Cortical processing of breathlessness in the athletic brain

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Key points

- Endurance athletes train to improve their respiratory system for enhanced exercise capacity and performance. However, it is unknown whether concurrent adaptation occurs in brain networks perceiving respiratory-related sensations, such as breathlessness.
- We have previously shown improved matching between changes in ventilation and perceptions of breathlessness in endurance athletes compared to sedentary controls (Faull *et al.*, 2016*a*). Here, we used functional brain scanning to investigate differences in brain activity during breathlessness tasks in these subjects.
- Athletes demonstrated a network of brain activity during anticipation of resistive inspiratory loading that corresponds to subjective breathlessness intensity, which was absent in sedentary controls. This may be related to improved brain synchronicity observed between primary sensorimotor cortices and task-positive brain networks in these athletes, and may underpin our previous findings of improved ventilatory interoception.
- Understanding brain changes in respiratory perceptions may help us to target both endurance training mechanisms and treatment of disease-related breathlessness symptomology.

Abstract

Exercise is associated with large increases in ventilation, which are consciously perceived as the sensation of breathlessness. We have previously demonstrated closer matching between changes in ventilation and corresponding perceptions of breathlessness in endurance athletes compared with sedentary controls (Faull *et al.*, 2016*a*), suggesting improved accuracy when interpreting respiratory sensations, or ventilatory interoception. Here, we sought to identify the mechanisms by which the processing of respiratory perception is optimised in these subjects.

Forty participants (20 athletes, 20 age/sex-matched sedentary participants) were scanned using a 7T Siemens Magnetom (Nova Medical 32 channel Rx, single channel birdcage Tx). Anticipation and breathlessness were induced with a previously trained delay-conditioned cue and an inspiratory resistance during fMRI scanning. Differences between group means and slope of subjective scores during task-based and resting fMRI were analysed using non-parametric statistical testing and independent component analysis.

Athletes demonstrated greater brain activity corresponding with intensity scores during anticipation of breathlessness, compared to sedentary controls. Athletes also exhibited greater functional connectivity (or communication) between a task-positive brain network closely matching breathlessness activity, and areas of primary sensorimotor cortices active during inspiratory resistance. These functional activity and connectivity differences in athletic brains may represent optimized processing of respiratory sensations, and contribute to improved ventilatory interoception in athletes. Furthermore, these brain mechanisms may be harnessed when exercise is employed in the treatment of breathlessness for 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 & Coyle, 1984; Jones AM, 2012). However, the role of the brain in perceiving and modulating changing sensations from the periphery, useful for processes such as pacing strategies and to maintain homeostasis, is often overlooked.

Ventilation during exercise is tightly controlled, balancing neurally-modulated feed forward ventilatory commands and peripheral feedback to stimulate appropriate ventilation for exercising needs (Kaufman & Forster, 1996; Waldrop *et al.*, 1996). Respiratory sensations are monitored to maintain homeostasis (Davenport & 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). While 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 interoceptive processing of ventilation might adapt or be altered in different states, such as here in athletes, and open the door to future work in disease models of altered breathlessness perceptions.

Importantly, prior experiences of strong respiratory sensations may also alter the way someone anticipates and perceives breathlessness (Faull *et al.*, 2017; Van den Bergh *et al.*, 2017;

Herigstad *et al.*, n.d.). 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 preventative actions (i.e. to avoid the hill), 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 & Joordens, 1997; Phelps *et al.*, 2006; Ling & Carrasco, 2006), reducing their anxiety (Spinhoven *et al.*, 1997; Bogaerts *et al.*, 2005; Tang & Gibson, 2005) or improving their interoceptive ability (Gray *et al.*, 2007; Critchley *et al.*, 2013; Mallorqui-Bague *et al.*, 2016; Garfinkel *et al.*, 2016*b*; 2016*a*). 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.*, n.d.), without concurrent improvements in lung function. It is possible that athletes may have different prior expectations and anticipations 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.*, 2016*a*). 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 during both conditioned anticipation and perception of a breathlessness stimulus, as well as any differences in the resting temporal coherence, or 'functional connectivity' (Gerstein & Perkel, 1969; Van Den Heuvel & Pol, 2010) of brain networks involved in attention towards sensory information. 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 (Faull *et al.*, 2016*a*) would demonstrate both altered functional breathlessness-related brain activity and connectivity 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 (20 males, 20 females; mean age ± SD, 26 ± 7 years), with no history of smoking or any respiratory disease. This cohort comprised two groups; 20 subjects who regularly participated in endurance sport (10 males, 10 females; mean age ± SEM, 26 ± 1.7 years) and 20 age- and sex-matched (±2 years) sedentary subjects (10 males, 10 females; mean age ± SEM, 26 ± 1.7 years). Prior to scanning, all subjects underwent breathlessness testing during exercise and chemostimulated hyperpnea, which have been presented elsewhere (Faull *et al.*, 2016*a*), and a combined whole-group analysis of fMRI data has been previously reported (Faull & Pattinson, 2017).

Stimuli and tasks

Subjects were trained using an aversive delay-conditioning paradigm to associate simple shapes with an upcoming breathlessness (inspiratory resistance) stimulus (Faull & Pattinson, 2017). 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. The 'unloaded breathing' symbol was presented for 20 s, and each condition was repeated 14 times in a semi-randomised order. A finger opposition task was also included in the protocol, where an opposition movement was conducted between the right thumb and fingers, with the cue 'TAP' presented for 15 s (10 repeats). Conscious associations between cue and threat level (cue contingencies) were required and verified in all subjects by reporting the meaning of each of the symbols following the training session and immediately prior to the MRI scan.

Rating scores of breathing perceptions were recorded after every symbol and at the beginning and end of the task, 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.

Breathing system and Physiological measurements

A breathing system was used to remotely administer periods of inspiratory resistive loading to induce breathlessness (as predicted by the conditioned cues), as previously described (Faull *et al.*, 2016*b*). End-tidal oxygen and carbon dioxide were maintained constant. The subject's nose was blocked using foam earplugs and they were asked to breathe through their mouth for the duration of the experiment. Physiological measures were recorded continuously during the training session and MRI scan as previously described (Faull *et al.*, 2016*b*).

153

154 **MRI scanning sequences**

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

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

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

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

170

171 **Physiological data analysis**

172 Values for mean and peak resistive loading, mean $P_{ET}CO_2$, $P_{ET}O_2$, respiratory rate and
173 respiratory volume per unit time (RVT) were calculated across each time block using custom
174 written scripts in MATLAB (R2013a, The Mathworks, Natick, MA). Measures were averaged
175 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, and high-pass temporal filtering (Gaussian-weighted least-squares straight line fitting; 120 s). B_0 field unwarping was conducted with a combination of FUGUE and BBR (Boundary-Based-Registration; part of FEAT: FMRI Expert Analysis Tool, version 6.0 (Greve & Fischl, 2009)). Data denoising was conducted using a combination of independent components analysis (ICA) and retrospective image correction (RETROICOR) (Harvey *et al.*, 2008; Brooks *et al.*, 2013), as previously described (Faull *et al.*, 2016*b*).

Image registration: Following preprocessing, the functional scans were registered to the MNI152 (1x1x1 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 & 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: Breathlessness periods (calculated from physiological pressure trace as onset to termination of each application of resistance); anticipation of breathlessness (calculated from onset of anticipation symbol to onset of resistance application); unloaded breathing (onset and duration of 'unloaded breathing' symbol); and finger opposition (onset and duration of finger opposition screen instruction). 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 extrapolating 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.*, 2009*a*; 2009*b*; Faull *et al.*, 2015; 2016*b*). 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, as well as the control condition of finger opposition (vs. baseline).

Functional voxelwise analysis incorporated HRF modeling using three FLOBS regressors 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. Voxelwise differences between mean group activity 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 & 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. Dual regression (Beckmann *et al.*, 2009) was then used to delineate subject-specific timecourses of these components, and their corresponding subject-specific spatial maps. Subject-specific spatial maps were again analysed non-parametrically using Randomise (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. Therefore, *p* threshold significance was adjusted to $p < 0.025$ using Bonferroni correction for multiple comparisons.

Results

Physiology and psychology of breathlessness

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 1. Group scores for breathlessness intensity and anxiety are presented in Table 2, with no mean differences observed between groups.

Task fMRI analysis

Anticipation of breathlessness: Mean activity during anticipation of breathlessness in each group is presented in Figure 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).

Resistive loading: Mean activity during breathlessness in each group is presented in Figure 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: Brain activity that correlated with breathlessness scores of intensity and anxiety were compared between groups. Athletes demonstrated widespread brain activity positively correlating with intensity scores during anticipation of breathlessness (Figure 2), whilst those same areas had a negative correlation in sedentary subjects (interaction). This included 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 amount of activity in the right putamen and caudate nucleus correlated with anxiety in sedentary subjects, but not in athletes during anticipation. No significant interactions between groups were present for either intensity of anxiety during breathlessness perception.

Finger opposition: Results of the control finger opposition task are provided in the Supplementary material.

Resting state network connectivity

Of the 25 resting state ‘networks’ identified in the group ICA analysis, 20 components were identified to represent relevant signal (19 cortical, 1 cerebellar) while the remaining 5 were

labeled as noise (see Supplementary Figure 1 for a summary the 20 resting networks). Two networks of interest were identified for group comparison analyses: 1) The network most representative of the typical 'default mode', which was closely represented by the whole group decrease in brain activity during anticipation of breathlessness, and 2) The network that displayed the most similarity to the task contrasts (anticipation and resistive loading) or 'task-positive' network, containing components of previously identified visual and dorsal attention networks (Vossel *et al.*, 2014) (Figure 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 active during resistive loading (Figure 3).

Discussion

Main findings

We have identified a cohesive brain network pertaining to subjective ratings of anticipated breathlessness intensity in athletes, which is absent in sedentary controls. Comparatively, sedentary subjects demonstrated anticipatory activity in the caudate nucleus and putamen corresponding to anxiety scores, which was not present in athletes. Athletes also demonstrated greater connectivity between an area of primary sensorimotor cortex that is active during inspiratory resistance, and a cingulo-opercular 'task-positive' network identified at rest. This network has strikingly similarities to the pattern of positive and negative BOLD changes induced during both anticipation and breathlessness, and may relate to attention and processing of sensory signals related to breathlessness. Increased connectivity between sensorimotor cortex

and the cingulo-opercular brain areas active during breathlessness tasks may underlie the observed differences in processing of respiratory signals during anticipation, and the improved ventilatory interoception previously reported in these endurance athletes (Faull *et al.*, 2016*a*).

Breathlessness processing in athletes

Endurance athletes have repeatedly elevated ventilation and perceptions of breathlessness as part of their training. In previously published results (Faull *et al.*, 2016*a*), we have demonstrated improved psychophysical matching between changes in chemostimulated hyperventilation and subjective breathlessness perceptions in these athletes compared to matched sedentary subjects. Therefore, whether by nature or nurture, these individuals appear to have improved ventilatory perception accuracy. A random relationship 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). Accordingly, a coherent network of brain activity was present corresponding to breathlessness intensity scores in athletes, incorporating key areas involved in sensorimotor control and interoception, such as the thalamus, insula and primary sensorimotor cortices (Feldman & Friston, 2010; Simmons *et al.*, 2012; Barrett & Simmons, 2015; Van den Bergh *et al.*, 2017). 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 preceding 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 (Barrett & 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 & Joordens, 1997; Phelps *et al.*, 2006; Ling & Carrasco, 2006) and interoceptive ability (Gray *et al.*, 2007; Critchley *et al.*, 2013; Mallorqui-Bague *et al.*, 2016; Garfinkel *et al.*, 2016*b*) are thought to influence this system, either by altering the prior expectations or incoming sensory information. Therefore, it is possible that repeated exercise training in athletes could develop breathlessness expectations (or priors) and direct attention towards breathing sensations, improving the robustness of the perceptual system to accurately infer the intensity of breathlessness.

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 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 & Perkel, 1969; Van Den Heuvel & 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). While properties of these resting state networks have been linked to lifestyle, demographic and psychometric factors (Smith *et al.*, 2015; Miller *et al.*, 2016), here we have found connectivity differences between athletes and sedentary subjects for a cingulo-opercular network that displays a very similar spatial distribution to the pattern of activity observed during the breathlessness tasks ('task-positive'), with (on average) a negative connectivity to primary sensorimotor cortices active during breathlessness (Figure 3). This task-positive network is also strikingly similar to previously reported networks of ventral and dorsal attention (Fox *et al.*, 2005; 2006). Here, we have demonstrated greater functional connectivity in athletes 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.*, 2009*b*; Faull *et al.*, 2015) and inspiratory resistances (Faull *et al.*, 2016*b*; Hayen *et al.*, 2017; Faull & Pattinson, 2017). It is possible that this greater connectivity in athletes between an attention network and primary sensorimotor cortex contributes to the processing of incoming and outgoing respiratory information, and thus may also be related to improved ventilatory interoception.

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 ventilatory interoception and perceptions of bodily sensations such as breathlessness.

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.*, n.d.). 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; n.d.). 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.*, n.d.). 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, ventilatory interoception and breathlessness perception may yield another treatment avenue (via interoception and more targeted exercise) 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

403 underpinned by increased functional connectivity between a task-positive network related to
404 breathlessness, and sensorimotor cortex that is active during ventilatory tasks. These differences
405 in brain activity and connectivity may relate to improvements in ventilatory interoception
406 previously reported between these subject groups (Faull *et al.*, 2016*a*), and open the door to
407 investigating exercise and interoception as a tool to manipulate brain processing of debilitating
408 symptoms, such as breathlessness in clinical populations.

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419 Competing interests

420 KP has acted as a consultant for Nektar Therapeutics. The work for Nektar has no bearing on the
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Tables

Table 1. 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.

	Unloaded breathing		**Anticipation**		**Breathlessness**	
	ATHLETE	SEDENTARY	ATHLETE	SEDENTARY	ATHLETE	SEDENTARY
Avg mouth pressure (cmH_2O)	0.54 (0.84)	0.15 (0.65)	0.71 (1.03)	0.21 (0.69)	6.22 (3.38)	5.17 (2.60)
$P_{ET}CO_2$ (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 2. Mean (±sd) physiological and psychological variables during breathlessness for both athletes and sedentary subjects.

	ATHLETE	SEDENTARY
Peak mouth pressure (cmH_2O)	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)

Figures

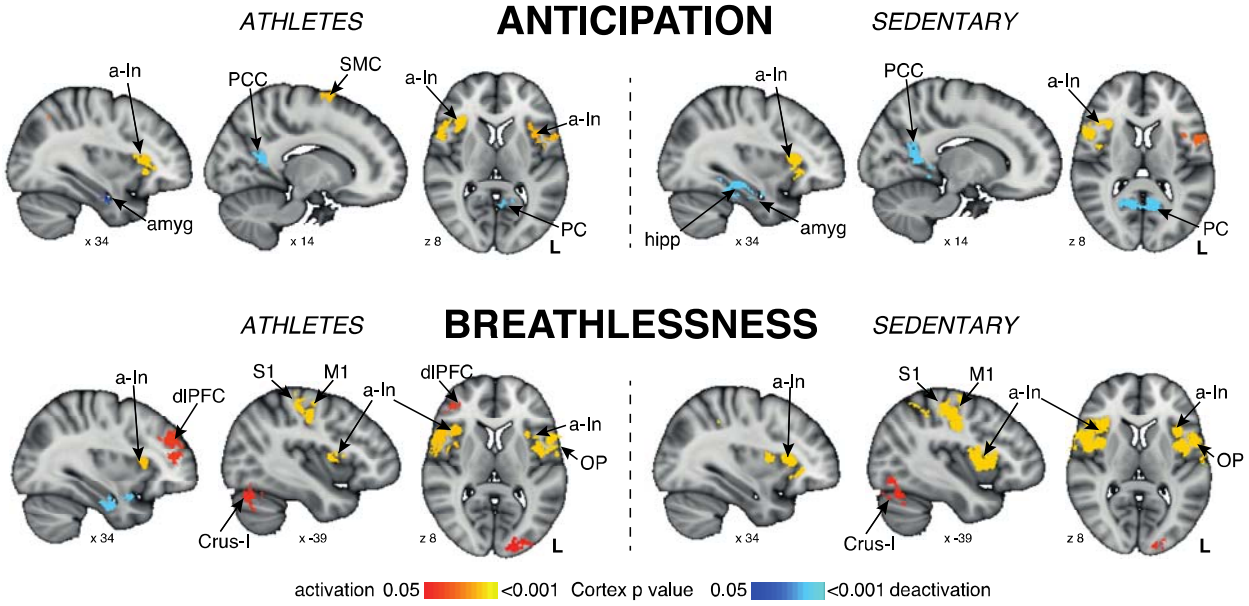


Figure 1. Mean BOLD activity in athletes and sedentary controls. Top: BOLD activity during conditioned anticipation of breathlessness. Bottom: BOLD activity during a breathlessness challenge, induced via inspiratory resistive loading. The images consist of a colour-rendered statistical map superimposed on a standard (MNI 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). 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.

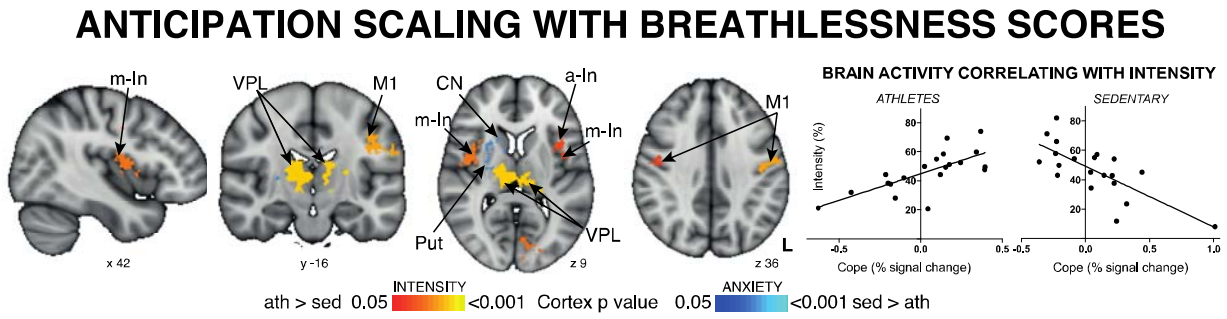


Figure 2. Interaction between groups and breathlessness scores. Left: BOLD activity during conditioned anticipation of breathlessness. Red-yellow = BOLD activity correlating with intensity scores in athletes > sedentary subjects; blue-light blue = BOLD activity correlating with anxiety scores in sedentary > athletic subjects. Right: Percentage BOLD signal change within the (red-yellow) intensity-correlated imaging mask against intensity scores, 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 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). Abbreviations: M1, primary motor cortex; a-In, anterior insula; m-In, middle insula; hipp, hippocampus; put, putamen; CN, caudate nucleus; VPL, ventral posterolateral thalamic nucleus. activation,

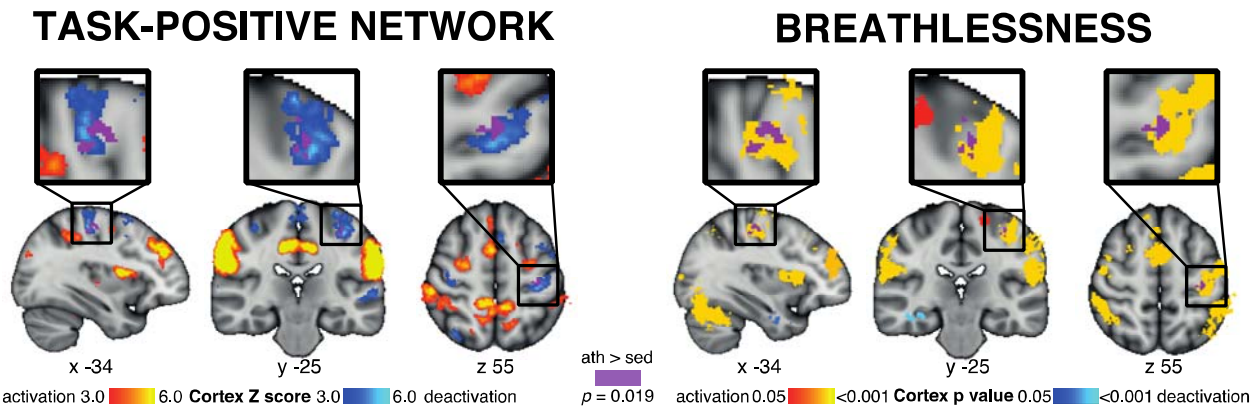
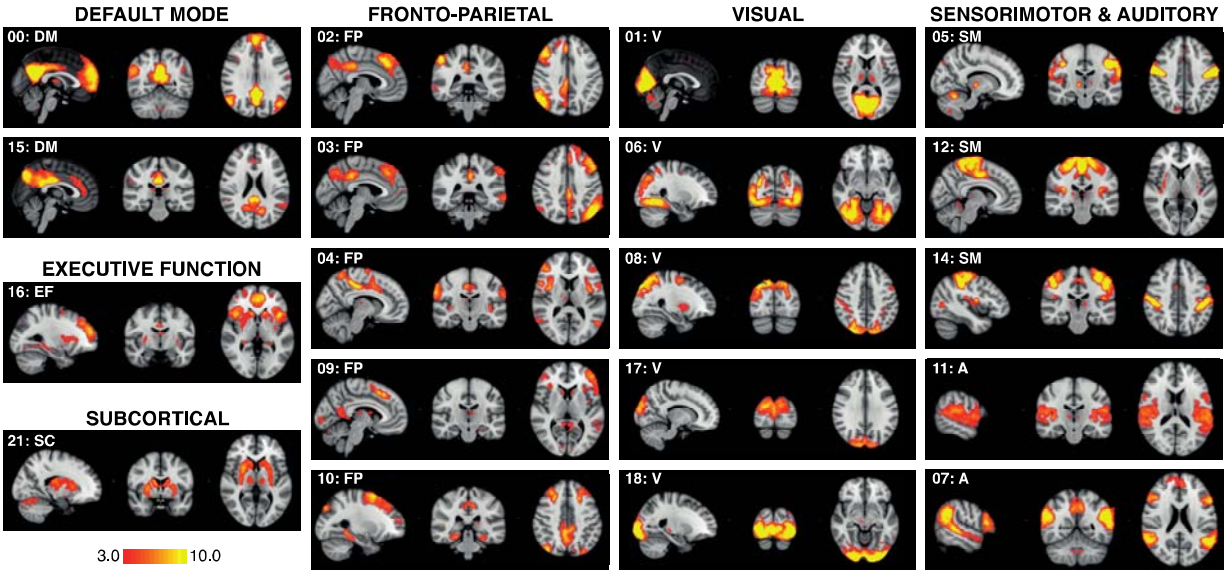


Figure 3. Differences in resting functional connectivity between athletes and sedentary subjects. Increased functional connectivity (purple) observed in athletes between an area of primary motor corticex that is active during breathlessness (right) and a cingulo-opercular task-positive network (left) identified at rest. 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).

SUPPLEMENTARY MATERIAL

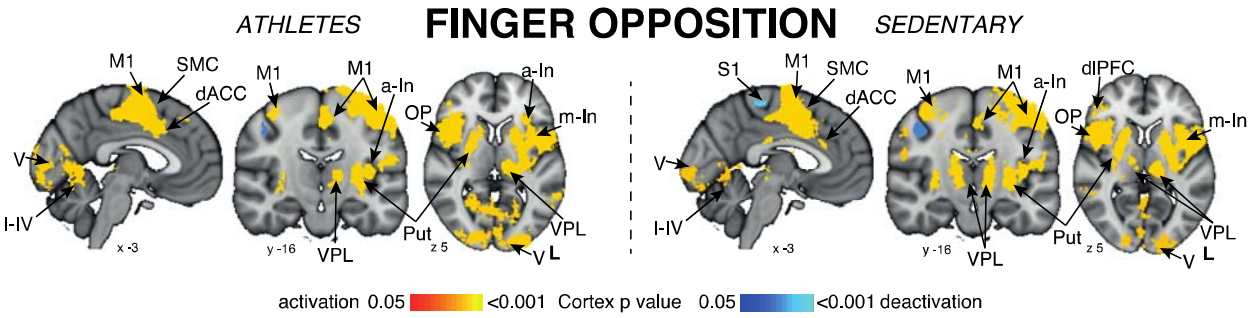
Resting state networks



Supplementary Figure 1. Overview of the twenty resting state networks identified using independent-component analysis in 40 subjects, using a constrained dimensionality of 25 networks. The images consist of a colour-rendered statistical map superimposed on a standard (MNI 1x1x1 mm) brain.

Finger opposition

Finger opposition resulted in significant signal increases in both the brainstem and cortex in both groups consistent with previous research (Pattinson *et al.*, 2009*a*; Faull *et al.*, 2015; 2016*b*), and are presented in the Supplementary material. In sedentary subjects, significantly increased BOLD activity was observed in the bilateral motor cortex (more extensive activation in the contralateral left motor cortex), supplementary motor cortex, dorsal anterior cingulate and paracingulate cortices, primary sensory cortex (more extensive activation in the contralateral left sensory cortex), superior parietal lobule, anterior insula cortex, operculum, caudate nucleus, putamen, left ventral posterolateral nucleus of the thalamus, bilateral cerebellum (VI and VIIIa lobules) and (sedentary only) right dorsolateral prefrontal cortex and right ventral posterolateral nucleus of the thalamus. Both subjects also revealed decreased BOLD activity in ipsilateral sensory and motor cortices.



Supplementary Figure 2. Mean BOLD response to a finger opposition task in each of two groups: 20 endurance athletes, and 20 age- and sex-matched sedentary control subjects. 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). Abbreviations: M1, primary motor cortex; S1, primary sensory cortex; SMC, supplementary motor cortex; dACC, dorsal anterior cingulate cortex; Put, putamen; dlPFC, dorsolateral prefrontal cortex; a-In, anterior insula; m-In, middle insula; OP, operculum; V, visual cortex; I-IV, cerebellar lobe; VPL, ventral posterolateral thalamic nucleus; activation, increase in BOLD signal; deactivation, decrease in BOLD signal.