who completed all three study visits. Variance is expression either in terms of standard deviation (SD) or interquartile range (IQR) depending on the normality of the underlying data distribution.* ***BMI*** *= Body Mass Index,* ***MRC*** *= Medical Research Council.* ***SpO_2%*** *= Peripheral Oxygen saturation, expressed as a percentage.* ***FEV*** *= Forced Expiratory Volume.* ***FVC*** *= Forced Vital Capacity. Also listed with prevalence in brackets are recorded comorbidities ordered by frequency*

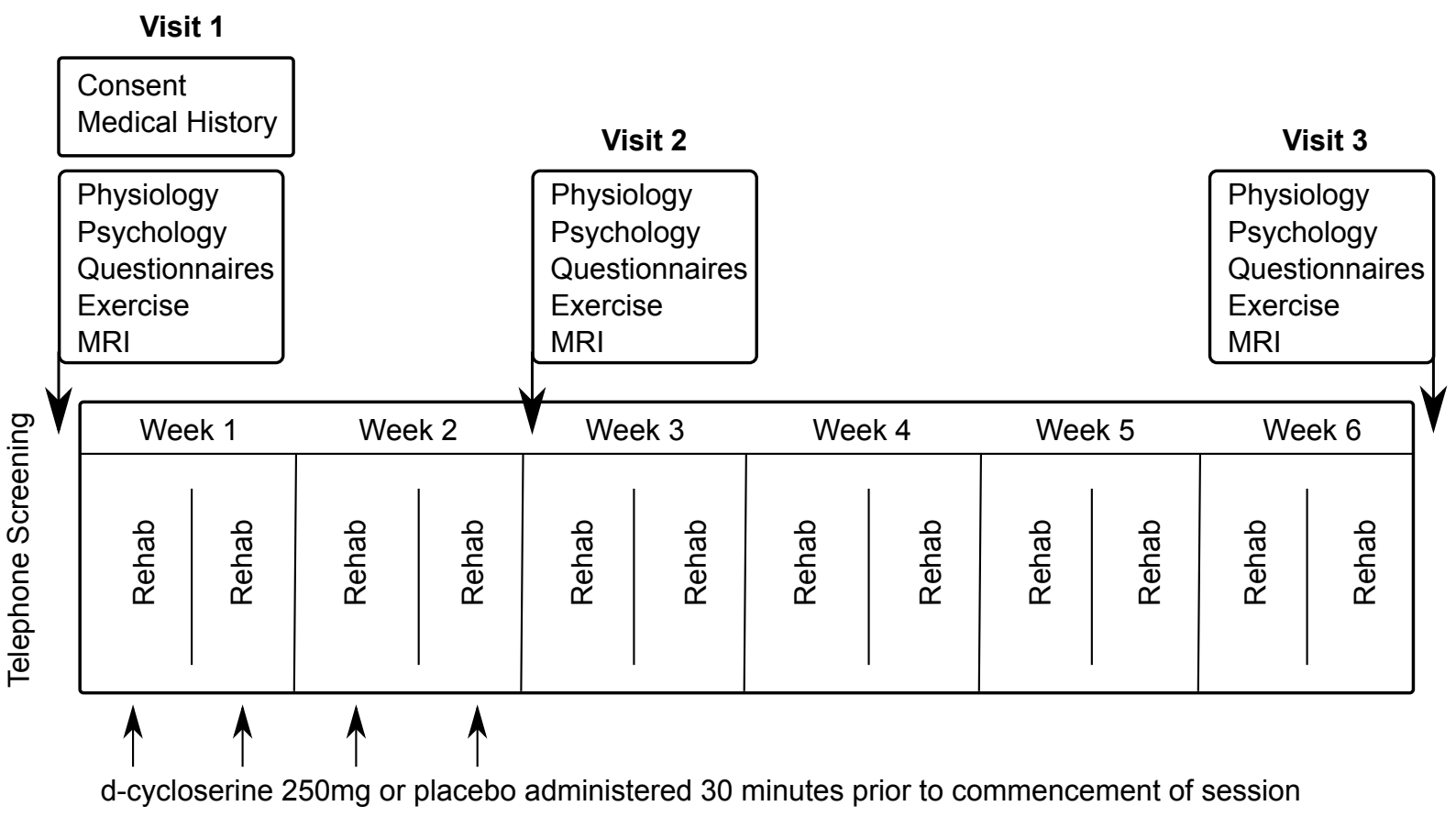
	Visit 1 (Pre-rehabilitation)	
	D-cycloserine	**Placebo**
Age (median years / range)	**71.0 / (47-81)**	**71.5 / (46-85)**
Smoking pack-years (years / IQR)	**34.0 / (25.6)**	**30.0 / (30.0)**
Duration of breathlessness (years / IQR)	**8.0 (17.0)**	**9.5 (10.0)**
Total exacerbations (number / IQR)	**0.0 (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 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)**	
FEV1% Predicted (IQR)		**0.56 / (0.13)**
GOLD	**51.7 (31.2)**	**66.8 (25.2)**
1 (A/B/C/D)	**10 (0/10/0/0)**	**6 (1/5/0/0)**
2 (A/B/C/D)	**14 (3/11/0/0)**	**14 (0/14/0/0)**
3 (A/B/C/D)	**12 (0/0/0/12)**	**11 (0/0/0/11)**
4 (A/B/C/D)	**1 (0/0/0/1)**	**3 (0/0/0/3)**
	Comorbidities (frequency)	
	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)

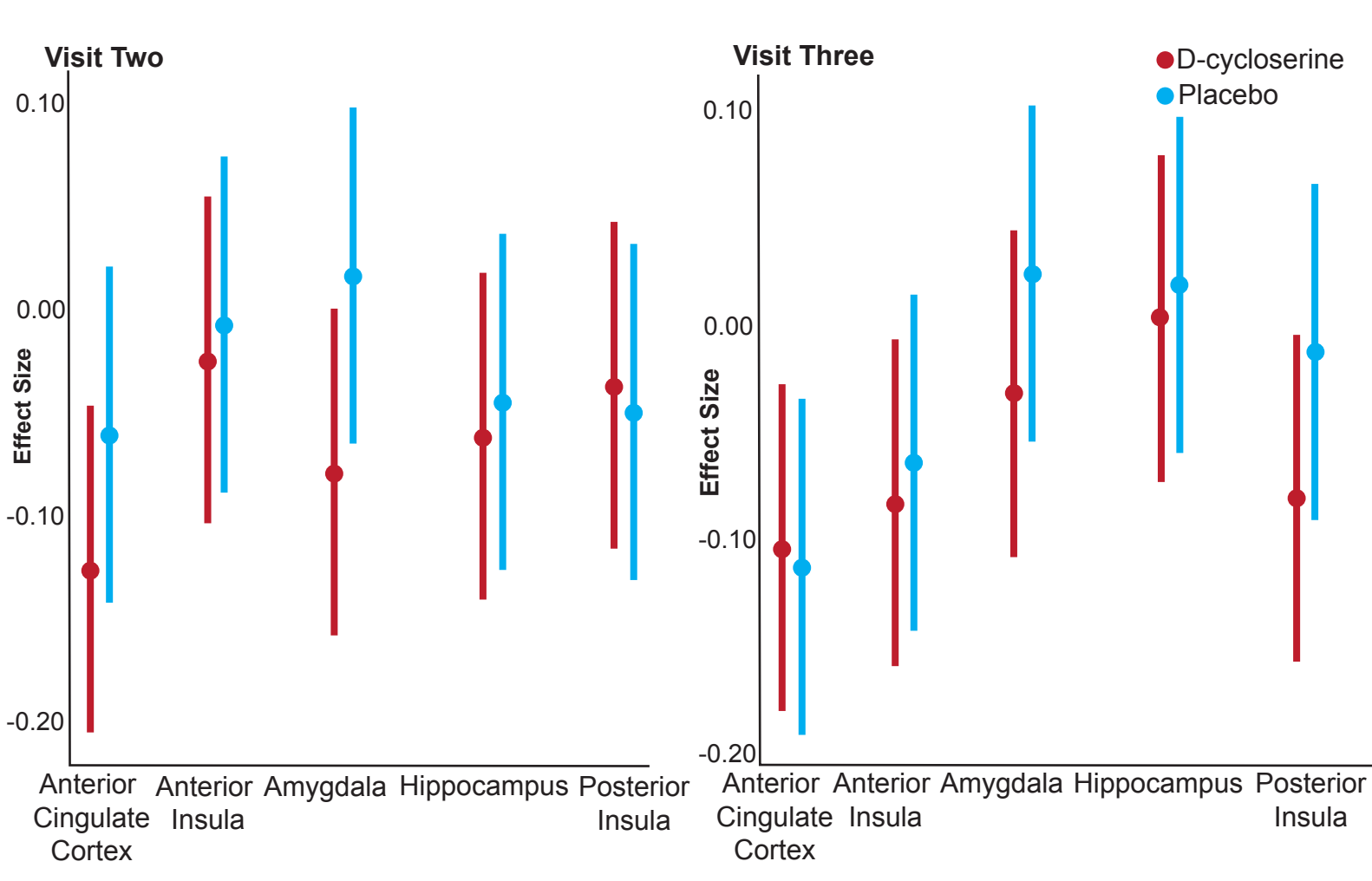
	Arrhythmia (3)	Arrhythmia (4)
	Inflammatory bowel disease (2)	Inflammatory bowel disease (3)
	Peptic ulcer (3)	Peptic ulcer (2)
	Heart failure (1)	Heart failure (1)
	Tuberculosis (1)	Neuromuscular weakness (2)

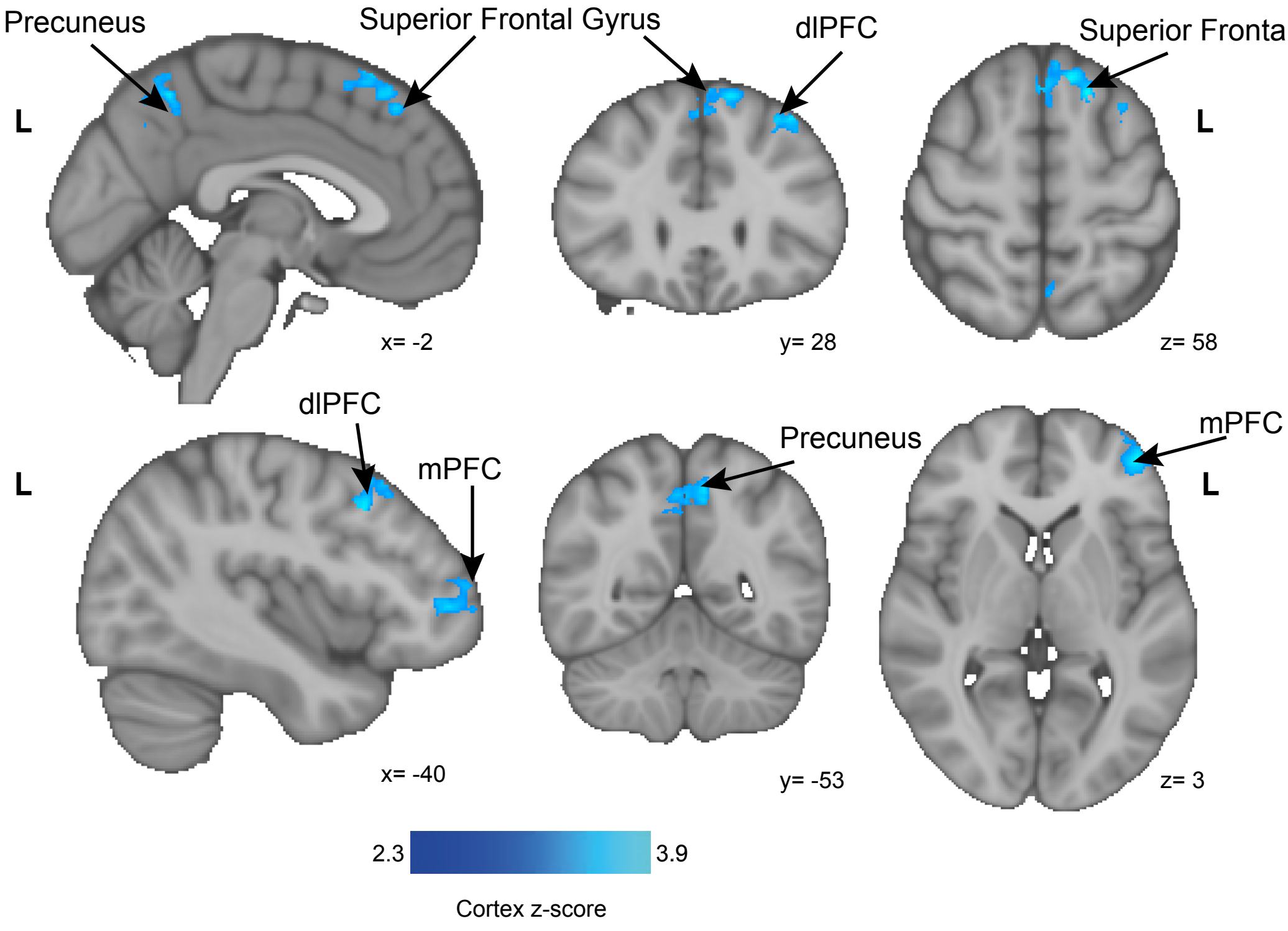
Table 2. *Significance of overall effect of D-cycloserine on mean brain activity within the five key regions of interest at visits two and three, having accounted for scores at visit one. Significance is reported as Family Wise Error (p<0.05) corrected p-values of the difference*

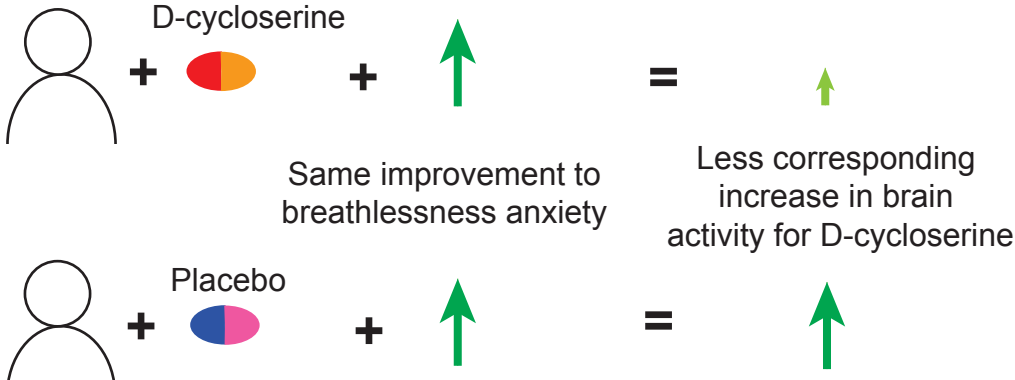
Visit	*Region of interest*	*Estimate*	*Std. Error*	*(p-value)*
Visit two	*Anterior cingulate cortex*	*0.066*	*0.059*	*0.70*
	Anterior insula	*0.019*	*0.058*	*0.98*
	Amygdala	*0.097*	*0.059*	*0.40*
	Hippocampus	*0.018*	*0.058*	*0.98*
	Posterior insula	*-0.013*	*0.059*	*0.98*
Visit three	*Anterior cingulate cortex*	*-0.009*	*0.056*	*0.98*
	Anterior insula	*0.019*	*0.055*	*0.98*
	Amygdala	*0.055*	*0.056*	*0.78*
	Hippocampus	*0.014*	*0.056*	*0.98*
	Posterior insula	*0.068*	*0.056*	*0.70*


Screened
Screened prior to eligibility assessment (n= 394)
Excluded (n= 293)
♦ Reasons (study exclusion criteria, scheduling difficulties)
Enrollment
Assessed for eligibility (n= 111)
Excluded (n= 20)
♦ Not meeting inclusion criteria (n= 20)
♦ Declined to participate (n=0)
Randomized (n=91)
Allocation (following visit 1)
Allocated to intervention (n=47)
♦ Received allocated intervention (n=43)
♦ Did not receive allocated intervention (illness, excluded from rehab) (n=4)
Allocated to intervention (n=44)
♦ Received allocated intervention (n=40)
♦ Did not receive allocated intervention (illness, declined further MRI) (n=4)
Visit 2
Completed visit 2 (n=39)
♦ Withdrew following visit 2 (illness) (n=4)
Completed visit 2 (n=37)
♦ Withdrew following visit 2 (illness, scanner error) (n=3)
Visit 3
Assessed for objective 1 (n= 37)
♦ Exclusion due to data quality (n=2)
Assessed for objective 1 (n= 34)
♦ Exclusion due to data quality (n=3)


Visit 1
Consent
Medical History
Physiology
Psychology
Questionnaires
Exercise
MRI
Visit 2
Physiology
Psychology
Questionnaires
Exercise
MRI
Visit 3
Physiology
Psychology
Questionnaires
Exercise
MRI
Telephone Screening
Week 1
Week 2
Week 3
Week 4
Week 5
Week 6
Rehab
d-cycloserine 250mg or placebo administered 30 minutes prior to commencement of session


Visit Two
Visit Three
D-cycloserine
Placebo
Effect Size
0.10
0.00
-0.10
-0.20
Anterior Cingulate Cortex
Anterior Insula
Amygdala
Hippocampus
Posterior Insula


Precuneus
Superior Frontal Gyrus
dlPFC
Superior Frontal Gyrus
L
L
x= -2
y= 28
z= 58
dlPFC
mPFC
Precuneus
mPFC
L
L
x= -40
y= -53
z= 3
2.3
3.9
Cortex z-score


D-cycloserine
Same improvement to breathlessness anxiety
Less corresponding increase in brain activity for D-cycloserine
Placebo

Supplementary Material

The effect of D-cycloserine on brain processing of breathlessness over pulmonary rehabilitation - an experimental medicine study

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Online Data Supplement

e-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.* ***SpO_2%*** *= 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**
Duration of breathlessness (years/IQR)	**8 / (4.5)**	**7.8 / (10.3)**	**8 / (10.8)**
Total exacerbations (number / IQR)	**2.2 (2)**	**1.3 (1.25)**	**2.2 (2.0)**
GOLD			
1 (A/B/C/D)	**25 (3/22/0/0)**	**12 (2/10/0/0)**	**13 (1/12/0/0)**
2 (A/B/C/D)	**33 (3/30/0/0)**	**15 (3/12/0/0)**	**18 (0/18/0/0)**
3 (A/B/C/D)	**27 (0/0/0/27)**	**12 (0/0/0/12)**	**15 (0/0/0/15)**
4 (A/B/C/D)	**6 (0/0/0/6)**	**1 (0/0/0/1)**	**5 (0/0/0/5)**
MRC breathlessness scale (IQR)	**3 (1)**	**3 (1)**	**3 (1)**
Resting SpO_2% (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 / (0.2)**	**0.54 / (0.2)**	**0.58 / (0.1)**
FEV1 % predicted (IQR)	**58 / (32.3)**	**66.7 (27.7)**	**54.1 (36.8)**

Comorbidities (frequency)

D-cycloserine	**Placebo**
Asthma (13)	Asthma (19)
Hypertension (11)	Hypertension (20)
Gastro-oesophageal reflux (10)	Gastro-oesophageal reflux (22)
Swelling of both ankles (11)	Swelling of both ankles (14)
Surgery to the chest (5)	Surgery to the chest (8)
Depression (5)	Depression (16)
Diabetes (4)	Diabetes (9)

Heart attack (3)	Heart attack (6)
Bronchiectasis (4)	Bronchiectasis (5)
Osteoporosis (2)	Osteoporosis (6)
Arrhythmia (2)	Arrhythmia (4)
Inflammatory bowel disease (2)	Inflammatory bowel disease (5)
Peptic ulcer (3)	Peptic ulcer (5)
Heart failure (1)	Heart failure (3)
Neuromuscular weakness (0)	Neuromuscular weakness (2)
Tuberculosis (1)	Tuberculosis (2)

Pulmonary rehabilitation details

Pulmonary rehabilitation was delivered by an experienced community pulmonary rehabilitation team. The full course ran for 6 weeks, with two sessions per week including an hour of exercises and an hour of education, as part of a standard pulmonary rehabilitation programme. Patients had been referred to pulmonary rehabilitation as part of their standard management. As pulmonary rehabilitation courses vary in duration and content we briefly describe the course that the patients in this study undertook. The Oxfordshire pulmonary rehabilitation programme is a cohort course which consists of two- hour sessions performed twice weekly for six weeks in an outpatient setting. This was run by Oxford Health NHS Foundation Trust. The Reading pulmonary rehabilitation programme differed in that it enrolled patients as part of a rolling intake rather than as a cohort. This was run by Berkshire Healthcare NHS. The Milton Keynes pulmonary rehabilitation programme enrolled patients as a cohort and took place within the hospital. This was run by Milton Keynes University Hospital NHS Foundation Trust. Content of the three courses was very similar.

Outpatient settings were non-medical facilities with the appropriate exercise equipment available, e.g. sports halls. Each session consisted of one hour of exercise (under supervision) and one hour of education.

Exercise sessions included both aerobic and strength exercises, tailored to the individual's ability. Aerobic exercises could include step-ups, walking (on the spot or treadmill) and exercising on a cycle ergometer. Strength exercises were conducted in sets (usually 3) of ten, and included sit-to-stand exercises, biceps curls, upright row and leg extensions.

Education sessions included items such as ‘introduction to rehabilitation’, ‘management of breathlessness’, ‘airway clearance’, ‘understanding your lung condition’, ‘home exercises’, medicine management’, ‘staying healthy’, ‘stress and relaxation’, ‘pacing and energy conservation’, ‘smoking cessation’, ‘continuing support’, ‘sexual function’ and ‘advanced care plans’. Supervised modified shuttle walking tests were used to measure patients’ improvement throughout the programme.

Randomisation Procedure

Study drugs were purchased from Ipswich Hospital Pharmacy Manufacturing Unit, Heath Road, Ipswich IP4 5PD, Tel: 01473 703603.

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. 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.

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) [1-4]**.** The most relevant paper (on treatment of snake phobia [5]**)** 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% around the mean) improvement in breathlessness-related anxiety, measured with our FMRI word task (pre-treatment mean score 38%, post treatment mean score 27%, difference 11%, SD15% around the mean) [6]. 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 a patient's breathlessness, using patient descriptors and incorporates both physical and affective aspects breathlessness. The questionnaire has been validated for use in patients with respiratory disease [7].

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 [8].

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" [9].

Fatigue Severity Scale: This 9-point questionnaire quantifies patient fatigue, which is well documented in its association with COPD [10].

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 [11].

Medical Research Council (MRC) breathlessness scale: The MRC scale quantifies perceived difficulty due to respiratory restrictions on a scale of 1 to 5 [12].

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) [13].

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 [14] [15].

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 [16] [15].

Physiological Measures

A trained respiratory nurse collected spirometry measures of FEV_1 and FVC using Association for Respiratory Technology and Physiology standards [17]. Participants performed two modified incremental shuttle walk tests (MSWT) [18], and heart rate and oxygen saturations (SpO_2) 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 (Go_2; Nonin Medical

Inc). Before and after the MWST participants also rated their breathlessness on a modified Borg scale [19]. 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, 7 minutes and 33 seconds duration and faces task - 168 volumes, 5 minutes and 42 seconds duration.

Field map scans of the B_0 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 [20]. 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 [20]. 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.

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 "XXXXXXXXXXXXXXX" with fixed length of 15 characters, and each time was presented for 7 seconds. No rating period followed these control blocks [6, 15, 20].

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 [21] and furthered by Young et al [22]. 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 [23, 24]. Neutral faces are not typically used as they can be interpreted as threatening or ambiguous in different settings [25].

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, [26, 27]) using the WhII.Standard.RData [28] 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) [29, 30]) 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 [31]; and fully detailed by [32]. 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 de-noising 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 [33]). Non-brain structures were removed using BET (Brain Extraction Tool [34]). 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 [35, 36] and BBR (Boundary Based Registration; part of the FMR Expert Analysis Tool, FEAT version 6.0 [37]). 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 [37]. 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 [38]).

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 [6] 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.

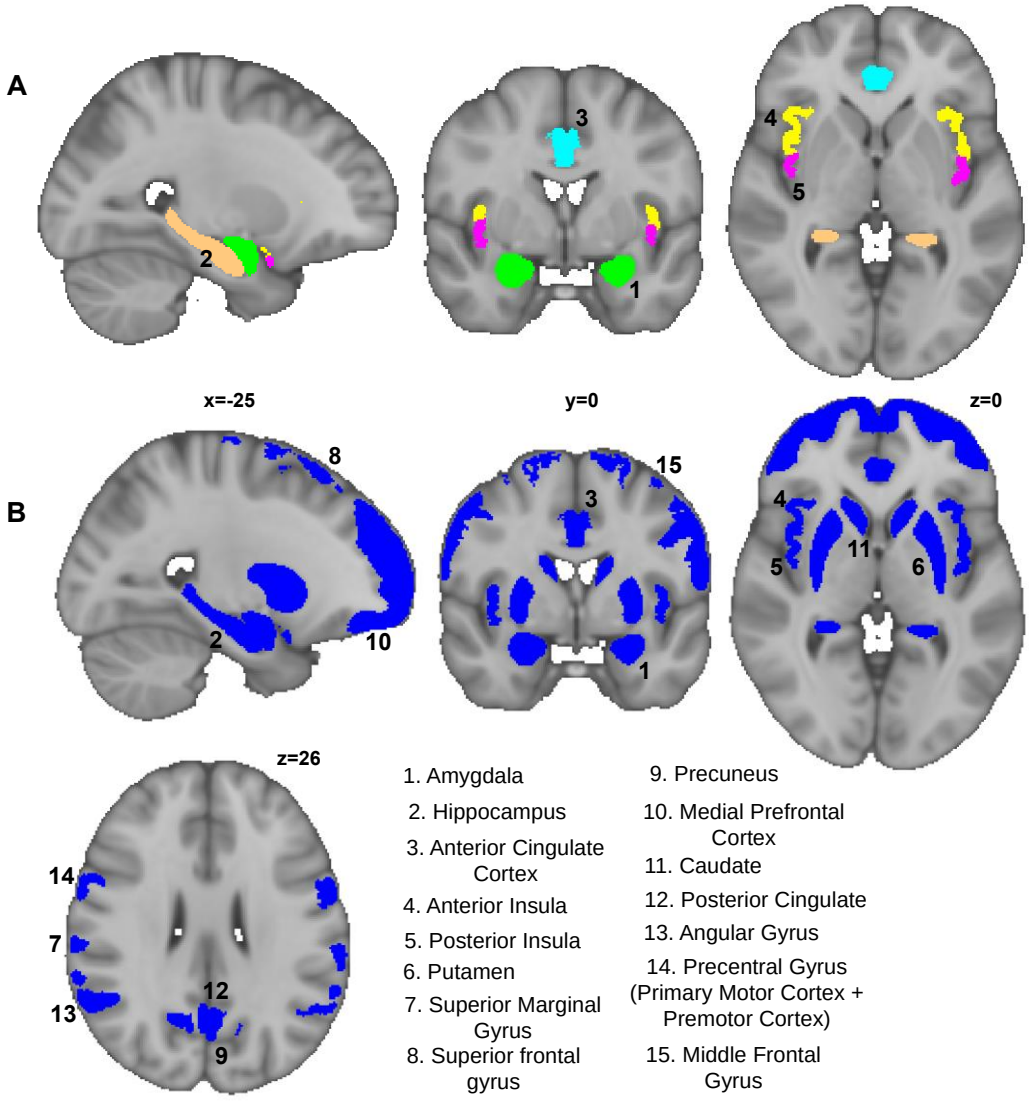
Left and right masks of bilateral regions of interests (anterior insula, posterior insula, anterior cingulate, amygdala and hippocampus) were added together to form one mask for each region of interest. Following registration each mask was re-thresholded at 40% probability to avoid interpolation errors before being binarised.

e-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.



e-Figure 1. *Panel A highlights the 5 key regions of interest while Panel B shows the*

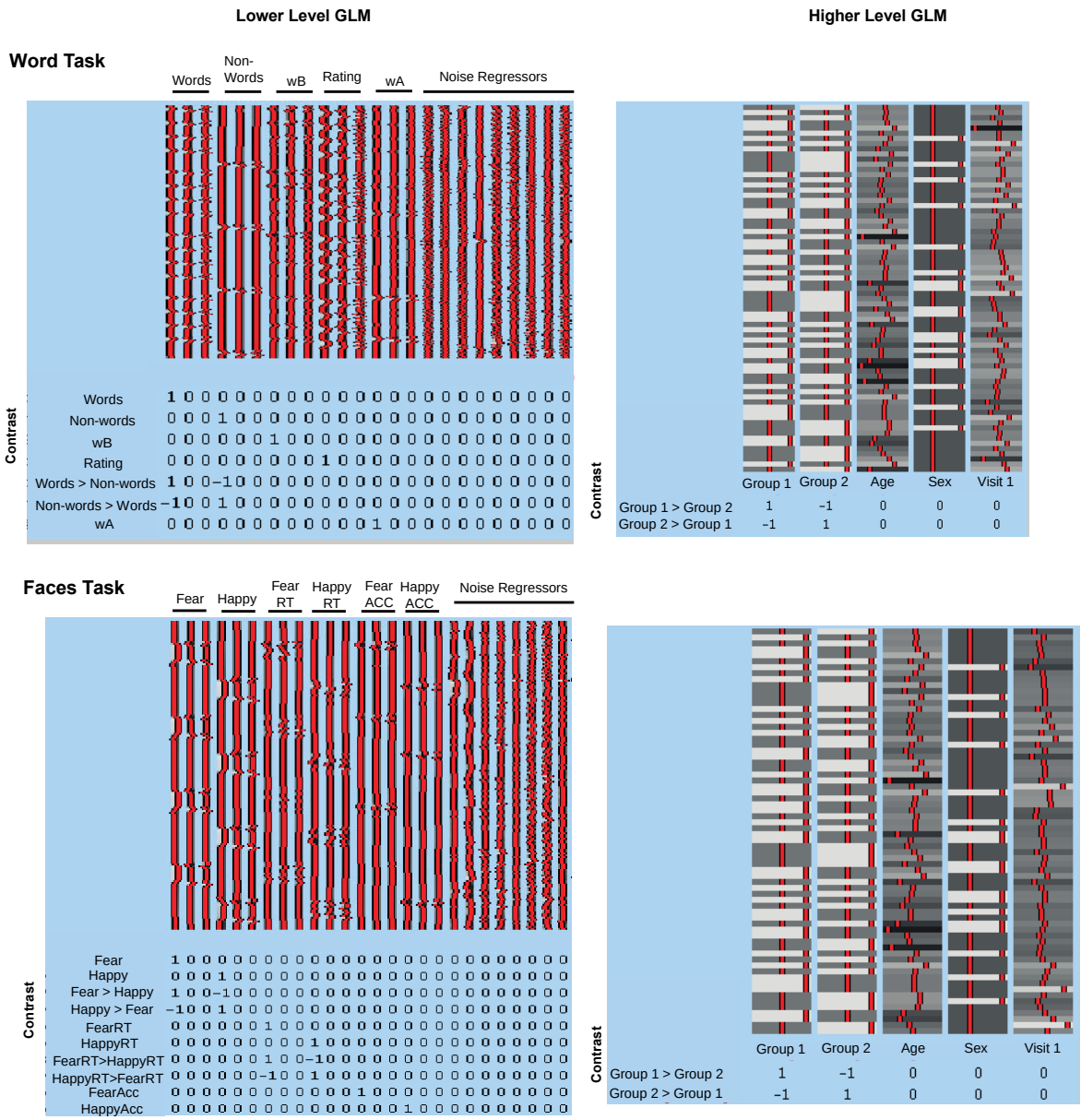
expanded region of interest map.

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.



e-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

Sensitivity Analysis

$$\triangle = \triangle_{CC} + Y_1 P_1 - Y_2 P_2$$

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

e-Table 3. *Missing data reported as the total percentage of data collected for that measure.* ***MSWT –*** *modified shuttle walk test;* ***HR –*** *Heart rate;* ***SpO_2% –*** *Oxygen saturation;* ***BMI –*** *Body mass index;* ***MRC –*** *Medical research council breathlessness scale*

	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	**11**	**9**	**14**	**3**	**14**	**9**
SpO_2% change	**11**	**9**	**14**	**3**	**14**	**9**
BMI	**-**	**-**	**8**	**3**	**30**	**21**
MRC	**-**	**-**	**-**	**-**	**3**	**3**

e-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 [1]standard deviation (SD) or [2]interquartile range (IQR).* ***BMI*** *= Body Mass Index,* ***MRC*** *= Medical Research Council clinical measure of breathlessness,* ***MSWT =*** *Modified Shuttle Walk Test,* ***HR*** *= Heart rate,* ***SpO_2%****= Peripheral Oxygen saturation, expressed as a*

	Visit One	Visit Two	Visit Three

percentage

	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 [1]
MRC	3 (1.0) [2]	3 (1.0) [2]	3 (1.0) [2]	2 (1.0) [2]	2 (1.3) [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 ***SpO_2%*** 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 ***SpO_2%*** 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]
FEV1/FVC	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]

e-Table 5. *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 [1]standard deviation (SD) or [2]interquartile range (IQR). Significance is reported as exploratory uncorrected p-values and as Family Wise*

		Visit Two	
	Cohort Change	**Uncorrected p-values**	**Corrected p-values**
Catastrophising	2.0 (5) [2]	**p<0.01***	**p=0.001***
Depression	2.2 ± 6.0 [1]	**p=0.004***	**p=0.01***
D12	3.0 ± 5.1 [1]	**p<0.001***	**p<0.001***
Fatigue	6.0 (11) [2]	**p<0.01***	**p=0.002***
BMI	-0.04 (0.6) [2]	p=0.52	p=0.61
MRC	0.0 (0) [2]	p=0.41	p=0.52
MSWT Distance (m)	0.0 (95.0) [2]	p=0.76	p=0.76
MSWT Borg Start	0.0 (1) [2]	p=0.40	p=0.52
MSWT Borg Change	0.5 (2) [2]	p=0.32	p=0.48
MSWT HR Start	0.5 ± 13.5 [1]	p=0.75	p=0.76
MSWT HR Change	-46 ± 24 [1]	p=0.22	p=0.35
MSWT *SpO_2%* Start	-0.2 ± 2.1 [1]	p=0.42	p=0.52
MSWT *SpO_2%* Change	0.0 (4) [2]	p=0.67	p=0.74
Vigilance	2.8 ± 12.1 [1]	p=0.05	p=0.12
FEV1/FVC	-0.01 (0.1) [2]	p=0.10	p=0.20
Avoidance - Alone	0.1 (0.3) [2]	**p=0.04***	p=0.09
Avoidance - Accompanied	0.05 (0.3) [2]	p=0.13	p=0.22
St George - Active	5.8 (12.0) [2]	**p=0.02***	**p=0.04***
St George – Impact	2.9 ± 7.9 [1]	**p=0.003***	**p=0.01***
St George – Symptom	4.2 ± 11.1 [1]	**p=0.002***	**p=0.01***
Trait	2.0 (6) [2]	**p=0.01***	**p=0.03***

Error (p<0.05) corrected p-values.

e-Table 6. *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 [1]standard deviation (SD) or [2]interquartile range (IQR). Significance is reported as exploratory uncorrected p-values and as Family Wise Error (p<0.05) corrected p-values.*

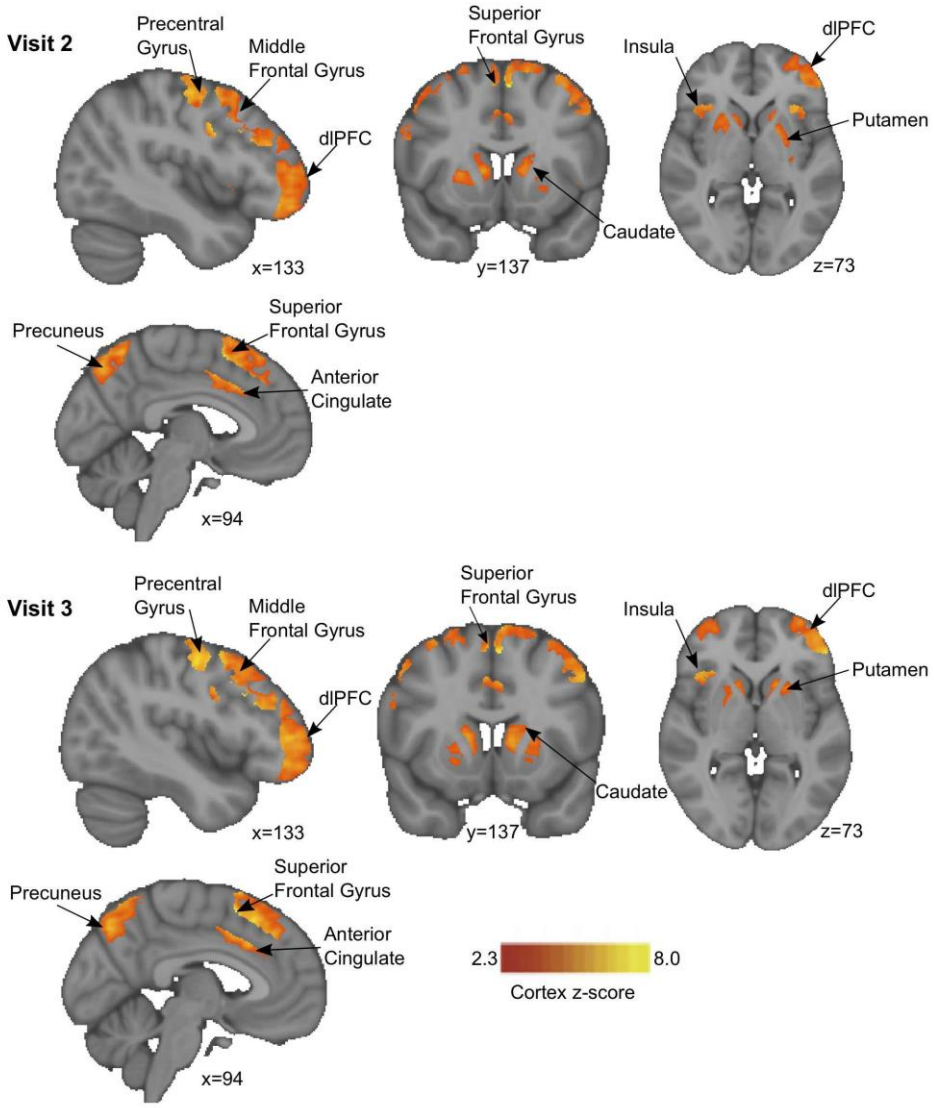
		Visit Three	
	Cohort Change	**Uncorrected p-values**	**Corrected p-values**
Catastrophising	3.0 (8) [2]	**p<0.001***	**p<0.001***
Depression	3.9 ± 6.0 [1]	**p<0.001***	**p<0.001***
D12	3.0 (7.6) [2]	**p<0.001***	**p<0.001***
Fatigue	7.8 ± 12.4 [1]	**p<0.001***	**p<0.001***
BMI	-0.04 (0.9) [2]	p=0.26	p=0.30
MRC	0.0 (1) [2]	**p=0.001***	**p=0.001***
MSWT Distance (m)	30 (80.0) [2]	**p<0.001***	**p=0.001***
MSWT Borg Start	0.0 (0.88) [2]	p=0.25	p=0.30
MSWT Borg Change	-0.01 ± 1.75 [1]	p=0.95	p=0.95
MSWT HR Start	2.6 ± 11.9 [1]	p=0.07	p=0.11
MSWT HR Change	-51 (27.8) [2]	p=0.09	p=0.12
MSWT *SpO_2%* Start	0.0 (3) [2]	p=0.38	p=0.40
MSWT *SpO_2%* Change	-1.0 (5.8) [2]	p=0.12	p=0.14
Vigilance	5.0 (11.8) [2]	**p=0.002***	**p=0.002***
FEV1/FVC	-0.01 (0.1) [2]	p=0.36	p=0.40
Avoidance - Alone	-0.1 (0.3) [2]	**p=0.01***	**p=0.02***
Avoidance - Accompanied	0.05 (0.3) [2]	**p=0.02***	**p=0.02***
St George - Active	6.1 (14.0) [2]	**p<0.001***	**p=0.001***
St George – Impact	5.1 (10.9) [2]	**p<0.001***	**p<0.001***
St George – Symptom	6.5 ± 12.5 [1]	**p<0.001***	**p<0.001***
Trait	3.0 ± 6.6 [1]	**p<0.001***	**p<0.001***

Control Task

e-Table 7. *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=0.05**	**p=0.05**
Happy	**-30.4 (102.8)**	**p=0.04**	**p=0.05**

Word Task



e-Figure 3. *Mean changes in Blood Oxygen Level Dependent (BOLD) activity at visit 2 (top panel) and visit 3 (bottom panel) over the course of pulmonary rehabilitation across both groups (drug/placebo) in response to breathlessness related words. Significant activity was observed in the following regions: Precentral gyrus, middle frontal gyrus, dorsolateral prefrontal cortex (dlPFC), superior frontal gyrus, caudate, insula, putamen and anterior cingulate with a cluster corrected threshold of z2.3 p<0.05.*

e-Table 8. *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*

		Visit Two	

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<0.001**	**p<0.001**
wB	**6.3 (14.9) [2]**	**p<0.001**	**p<0.001**

Effect of D-cycloserine

e-Table 9. *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,* ***SpO_2%****= Peripheral Oxygen saturation, expressed as a percentage*

	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	3.0 (5.5) [2]	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
D12	3.0 (13.3) [2]	2.5 (8) [2]	0.86
MSWT Distance (m)	0.0 (11.3) [2]	5.0 (7.0) [2]	0.85
Fatigue	9.7 ± 12.5 [1]	5.7 ± 12.0 [1]	0.86
MSWT Borg Start	0 (1)	0 (1)	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 SpO_2% Start	-0.30 ± 2.2 [1]	-0.1 ± 2.2 [1]	0.85
MSWT SpO_2% Change	0 (5) [2]	0 (4.0) [2]	0.91
Vigilance	3.8 ± 10.6 [1]	1.8 ± 13.6 [1]	0.85
FEV1/FVC	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		

Visit Three
D-cycloserine Placebo p-value

Table 10. *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,* ***SpO_2%*** *= Peripheral Oxygen saturation, expressed as a percentage.*

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 ***SpO_2%*** Start	**0.0 (2)** [2]	**0.0 (3)** [2]	**0.86**
MSWT ***SpO_2%*** 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**		

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.

e-Table 11. 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 stimulate 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

e-Table 12. 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 stimulate 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. 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.
2. 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.
3. 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.
4. 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.
5. 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.
6. 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).
7. Yorke, J., et al., *Quantification of dyspnoea using descriptors: development and initial testing of the Dyspnoea-12.* 2010. **65**(1): p. 21-26.
8. Radloff, L.S., *The CES-D Scale:A Self-Report Depression Scale for Research in the General Population.* 1977. **1**(3): p. 385-401.
9. Spielberger, C.D., *State-Trait Anxiety Inventory*, in *The Corsini Encyclopedia of Psychology*. 2010.
10. 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.
11. 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.
12. 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.
13. Chambless, D.L., et al., *The Mobility Inventory for Agoraphobia.* Behav Res Ther, 1985. **23**(1): p. 35-44.
14. De Peuter, S., et al., *Illness-specific catastrophic thinking and overperception in asthma.* Health Psychol, 2008. **27**(1): p. 93-9.
15. 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.
16. 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.
17. 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 1 www.gpiag.org 2 www.artp.org 3 www.educationforhealth.org.uk. Prim Care Respir J, 2009. **18**(3): p. 130-47.
18. Bradley, J., et al., *Validity of a modified shuttle test in adult cystic fibrosis.* Thorax, 1999. **54**(5): p. 437-9.
19. 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.
20. 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.
21. Ekman, P.F.W., *Pictures of Facial Affect.* Consult. Psychol. Press, 1975.
22. Young, A.W., et al., *Facial expression megamix: Tests of dimensional and category accounts of emotion recognition.* Cognition, 1997. **63**(3): p. 271-313.
23. Godlewska, B.R., et al., *Short-term SSRI treatment normalises amygdala hyperactivity in depressed patients.* Psychological Medicine, 2012. **42**(12): p. 2609-2617.
24. 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.
25. 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.
26. Griffanti, L., et al., *ICA-based artefact removal and accelerated fMRI acquisition for improved resting state network imaging.* Neuroimage, 2014. **95**: p. 232-47.
27. 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.
28. Filippini, N., et al., *Study protocol: the Whitehall II imaging sub-study.* BMC Psychiatry, 2014. **14**(1): p. 159.
29. 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.
30. Brooks, J.C., et al., *Physiological noise modelling for spinal functional magnetic resonance imaging studies.* Neuroimage, 2008. **39**(2): p. 680-92.
31. Faull, O.K., et al., *Conditioned respiratory threat in the subdivisions of the human periaqueductal gray.* Elife, 2016. **5**.
32. Hayen, A., et al., *Opioid suppression of conditioned anticipatory brain responses to breathlessness.* Neuroimage, 2017. **150**: p. 383-394.
33. 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.
34. Smith, S.M., *Fast robust automated brain extraction.* Hum Brain Mapp, 2002. **17**(3): p. 143-55.

35. 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.
36. Jenkinson, M., *Fast, automated, N-dimensional phase-unwrapping algorithm.* Magn Reson Med, 2003. **49**(1): p. 193-7.
37. Greve, D.N. and B. Fischl, *Accurate and robust brain image alignment using boundary-based registration.* Neuroimage, 2009. **48**(1): p. 63-72.
38. Andersson, J., *Non-Linear registration, aka spatial normalisation.* FMRIB technical report, 2010. **TR07JA2**.