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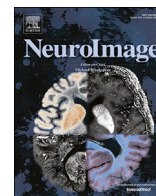
Cortical processing of breathing perceptions in the athletic brain

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A B S T R A C T

Athletes regularly endure large increases in ventilation and accompanying perceptions of breathlessness. Whilst breathing perceptions often correlate poorly with objective measures of lung function in both healthy and clinical populations, we have previously demonstrated closer matching between subjective breathlessness and changes in ventilation in endurance athletes, suggesting that athletes may be more accurate during respiratory interoception. To better understand the link between exercise and breathlessness, we sought to identify the mechanisms by which the brain processing of respiratory perception might be optimised in athletes.

Twenty endurance athletes and twenty sedentary controls underwent 7 T functional magnetic resonance imaging. Inspiratory resistive loading induced conscious breathing perceptions (breathlessness), and a delay-conditioning paradigm was employed to evoke preceding periods of breathlessness-anticipation. Athletes demonstrated anticipatory brain activity that positively correlated with resulting breathing perceptions within key interoceptive areas, such as the thalamus, insula and primary sensorimotor cortices, which was negatively correlated in sedentary controls. Athletes also exhibited altered connectivity between interoceptive attention networks and primary sensorimotor cortex. These functional differences in athletic brains suggest that exercise may alter anticipatory representations of respiratory sensations. Future work may probe whether these brain mechanisms are harnessed when exercise is employed to treat breathlessness within chronic respiratory disease.

Introduction

Athletes are able to undertake incredible feats of human achievement, with faster, higher and stronger performances recorded each year. Whilst exercise training is known to induce widespread physiological changes in the periphery, the concurrent changes in the structure and function of the athletic brain are less well investigated. For endurance athletes, exercise training is targeted to improve the ability of tissues to utilize oxygen in the combustion of fuels such as fat and carbohydrate, producing the energy required for repeated skeletal muscle contraction (Holloszy and Coyle, 1984; Jones and Carter, 2000). However, the role of the brain in perceiving and modulating changing sensations from the periphery, which is useful for maintenance of homeostasis during situations of perturbed physiology, is often overlooked.

Ventilation during exercise is tightly controlled, balancing neurally-modulated feed forward commands and peripheral feedback to stimulate appropriate ventilation for exercising needs (Kaufman and Forster, 1996; Waldrop et al., 2010). Interoceptive monitoring of respiratory sensations contributes to the maintenance of homeostasis (Davenport

and Vovk, 2009), and with sufficient exercise intensity, the strain of immense increases in ventilation induces perceptions of breathlessness (El-Manshawi et al., 1986; Takano et al., 1997; Lansing et al., 2000; Borg et al., 2010). Whilst endurance athletes are repeatedly exposed to these respiratory sensations and breathlessness, it is as yet unknown whether brain networks involved in these perceptions may also adapt to better cope with exercise demands. This understanding would allow us to explore how processing of ventilatory signals might be altered in different physiological states, such as in athletes or conversely in chronic respiratory disease, where subjective reports of breathlessness are often discordant with objective measures of lung function and ventilation (Herigstad et al., 2017).

Whilst exteroception can be thought of as the brain's processing of external sensations (such as vision, audition and touch), 'interoception' is the processing of homeostatic sensations from the body's *internal milieu* (Craig, 2002;2003; Kleckner et al., 2017). As such, breathlessness can be thought of an extreme interoceptive perception of breathing, where it is monitored as a potentially life-threatening perturbation of homeostasis. Previous neuroimaging work has investigated the brain correlates of

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these salient perceptions of breathlessness, where an extensive limbic and paralimbic cortical network has been revealed during breathlessness induced via carbon dioxide inhalation (Liotti et al., 2001; Banzett et al., 2000; Evans et al., 2002), and both a sensorimotor and limbic brain network during breathlessness induced via inspiratory loading (Peiffer et al., 2001; von Leupoldt et al., 2008; Paulus et al., 2012; Faull et al., 2016b; Faull and Pattinson, 2017; Hayen et al., 2017). Furthermore, cortical differences have previously been demonstrated between endurance athletes and sedentary individuals during resistive inspiratory loading, postulated to represent a reduction in body prediction error, or mismatch between expected and actual breathing perceptions (Paulus et al., 2012).

Here, we sought to better understand these body predictions regarding breathing in endurance athletes. Importantly, prior experiences of strong respiratory sensations may alter the way someone predicts, anticipates and perceives their breathing (Faull et al., 2017; Van den Bergh et al., 2017; Herigstad et al., 2017). Expectations regarding upcoming respiratory sensations from conditioned cues (Pavlov et al., 2003), for example the breathlessness associated with an approaching hill whilst running, can be an important influence on both threat behaviours and preventative actions (i.e. to avoid the hill) (Lang et al., 2011), or on the perception itself (Price et al., 1999; Porro et al., 2002; Wager et al., 2004). Repeated breathlessness exposure may alter this anticipation in athletes, focusing their attention towards respiratory sensations (Merikle and Joordens, 1997; Phelps et al., 2006; Ling and Carrasco, 2006), reducing their anxiety (Spinhoven et al., 1997; Bogaerts et al., 2005; Tang and Gibson, 2005) or improving their interoceptive ability (Gray et al., 2007; Critchley et al., 2013; Mallorqui-Bague et al., 2016; Garfinkel et al., 2016b, 2016a). Interestingly, exercise therapy is currently the most effective treatment for breathlessness associated with chronic obstructive pulmonary disease (COPD), improving breathlessness intensity and anxiety (Carrieri-Kohlman et al., 1996, 2001; Herigstad et al., 2017) without concurrent improvements in lung function. It is possible that athletes may have different prior expectations and anticipation of breathlessness, although this has yet to be investigated.

In previous work we have observed closer matching between changes in ventilation and perceptions of breathlessness in endurance athletes compared to sedentary individuals (Faull et al., 2016a). Here, we sought to identify how the brain processing of both anticipation and perception of respiratory sensations may be altered in these athletes, to better understand potential contributors to ventilatory interoception. We investigated functional brain activity using magnetic resonance imaging (fMRI) during both conditioned anticipation and perception of a breathlessness stimulus. We also examined potential differences in the resting temporal coherence, or 'functional connectivity' (Gerstein and Perkel, 1969; Van Den Heuvel and Pol, 2010) of brain networks involved in attention towards sensory information, allostasis (the maintenance of homeostasis through physiological or behavioural changes) and interoception (Kleckner et al., 2017). Differences in underlying functional connectivity may help us to understand how the athlete brain may be altered to facilitate accurate respiratory perceptions, and we hypothesized that these athletes would demonstrate both altered functional breathlessness-related brain activity and connectivity compared to their sedentary counterparts.

Materials and methods

Subjects

The Oxfordshire Clinical Research Ethics Committee approved the study and volunteers gave written, informed consent. Forty healthy, right-handed individuals undertook this study, with no history of smoking or any respiratory disease. This cohort comprised two groups; 20 subjects who regularly participated in endurance sport, and 20 age- and sex-matched (± 2 years) sedentary subjects (in each group: 10 males, 10 females; mean age $\pm$ sd: athletes 25.8 ± 7.5 years, sedentary

25.7 ± 7.4 years). Athletes were active participants in endurance sports (cycling, rowing and endurance running), with training sessions conducted at least 5 times per week, and had been performing endurance activity for a mean of 8.4 ± 6.5 years. Sedentary subjects did not partake in any regular exercise or sport.

Stimuli and tasks

Prior to scanning, all subjects underwent breathlessness testing during exercise and chemostimulated hyperpnea, which have been presented elsewhere (Faull et al., 2016a). Briefly, this test involved stimulating ventilation and recording perceptions of breathlessness intensity and anxiety by elevating arterial pressure of arterial carbon dioxide (PCO2) levels, termed 'hypercapnia'. This was induced by breathing a gas mixture that contained supplementary CO_2, with the amount titrated to achieve specific increases in the measured exhaled CO_2 (pressure of end-tidal carbon dioxide, $P_{ET}CO_2$) to approximate arterial PCO2. Two levels of hypercapnia were employed, with a 3 min elevation in $P_{ET}CO_2$ of 0.8% (6.1 mm Hg) and 1.5% (11.2 mm Hg) above baseline induced in a randomised order, separated by 4 min of breathing medical air. To raise $P_{ET}CO_2$, the composition of the delivered gas mixture was adjusted by the experimenter via titration of a gas mixture of 20% CO_2, 21% O_2, 59% N_2 (nitrogen). To maintain pressure of end-tidal oxygen ($P_{ET}O_2$) constant at baseline levels (measured when breathing room air) throughout the protocol, a second gas mixture consisting of 7% O_2, 93% N_2 was also titrated into the delivered gas mix, to maintain consistent $P_{ET}O_2$ despite the increased ventilation induced by elevated $P_{ET}CO_2$ (which would otherwise lead to increases in $P_{ET}O_2$).

Whilst no mean differences in ventilatory or subjective breathlessness responses were found, this test revealed between-group differences in the relationship between subjective responses and physiological changes in ventilation. The correlations between percentage change in ventilation and percentage change in breathlessness intensity/anxiety scores were calculated for each group at each level of hypercapnia, and the difference between group regression slopes were compared at each hypercapnic level by calculating a p value (two-tailed) testing the null hypothesis that the slopes are all identical (the lines are parallel). During both mild and moderate hypercapnia, the athlete group showed a positive linear correlation between change in ventilation and change in breathing anxiety that was significantly different from sedentary subjects (slope difference: mild $p = 0.018$; moderate $p = 0.011$). Athletes also demonstrated significant positive correlations for breathlessness intensity against change in ventilation, where the slope was significantly different to sedentary subjects in moderate ($p = 0.047$) but not mild ($p = 0.177$) hypercapnia (Faull et al., 2016a) (full results supplied in the supplementary material).

For the breathlessness task in this study, subjects were trained using an aversive delay-conditioning paradigm the day prior to scanning, to associate simple shapes with an upcoming breathlessness (inspiratory resistance) stimulus (Faull and Pattinson, 2017). A breathing system was used to remotely administer periods of inspiratory resistive loading to induce breathlessness (as predicted by the conditioned cues). The breathing system contained an inspiratory resistance arm (using a porous glass disk) with a non-rebreathing valve connected to a mouth piece, which could be periodically applied using the addition or removal of medical air through an alternative inspiratory non-rebreathing arm (detailed elsewhere (Faull et al., 2016b; Faull and Pattinson, 2017)). Mean peak inspiratory resistance was recorded at 14.7 (± 8.3) cmH2O for the loading periods across subjects, and group values are presented in Tables 2 and 3. The subject's nose was blocked using foam earplugs and they were asked to breathe through their mouth for the duration of the experiment.

Two conditions were trained: 1) A shape that always predicted upcoming breathlessness (100% contingency pairing), and 2) A shape that always predicted unloaded breathing (0% contingency pairing with inspiratory resistance). The 'certain upcoming breathlessness' symbol was presented on the screen for 30 s, which included a varying 5–15 s

anticipation period before the loading was applied, with the remainder of the symbol presentation time (15–25 s) consisting of inspiratory loading ('breathlessness' period). The anticipation and breathlessness periods were consistent between both groups. The 'unloaded breathing' symbol was presented for 20 s to correspond to the average breathlessness period, and each condition was repeated 14 times in a randomised order. The inter-trial interval varied between 9 and 20 s, to allow for adequate recovery between stimuli. Conscious associations between cue and threat level (cue contingencies) were required and verified in all subjects by reporting (in writing) the meaning of each of the symbols both following the training session the day before scanning, and immediately prior to the MRI scan.

Rating scores of breathing intensity were recorded after every stimulus, using a visual-analogue scale (VAS) with a sliding bar to answer the question 'How difficult was the previous stimulus?' where the subjects moved between 'Not at all difficult' (0%) and 'Extremely difficult' (100%). Subjects were also asked to rate how anxious each of the symbols made them feel ('How anxious does this symbol make you feel?') using a VAS between 'Not at all anxious' (0%) and 'Extremely anxious' (100%) immediately following the functional MRI protocol.

Physiological measurements

We used established methods to decorrelate the effects of hypercapnia from the localised BOLD responses associated with breathing against an inspiratory resistance, where additional, matched carbon dioxide (CO_2) boluses were interspersed during rest periods in the fMRI protocols (Pattinson et al., 2009b; Faull et al., 2015, 2016b). An example physiological file demonstrating $P_{ET}CO_2$ traces across breathlessness and rest periods can be found in the supplementary material. In addition, a mildly hyperoxic state was achieved through a constant administration of oxygen at a rate of 0.5 L/min, to minimise fluctuations in $P_{ET}O_2$ (Table 2). Physiological measures were recorded continuously using respiratory bellows surrounding the chest, and heart rate was measured using a pulse oximeter (9500 Multigas Monitor, MR Equipment Corp., NY, USA) during the training session and MRI scan, as previously described (Faull et al., 2016b).

MRI scanning sequences

MRI was performed with a 7 T S Magnetom scanner, with 70 mT/m gradient strength and a 32 channel Rx, single channel birdcage Tx head coil (Nova Medical).

BOLD scanning: A T2*-weighted, gradient echo EPI was used for functional scanning. The field of view (FOV) covered the whole brain and comprised 63 slices (sequence parameters: TE, 24 ms; TR, 3 s; flip angle, 90°; voxel size, $2 \times 2 \times 2$ mm; field of view, 220 mm; GRAPPA factor, 3; echo spacing, 0.57 ms; slice acquisition order, descending; orientation, axial tilted), with 550 vol (scan duration, 27 min 30 s) for the task fMRI, and 190 vol (scan duration, 9 min 30 s) for a following resting-state acquisition (eyes open).

Structural scanning: A T1-weighted structural scan (MPRAGE, sequence parameters: TE, 2.96 ms; TR, 2200 ms; flip angle, 7°; voxel size, $0.7 \times 0.7 \times 0.7$ mm; field of view, 224 mm; inversion time, 1050 ms; bandwidth; 240 Hz/Px) was acquired. This scan was used for registration of functional images.

Additional scanning: Fieldmap scans (sequence parameters: TE1, 4.08 ms; TE2, 5.1 ms; TR, 620 ms; flip angle, 39°; voxel size, $2 \times 2 \times 2$ mm) of the B_0 field were also acquired to assist distortion-correction.

Physiological data analysis

Values for $P_{ET}CO_2$ were extrapolated for use as a noise regressor in fMRI analysis (explained below). Respiratory waveforms, respiratory volume per unit time (RVT) and cardiac pulse oximetry triggers were included in the image denoising procedures (explained below). RVT values were calculated per breath as the respiration amplitude divided by the respiratory period, and 'baseline' RVT was the average across all non-stimulus (rest) periods, i.e., during the presentation of the fixation cross. Values for mean and peak resistive loading, mean $P_{ET}CO_2$ and $P_{ET}O_2$, respiratory rate and RVT were calculated separately across each stimulus using custom written scripts in MATLAB (R2013a, The Mathworks, Natick, MA). Measures were averaged across each subject in each condition (unloaded breathing, anticipation and breathlessness). Peak mouth pressure was also calculated in each block and averaged in each subject for the resistive loading condition. Mean peak mouth pressure, breathlessness intensity and breathlessness anxiety ratings were then compared between the two groups using a student's paired T-test.

Imaging analysis

Preprocessing: Image processing was performed using the Oxford Centre for Functional Magnetic Resonance Imaging of the Brain Software Library (FMRIB, Oxford, UK; FSL version 5.0.8; http://www.fmrib.ox.ac.uk/fsl/). The following preprocessing methods were used prior to statistical analysis: motion correction and motion parameter recording (MCFLIRT (Jenkinson et al., 2002)), removal of the non-brain structures (skull and surrounding tissue) (BET (Smith, 2002)), spatial smoothing using a full-width half-maximum Gaussian kernel of 2 mm (to maintain the resolution afforded to us by 7 T scanning, and as we were not constrained by any minimal smoothing requirements imposed by the use of gaussian random field theory in parametric cluster thresholding analyses), and high-pass temporal filtering (Gaussian-weighted least-squares straight line fitting; 120 s) (Smith et al., 2004). B_0 field unwarping was conducted with a combination of FUGUE (FMRIB's Utility for Geometrically Unwarping EPIs) and BBR (Boundary-Based-Registration; part of FEAT: FMRI Expert Analysis Tool, version 6.0 (Greve and Fischl, 2009)). Data denoising was conducted using a combination of independent components analysis (ICA) and retrospective image correction (RETRO-ICOR) (Harvey et al., 2008; Glover et al., 2000; Brooks et al., 2013) using the externally recorded physiological measures (Faull et al., 2016b), and included simultaneous regression of motion parameters. Noise components from the ICA decomposition were identified via established noise properties pertaining to spatial location, frequency and timeseries patterns (Griffanti et al., 2017).

Image registration: Following preprocessing, the functional scans were registered to the MNI152 ($1 \times 1 \times 1$ mm) standard space (average T1 brain image constructed from 152 normal subjects at the Montreal Neurological Institute (MNI), Montreal, QC, Canada) using a two-step process: 1) Registration of subjects' whole-brain EPI to T1 structural image was conducted using BBR (6 DOF) with (nonlinear) fieldmap distortion-correction (Greve and Fischl, 2009), and 2) Registration of the subjects' T1 structural scan to 1 mm standard space was performed using an affine transformation followed by nonlinear registration (FNIRT) (Andersson et al., 2007).

Functional voxelwise and group analysis: Functional data processing was performed using FEAT (FMRI Expert Analysis Tool), part of FSL. The first-level analysis in FEAT incorporated a general linear model (Woolrich et al., 2004), with the following regressors: Resistive loading periods (calculated from physiological pressure trace from the onset of added inspiratory pressure at the mouth, through to the termination of each inspiratory load); anticipation of breathlessness (calculated from onset of anticipation symbol to onset of resistance application); and unloaded breathing (onset and duration of 'unloaded breathing' symbol). An example physiological trace demonstrating inspiratory loading can be found in the supplementary material. Additional regressors to account for relief from breathlessness, periods of rating using the button box, demeaned ratings of intensity between trials, and a period of no loading following the final anticipation period (for decorrelation between anticipation and breathlessness) were also included in the analysis. A final $P_{ET}CO_2$ regressor was formed by linearly interpolating between

end-tidal CO_2 peaks, and included in the general linear model to decorrelate any $P_{ET}CO_2$-induced changes in BOLD signal from the respiratory tasks (McKay et al., 2008; Pattinson et al., 2009a, 2009b; Faull et al., 2015, 2016b). An example general linear model at the subject level can be found in the supplementary material. Contrasts for breathlessness (vs. baseline) and differential contrasts of anticipation of breathlessness > unloaded breathing (referred to as 'anticipation' or 'anticipation of breathlessness') were investigated at the group level.

Functional voxelwise analysis incorporated haemodynamic response function (HRF) modelling of all regressors (including $P_{ET}CO_2$) using a set of three linear optimal basis functions (FMRIB's linear optimal basis set: FLOBS) to produce three input regressors for each explanatory variable. These FLOBS regressors help to account for any HRF differences caused by slice-timing delays, differences across the brainstem and cortex, or between individuals (Handwerker et al., 2004; Devonshire et al., 2012). Time-series statistical analysis was performed using FILM, with local autocorrelation correction (Woolrich et al., 2001). The second and third waveforms were orthogonalised to the first to model the 'canonical' HRF, of which the parameter estimate was then passed up to the group analysis in a mixed-effects analysis. Group analysis was conducted using rigorous permutation testing of a General Linear Model (GLM) using FSL's Randomize tool (Winkler et al., 2014), where the GLM consisted of group mean BOLD activity for each group, and demeaned, separated breathlessness intensity and anxiety covariates for each group. Including breathlessness scores into the anticipation contrast allows us to identify preparatory brain activity that predicts the subsequent breathlessness perception when the stimulus is applied. A combined whole-group analysis of fMRI data has been previously reported (Faull and Pattinson, 2017), while this focus of this analysis was to compare between the groups. Mean voxelwise differences between groups were calculated, as well as the interactions between group and breathlessness intensity/anxiety scores. A stringent initial cluster-forming threshold of $t = 3.1$ was used, in light of recent reports of lenient thresholding previously used in fMRI (Eklund et al., 2016), and images were family-wise-error (FWE) corrected for multiple comparisons. Significance was taken at $p < 0.05$ (corrected).

Resting functional connectivity analysis: Following preprocessing and image registration, resting state scans from all subjects were temporally concatenated and analysed using independent component analysis (ICA) using MELODIC (Beckmann and Smith, 2004), part of FSL. ICA decomposes the data into a set of spatial maps and their associated timecourses, referred to as 'functional networks'. Model order in the group ICA was set to 25 spatially independent components, and ICA components were identified across both groups to ensure component correspondence. Dual regression (Beckmann et al., 2009) was then used to delineate subject-specific timecourses of these components, and their corresponding subject-specific spatial maps. Group differences were then identified via these subject-specific spatial maps, which were again analysed non-parametrically using Randomize (part of FSL) (Winkler et al., 2014) with the same GLM and significance thresholds previously applied to the functional task group analysis. Twenty components were identified as signal, and two components of interest ('default mode' network and 'task positive' network) were considered for group differences, in accordance with recent interoceptive research (Kleckner et al., 2017). Therefore, p threshold significance was adjusted to $p < 0.025$ using Bonferroni correction for multiple comparisons.

Results

Physiology and psychology of breathlessness

Baseline physiological and psychological measures for each group are included Table 1. Mean physiological values for each group for mouth pressure, $P_{ET}CO_2$, $P_{ET}O_2$, RVT, respiratory rate and RVT are presented in Table 2. The only differences observed between the groups in any condition were respiratory rate during unloaded breathing and anticipation,

however, when ventilatory changes are corrected to percentage change from baseline (as in the RVT measure), no differences were observed between the groups. Group scores for breathlessness intensity and anxiety are presented in Table 3, with no mean differences observed between groups. Previously, we have reported a difference in the accuracy between subjective breathlessness scores and changes in ventilation induced via a hypercapnic challenge (Faull et al., 2016a) in the same subjects used in the current study. For clarity, we have reproduced these results in Supplementary Fig. 1.

Task fMRI analysis

Mean group differences: Significant clusters of activity during anticipation of breathlessness in each group are presented in Fig. 1. In sedentary subjects, significantly increased BOLD activity was observed in the right anterior insula, operculum and bilateral primary motor cortex, and decreased BOLD activity in bilateral posterior cingulate cortex, precuneus, lateral occipital cortex, hippocampus, parahippocampal gyrus and amygdala. In athletes, increased BOLD activity was observed in bilateral anterior insula, operculum and primary motor cortex, and right supplementary motor cortex, and decreased BOLD activity in bilateral precuneus, hippocampus, parahippocampal gyrus and amygdala. No statistically significant voxelwise differences were observed between group mean activities during anticipation of breathlessness (differentially contrasted against unloaded breathing).

Significant clusters of BOLD activity during breathlessness in each group are also presented in Fig. 1. In sedentary subjects, significantly increased BOLD activity was observed in the bilateral anterior and middle insula, operculum, primary sensory and motor cortices, supplementary motor cortex, supramarginal gyrus and cerebellar VI, and decreased BOLD activity in bilateral precuneus. In athletes, significantly increased BOLD activity was observed in the right dorsolateral prefrontal cortex, bilateral anterior and middle insula, operculum, primary sensory and motor cortices, supplementary motor cortex, left visual cortex and cerebellar Crus-I, and decreased BOLD activity in right amygdala, hippocampus and superior temporal gyrus. No statistically significant voxelwise differences were observed between group mean activities during breathlessness.

Subjective breathlessness scores: The brain activity that correlated with breathlessness scores of intensity and anxiety was considered separately and compared between groups, to identify any interaction effects (group x subjective score). When groups were considered separately, athletes

Table 1
Mean ($\pm$SD) baseline group physiology measures and questionnaires. Abbreviations: BMI, body mass index; FVC, forced vital capacity; FEV1/FVC, forced expiratory volume in 1 s as a fraction of forced vital capacity; MVV, maximal voluntary ventilation. Exercise exposure ('Exercise') was measured as the number of hours per week x intensity of exercise (1–3: easy, moderate, intense). *Significantly different ($p < 0.05$) between groups. Table is adapted from previously published data (Faull et al., 2016a) under the Creative Commons license.

	Athlete	Sedentary	
Number of females/males	10/10	10/10	
Age (years)	25.8 (7.5)	25.7 (7.4)	$p = 0.95$
Height (m)	1.8 (0.9)	1.7 (0.1)	$p = 0.01$*
Weight (kg)	75.2 (10.1)	68.7 (13.6)	$p = 0.09$
BMI (kg/m2)	23.1 (2.8)	23.3 (3.5)	$p = 0.87$
FVC (L)	5.7 (0.9)	4.2 (1.2)	$p < 0.01$*
FVC (% predicted)	109.5 (9.4)	91.0 (19.5)	$p < 0.01$*
FEV1/FVC (%)	78.2 (7.0)	81.3 (4.6)	$p = 0.10$
MVV (L/min)	150.9 (42.8)	113.0 (39.5)	$p = 0.01$*
Exercise (volume x intensity)	20.3 (6.0)	1.8 (1.9)	$p < 0.01$*
Trait anxiety	29.6 (5.9)	30.8 (6.8)	$p = 0.54$
Pre-exercise state anxiety	27.8 (6.5)	25.6 (5.4)	$p = 0.25$
Anxiety sensitivity index	13.5 (6.1)	16.1 (7.7)	$p = 0.24$
Depression	6.4 (4.2)	7.6 (4.7)	$p = 0.40$

Table 2

Mean (±SD) physiological variables across conditioned respiratory tasks. *Significantly ($p < 0.05$) different from sedentary group. Abbreviations: $P_{ET}CO_2$, pressure of end-tidal carbon dioxide; $P_{ET}O_2$, pressure of end-tidal oxygen; RVT, respiratory volume per unit time; bpm, beats per minute.

	Unloaded breathing		Anticipation		Breathlessness	
	ATHLETE	SEDENTARY	ATHLETE	SEDENTARY	ATHLETE	SEDENTARY
$P_{ET}CO_2$ (mmHg)	35.96 (5.56)	35.08 (3.20)	35.50 (5.81)	34.76 (3.60)	36.34 (6.23)	35.40 (3.92)
$P_{ET}O_2$ (mmHg)	129.68 (6.41)	134.09 (15.15)	129.55 (6.75)	133.59 (13.47)	131.18 (6.83)	137.55 (16.42)
Respiratory rate (min^{-1})	10.15 (2.59)*	13.35 (3.51)	9.99 (2.63)*	12.93 (4.29)	9.40 (3.58)	11.54 (5.11)
RVT (% change from baseline)	−4.06 (5.70)	−0.56 (7.94)	−0.03 (12.14)	6.07 (18.78)	−20.00 (24.88)	−13.23 (28.54)

Table 3

Mean (±SD) physiological and psychological variables during breathlessness for both athletes and sedentary subjects.

	ATHLETE	SEDENTARY
Peak mouth pressure (cmH$_2$O)	14.4 (8.5)	12.0 (5.8)
Breathlessness intensity rating (%)	46.3 (14.1)	46.7 (18.1)
Breathlessness anxiety rating (%)	31.9 (17.8)	36.1 (20.0)
Unloaded breathing intensity rating (%)	2.3 (3.5)	3.4 (3.4)
Unloaded breathing anxiety rating (%)	2.8 (4.8)	2.2 (2.7)

demonstrated brain activity during anticipation of breathlessness in the right ventral posterolateral nucleus of the thalamus, which positively correlated with their subsequent breathlessness intensity scores (Fig. 2A). In comparison, sedentary subjects only demonstrated negative correlations with intensity scores during anticipation of breathlessness, and this was apparent in the bilateral ventral posterolateral nucleus of the thalamus, right middle and anterior insula, and bilateral primary motor and sensory cortices (Fig. 2B). No significant results were observed within groups for brain correlates of anxiety during anticipation of breathlessness, nor for either intensity or anxiety during breathlessness perception.

Secondly, the comparison of brain activity correlating with subjective scores between groups produces an interaction effect, demonstrating how the difference between groups varies as a function of the covariate (subjective scores). Within this interaction, athletes demonstrated widespread significant clusters of brain activity that positively correlated with (predicted) intensity scores during anticipation of breathlessness (Fig. 2C), whilst those same clusters demonstrated a negative correlation in sedentary subjects (interaction). This included clusters of activity in the bilateral ventral posterolateral nucleus of the thalamus, middle insula, and primary motor and sensory cortices, as well as left anterior insula. In contrast, a small cluster of activity in the right putamen and caudate nucleus correlated with anxiety in sedentary subjects, which was reversed in athletes during anticipation (BOLD signal change values not displayed). There were no correlations between breathlessness intensity/anxiety scores and years of training, nor training volume/intensity within the athlete group.

Resting state network connectivity

Of 25 independent components produced in the group ICA analysis, 20 components were identified to represent relevant signal (19 cortical, 1
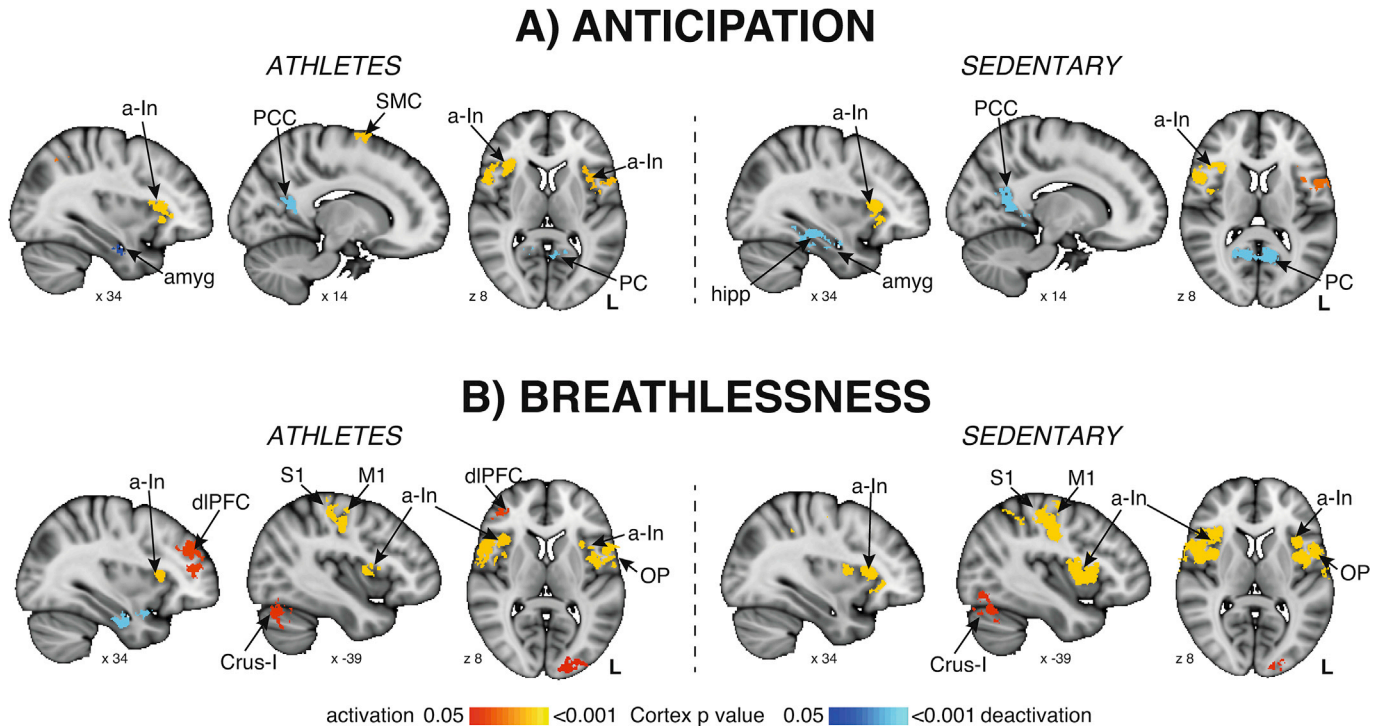

Fig. 1. Significant clusters of BOLD activity in athletes and sedentary controls. A) Significant clusters of BOLD activity during conditioned anticipation of breathlessness. B): Significant clusters of BOLD activity during a breathlessness challenge, induced via inspiratory resistive loading. The images consist of a colour-rendered statistical map superimposed on a standard (MNI 1 × 1x1 mm) brain, and significant regions are displayed with a non-parametric cluster probability threshold of $t < 3.1$; $p < 0.05$ (corrected for multiple comparisons). Abbreviations: M1, primary motor cortex; SMC, supplementary motor cortex; dACC, dorsal anterior cingulate cortex; PCC, posterior cingulate cortex; dlPFC, dorsolateral prefrontal cortex; a-In, anterior insula; OP, operculum; amyg, amygdala; hipp, hippocampus; Crus-I, cerebellar lobe; activation, increase in BOLD signal; deactivation, decrease in BOLD signal.
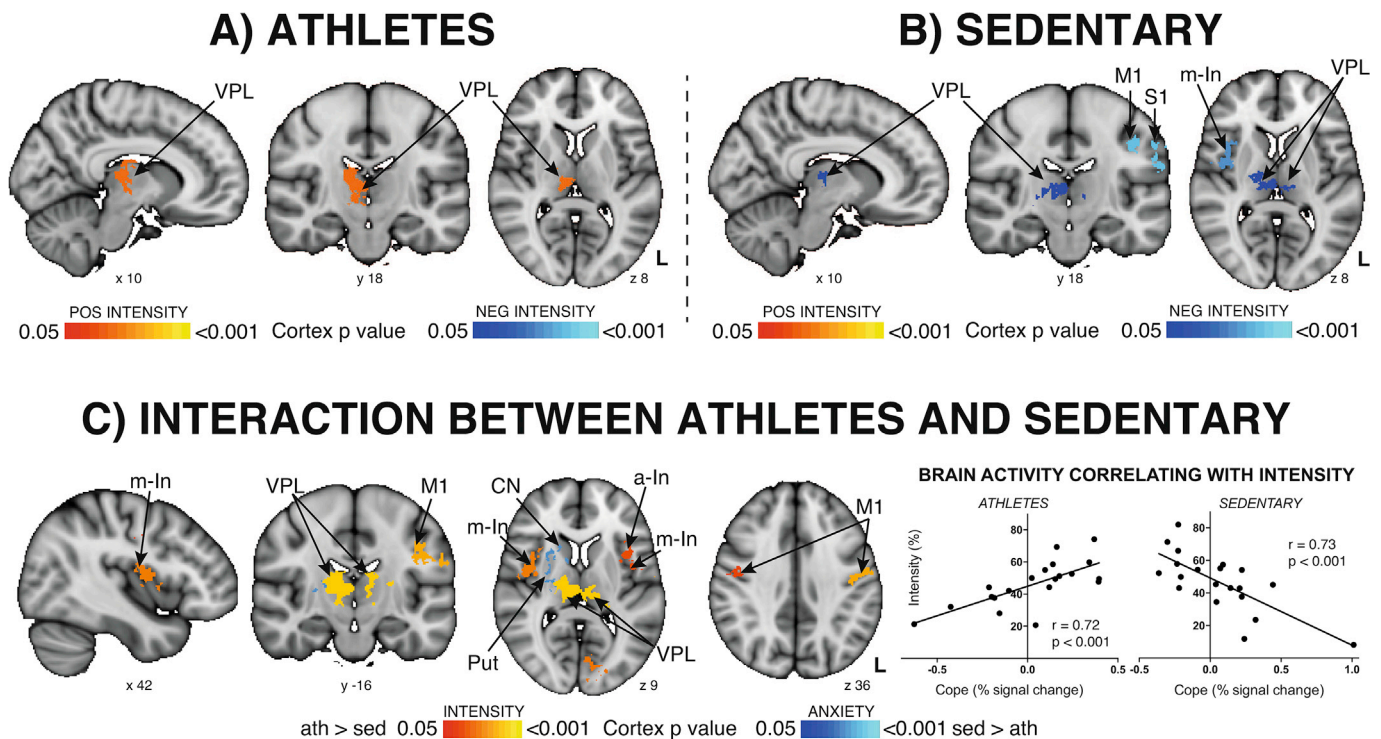
Fig. 2. Interaction between groups and breathlessness scores during anticipation of breathlessness. A) Significant clusters of BOLD activity correlating with subsequent breathlessness intensity scores in athletes. B) Significant clusters of BOLD activity correlating with subsequent breathlessness intensity scores in sedentary subjects. C) Left: Clusters of BOLD activity significantly different between athletes and sedentary subjects (interaction effect) are displayed in red-yellow for intensity scores, and difference in correlations between brain clusters and anxiety scores are displayed in blue-light blue. Right: Percentage BOLD signal change within the significant clusters of brain activity that correlated with intensity scores (red-yellow map), demonstrating a positive, linear correlation in athletes and a negative relationship in sedentary subjects. The images consist of a colour-rendered statistical map superimposed on a standard (MNI $1 \times 1x1$ mm) brain, and significant regions are displayed with a non-parametric cluster probability threshold of $t < 3.1$; $p < 0.05$ (corrected for multiple comparisons). Abbreviations: M1, primary motor cortex; S1, primary sensory cortex; a-In, anterior insula; m-In, middle insula; hipp, hippocampus; put, putamen; CN, caudate nucleus; VPL, ventral posterolateral thalamic nucleus.

cerebellar) while the remaining 5 were labeled as noise (see supplementary material for a summary of the 20 resting networks). Two networks of interest were previously identified by Kleckner et al. (2017) to be important for interoceptive processing in the brain (for instance during processing of breathlessness perceptions), which we utilized for a comparison of connectivity between athletes and sedentary controls. These networks consisted of: 1) The network most representative of the typical 'default mode', and 2) A network containing components of previously identified visual and dorsal attention networks (Vossel et al., 2014), which we have labeled as a 'task-positive' network due to its similarity to the clusters of brain activity identified during perceptions of breathlessness (Fig. 3).

When network connectivity was compared between athletes and controls, athletes were found to have significantly greater ($p = 0.019$) connectivity of the task-positive network to an area of primary motor cortex. As this area demonstrating a difference between groups was primarily overlapping with an area of negative connectivity within the resting state map (Fig. 3), this group difference represents a reduced negative correlation in athletes compared to sedentary controls. This area of altered resting connectivity in athletes was found to be within an area of primary motor task that was active during breathlessness induced via the task fMRI contrasts described above (Fig. 3).

Discussion

Main findings

We have identified a cohesive anticipatory brain network that predicts upcoming subjective ratings of breathlessness in athletes.
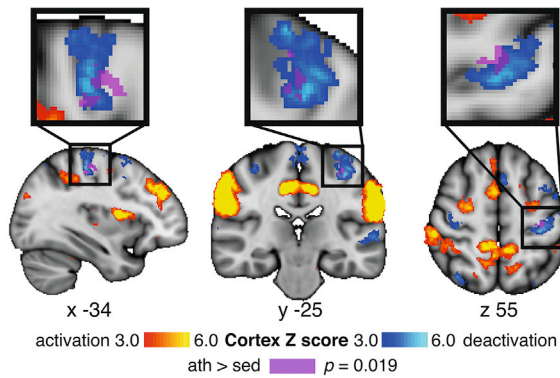
Comparatively, this brain activity was reversed (i.e. negatively correlated with upcoming breathlessness ratings) in sedentary controls. Furthermore, at rest, athletes demonstrated altered connectivity between a 'task-positive' attention network and an area of primary motor cortex that is active during breathlessness tasks. This task-positive network was strikingly similar to that recently identified to be involved in allostatic-interoceptive processing (Kleckner et al., 2017), and thus may also be integral within the attention and processing of interoceptive signals related to breathing. Furthermore, altered connectivity between sensorimotor cortex and this brain network may be related to the observed differences in anticipatory processing of respiratory signals in the brain, and the improved ventilatory perceptive accuracy found in these endurance athletes.

Breathlessness processing in athletes

Endurance athletes have repeated episodes of elevated ventilation and perceptions of breathlessness as part of their training. In previously published results (Faull et al., 2016a), we have demonstrated improved psychophysical matching between changes in chemostimulated hyperventilation and subjective breathlessness perceptions in these athletes compared to matched sedentary subjects (Supplementary Fig. 1). Therefore, whether by nature or nurture, these individuals appear to have improved ventilatory perception accuracy. The reduced correlation between changes in ventilation and perceptions of breathlessness demonstrated in sedentary subjects implies a worsened ability to process respiratory sensations, which may be a risk factor for symptom discordance in disease (Van den Bergh et al., 2017).

In accordance with behavioural findings, here we have observed

A) TASK-POSITIVE NETWORK

x -34   y -25   z 55

activation 3.0 ███ 6.0 **Cortex Z score** 3.0 ███ 6.0 deactivation

ath > sed ███ *p* = 0.019

B) BREATHLESSNESS

x -34   y -25   z 55

activation 0.05 ███ <0.001 **Cortex p value** 0.05 ███ <0.001 deactivation
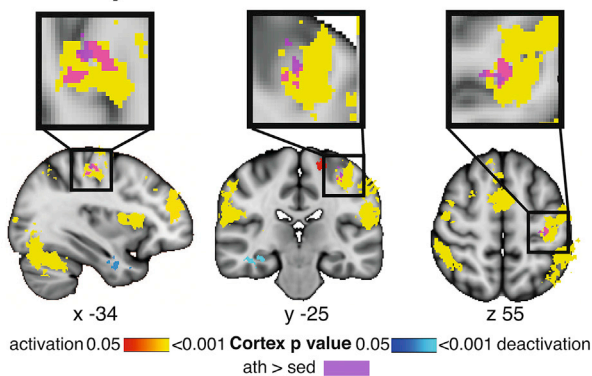
ath > sed ███

Fig. 3. Differences in resting functional connectivity between athletes and sedentary subjects. A) The resting state network (produced from the combination of both groups via independent component analysis) that was identified as the 'task positive' network (displayed as a brain-wide Z-score map), and overlaid on this map (in purple) is the area of altered functional connectivity (reduced negative correlation) in athletes compared to sedentary controls (displayed as a *p* value) within this network. This purple area is transparent, and thus darker purple displays overlap with underlying (blue) deactivation within the resting state map. B) To demonstrate the importance of the location of this area of altered connectivity in athletes to breathlessness, we have overlaid the connectivity difference (transparent purple – for illustration only, no new statistical test was performed and thus no p value is reported here) from panel A over the significant clusters of brain activity identified during the breathlessness task, averaged across both groups. Lighter purple areas demonstrate overlap with underlying yellow activity. The images consist of a colour-rendered statistical map superimposed on a standard (MNI 1x1x1 mm) brain, and significant regions are displayed with a non-parametric cluster probability threshold of $t < 3.1$; $p < 0.05$ (corrected for multiple comparisons).

differences in the brain processing of breathing perceptions in athletes. Specifically, a coherent network of brain activity corresponding to breathlessness intensity scores was observed during anticipation in athletes, which was reversed (i.e. negatively correlated) with subjective scores in sedentary subjects. This network incorporates key areas involved in sensorimotor control and interoception, such as the thalamus, insula and primary sensorimotor cortices (Feldman and Friston, 2010; Simmons et al., 2012; Feldman Barrett and Simmons, 2015; Van den Bergh et al., 2017). The opposing relationship between brain activity and subjective scores in athletes and sedentary subjects indicates a fundamental difference in preparatory, anticipatory brain activity directed towards subjective perceptions between these groups, which occurs without any difference in overall group mean brain activity. Conversely, sedentary subjects demonstrated activity corresponding to

anxiety scores in the ventral striatum (caudate nucleus and putamen) during anticipation of breathlessness. The striatum has been previously linked with cardiovascular responses resulting from social threat (Wager et al., 2009), and may represent heightened threat responses in sedentary subjects.

Interestingly, the intensity-related differences in brain activity were observed during the anticipation period that preceded the actual perception of breathlessness. It is possible that repeated increases in ventilation and breathlessness during training helps athletes improve the accuracy of their breathing expectations for an upcoming stimulus, such as expecting to run up a hill. Recent theories of symptom perception have proposed a comprehensive, Bayesian model (Feldman Barrett and Simmons, 2015; Van den Bergh et al., 2017), which includes a set of perceptual expectations or 'priors'. These expectations are combined with sensory information from the periphery, for the brain to probabilistically produce the most likely resulting perception. Furthermore, factors such as attention (Merikle and Joordens, 1997; Phelps et al., 2006; Ling and Carrasco, 2006) and interoceptive ability (Gray et al., 2007; Critchley et al., 2013; Mallorqui-Bague et al., 2016; Garfinkel et al., 2016b) are thought to influence this system, either by altering the prior expectations or incoming sensory information.

Whilst previous research had identified reduced anterior insula activity during loaded breathing in endurance athletes (Paulus et al., 2012), we have not reproduced these findings when employing more stringent fMRI statistics. Nevertheless, the proposal by Paulus and colleagues (Paulus et al., 2012) that athletes demonstrate more efficient minimization of the body prediction error remains a very plausible possibility. Here, instead, we have observed functional perception-related differences during anticipation of loaded breathing in endurance athletes. Therefore, repeated exercise training in athletes may develop breathlessness expectations (or priors) and better direct attention towards breathing sensations, improving the robustness of the perceptual system to accurately infer the intensity of breathlessness and minimize body prediction error.

Differences in functional connectivity within the athletic brain

Understanding differences in underlying communication between functional brain regions may inform us as to why differences in functional activity, such as that observed in these athletes during anticipation of breathlessness, may arise. The temporal synchronicity of seemingly spontaneous fluctuations in brain activity across spatially distinct regions can inform us of how 'functionally connected' these disparate regions may be, and is thought to be related to the temporal coherence of neuronal activity in anatomically distinct areas (Gerstein and Perkel, 1969; Van Den Heuvel and Pol, 2010).

It is now well established that the brain can be functionally parsed into resting state 'networks', where distinct brain regions are consistently shown to exhibit temporally similar patterns of brain activity (Smith et al., 2009; Miller et al., 2016). Properties of these resting state networks have been linked to lifestyle, demographic and psychometric factors (Smith et al., 2015; Miller et al., 2016), and here we have found connectivity differences between athletes and sedentary subjects for a cingulo-opercular network. This network displays a very similar spatial distribution to the pattern of activity observed during the breathlessness tasks ('task-positive') (Fig. 3), as well as the allostatic-interoceptive network recently identified by Kleckner and colleagues (Kleckner et al., 2017), and to previously reported networks of ventral and dorsal attention (Fox et al., 2005, 2006).

Here, we have demonstrated altered functional connectivity in athletes (in the form of a reduction in negative correlation) between an area of primary sensory and motor cortices that has consistently been identified as active during tasks such as breath holds (Pattinson et al., 2009b; Faull et al., 2015) and inspiratory resistances (Faull et al., 2016b; Faull and Pattinson, 2017; Hayen et al., 2017). This resting connectivity difference between groups is primarily located within an area of negative

connectivity of this cingulo-opercular network, representing a typical decorrelation between these areas. Therefore, a reduction in this decorrelation in athletes may represent a qualitative as well as quantitative difference in networks, whereby this area may have a tendency to be recruited by this network instead of being as heavily deactivated, as it appears to be in sedentary subjects. As such, it is possible that this altered connectivity between an interoceptive attention network and primary sensorimotor cortex contributes to the processing of incoming and outgoing respiratory information, and thus may also be related to more accurate ventilatory perceptions in athletes.

Whilst this cross-sectional study is unable to determine whether endurance exercise training *induces* these differences in brain function and connectivity, or whether these individuals are biased towards training for endurance sports, this work provides intriguing preliminary insight that the brain may undergo adaptation in conjunction with the periphery, to more accurately process perceptions of bodily sensations such as breathlessness. Further work is needed to more extensively investigate potential qualitative differences in resting state networks as a result of exercise, and how these directly relate to any changes in ventilatory interoception. This could be achieved through longitudinal studies utilising exercise as an intervention.

Neuroimaging statistical considerations

Extensive efforts were made within the analysis of this dataset to ensure only the most robust and reliable results were reported. Firstly, physiological noise and potential motion artifacts need to be specifically addressed when using breathing-related tasks, and these can be further compounded at higher field strengths (Brooks et al., 2013). Here we employed rigorous noise correction procedures, combining retrospective image correction of physiological parameters (heart rate, ventilation and end-tidal carbon dioxide) with both motion parameter regression and independent component analysis de-noising (Faull et al., 2016b; Hayen et al., 2017). Secondly, recent work has revealed the potential leniency of previous fMRI statistical methodologies and thresholds (Eklund et al., 2016). In this manuscript, we have utilized minimal (2 mm) spatial smoothing to maintain accurate localization of brain activity, and employed non-parametric permutation testing with a robust cluster threshold of 3.1 (Eklund et al., 2016), to represent only the most reliable statistical results. Whilst these approaches forsake much of our previously-reported activity within these breathing-related tasks (Faull and Pattinson, 2017), we can have greater confidence in our reported differences between brain and behavior in athletes and sedentary subjects.

Potential clinical implications of altering breathlessness processing

As discussed, prior expectations of breathlessness are now considered to be a major contributor to symptom perception (Hayen et al., 2013; Faull et al., 2017; Van den Bergh et al., 2017; Geuter et al., 2017; Herigstad et al., 2017). Altering the accuracy of breathlessness perception using exercise training may be of interest when treating individuals with habitual symptomology, such as those with chronic obstructive pulmonary disease (COPD) or asthma. Recent research has shown exercise training to reduce breathlessness intensity and anxiety in patients with COPD, with corresponding changes in the brain's processing of breathlessness-related words (Herigstad et al., 2016, 2017). It has been proposed that exercise exposure alters breathlessness expectations and priors in these patients, modifying symptom perception when it has become discordant with physiology in chronic disease (Parshall et al., 2012; Herigstad et al., 2017). It is also possible that exercise helps improve the processing of respiratory signals for more accurate ventilatory interoception in these patients, allowing breathlessness perception to better match respiratory distress. Future work investigating the link

between exercise, ventilation and breathlessness perception may yield another treatment avenue (via targeted exercises) to improve patient quality of life in the face of chronic breathlessness.

Conclusions

In this study, we have demonstrated altered anticipatory brain processing of breathlessness intensity in athletes compared to sedentary subjects. This altered functional brain activity may be underpinned by changes in functional connectivity between an interoceptive network related to breathlessness, and sensorimotor cortex that is active during ventilatory tasks. These differences in brain activity and connectivity may also relate to improvements in ventilatory perception previously reported between these subject groups (Faull et al., 2016a), and open the door to investigating exercise as a tool to manipulate brain processing of debilitating breathlessness in clinical populations.

Conflicts of interest

KP has acted as a consultant for Nektar Therapeutics. The work for Nektar has no bearing on the contents of this manuscript. KP is named as a co-inventor on a provisional UK patent application titled "Use of cerebral nitric oxide donors in the assessment of the extent of brain dysfunction following injury."

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.neuroimage.2018.06.021.

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