

Altered interoceptive activation before, during, and after aversive breathing load in women remitted from anorexia nervosa

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Background. The neural mechanisms of anorexia nervosa (AN), a severe and chronic psychiatric illness, are still poorly understood. Altered body state processing, or interoception, has been documented in AN, and disturbances in aversive interoception may contribute to distorted body perception, extreme dietary restriction, and anxiety. As prior data implicate a potential mismatch between interoceptive expectation and experience in AN, we examined whether AN is associated with altered brain activation before, during, and after an unpleasant interoceptive state change.

Methods. Adult women remitted from AN (RAN; $n = 17$) and healthy control women (CW; $n = 25$) underwent functional magnetic resonance imaging during an inspiratory breathing load paradigm.

Results. During stimulus anticipation, the RAN group, relative to CW, showed reduced activation in right mid-insula. In contrast, during the aversive breathing load, the RAN group showed increased activation compared with CW in striatum and cingulate and prefrontal cortices (PFC). The RAN group also showed increased activation in PFC, bilateral insula, striatum, and amygdala after stimulus offset. Time course analyses indicated that RAN responses in interoceptive processing regions during breathing load increased more steeply than those of CW. Exploratory analyses revealed that hyperactivation after breathing load was associated with markers of past AN severity.

Conclusions. Anticipatory deactivation with a subsequent exaggerated brain response during and after an aversive body state may contribute to difficulty predicting and adapting to internal state fluctuation. Because eating changes our interoceptive state, restriction may be one method of avoiding aversive, unpredictable internal change in AN.

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Introduction

Anorexia nervosa (AN) is a serious, often chronic eating disorder of unknown etiology. Aberrant interoception, or the perception and integration of signals relating to body homeostasis (e.g. hunger, heartbeat, respiration), has been postulated to contribute to AN symptoms including body image distortion, extreme restriction despite starvation, and alexithymia (Pollatos *et al.* 2008; Nunn *et al.* 2011; Zucker *et al.* 2013; Kerr *et al.* 2016). In support of this notion, reported sensory over-responsiveness (Brand-Gothelf *et al.* 2016) and altered neural responses to taste stimuli have been

documented in individuals ill with and remitted from AN (RAN) (Cowdrey *et al.* 2011; Oberndorfer *et al.* 2013b).

Signaling of both predicted and actual interoceptive experience may be particularly relevant for understanding AN. Learning depends on associations between anticipated and actual outcomes (Schultz *et al.* 1997), and differences between the expected and observed interoceptive state, or prediction errors, may promote avoidance learning and behavior (Paulus & Stein, 2006). Women with AN have difficulty distinguishing actual from anticipated sensations (Khalsa *et al.* 2015), and RAN women show altered neural activation during anticipation and receipt of sucrose tastes (Oberndorfer *et al.* 2013b; Frank *et al.* 2016). Return to homeostasis after state changes may also be impaired in AN: Interoceptive accuracy in AN decreases after eating (Khalsa *et al.* 2015), and habituation to fullness is protracted (Zucker *et al.*

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2013). Altered AN brain response before, during, and after interoceptive events could contribute to problematic learning from experience about body-related changes, thereby perpetuating maladaptive avoidance.

Many AN behaviors involve avoidance of unpleasant physical sensations (e.g. feelings of fullness; Courty *et al.* 2015), suggesting that brain responses before and during aversive interoceptive experiences may be closely associated with AN symptoms. In individuals with AN, unlike healthy individuals, eating and anticipating eating stimulate dysphoric mood (Steinglass *et al.* 2010) and aversive sensations (e.g. dyspnea; Khalsa *et al.* 2015). ‘Noisy’ interoceptive signals may interfere with accurate prediction of the impact of future aversive stimuli, contributing to anxiety (Paulus & Stein, 2006, 2010; Barrett & Simmons, 2015). RAN women show altered insular and prefrontal activation in response to aversive tastes (Cowdrey *et al.* 2011) and before and during brief pain (Strigo *et al.* 2013). However, brief stimulation may not adequately capture the altered responsivity to prolonged aversive homeostatic state changes that could contribute to AN. Eating, for example, involves non-painful hunger-to-satiety state change. No study to date has examined anticipation of and adaptation to prolonged, non-painful, aversive state changes in AN.

Non-painful aversive interoception recruits circuitry that includes the insula, cingulate, lateral prefrontal cortices (PFC), striatum, and amygdala. Future events are predicted in the anterior insula (Craig, 2002, 2003), and direct sensation processing involves posterior insula. The difference between anticipated and experienced sensation is computed in the mid- to posterior insula, with afferents and efferents to anterior and posterior insula (Barrett & Simmons, 2015). The amygdala and striatum are involved in evaluation of the rewarding or aversive aspects of the interoceptive experience (Craig, 2002; Khalsa *et al.* 2009; Hayes & Northoff, 2012; Simmons *et al.* 2013). Projections from anterior and mid-cingulate and orbitofrontal and lateral PFC to anterior insula may modulate cognitive reactions during the stimulus (Craig, 2002; Critchley *et al.* 2004; Khalsa *et al.* 2009; Hayes & Northoff, 2012; Simmons *et al.* 2013). Finally, return to homeostasis after aversive interoceptive experience is associated with activation in rostral anterior cingulate, lateral PFC, and anterior insula (Peiffer, 2009; Leknes *et al.* 2011).

In this study, we used an inspiratory breathing load paradigm and functional magnetic resonance imaging (fMRI) to examine whether AN is associated with altered brain response before, during, and after aversive interoceptive state changes. We studied remitted participants to avoid confounding effects of malnutrition on neural function. The well-validated task has been shown to activate regions of the insula, PFC,

striatum, and anterior cingulate (Paulus *et al.* 2012; Galli *et al.* 2013; Stewart *et al.* 2014; Berk *et al.* 2015). The majority of prior data from this paradigm suggests that less healthy or less resilient individuals tend to show reduced activation during anticipation and increased activation during breathing load (Paulus *et al.* 2012; Haase *et al.* 2014, 2015, 2016; Berk *et al.* 2015; see online Supplementary Table S1 for summary). Unlike brief pain, taste, or food picture tasks previously used to study RAN (Cowdrey *et al.* 2011; Oberndorfer *et al.* 2013a; Strigo *et al.* 2013), the breathing load paradigm uses prolonged, aversive stimulation to induce a negative interoceptive state while importantly avoiding the confounds of symptom-specific responses to food stimuli. We therefore preliminarily predicted that RAN, similar to less healthy or less resilient individuals in prior breathing load studies, would show increased activation in insular and prefrontal cortices during prolonged aversive stimulation and decreased activation in the anterior and mid-insula during anticipation relative to controls. We also predicted that the time course of the RAN response would differ from that of controls. Exploratory analyses examined associations between task-related activation and clinical symptoms.

Materials and methods

Participants

Twenty RAN women and 26 healthy control women (CW) participated in this study. CW participants were recruited from San Diego, and RAN participants were recruited nationally. Masters-level or higher research staff administered standard structured interviews to confirm past eating disorder diagnosis and exclude current eating disorder diagnoses (see online Supplement for further detail). AN remission was defined as in prior studies (Bailer *et al.* 2004, 2005; Frank *et al.* 2005; Wagner *et al.* 2007; Wagner *et al.* 2008; Strigo *et al.* 2013; Wierenga *et al.* 2015). Women from both groups were excluded if they met diagnostic criteria for a current *Diagnostic and Statistical Manual of Mental Disorders: Fourth Edition (DSM-IV)* Axis I diagnosis, and CW participants with a history of any eating disorders were also excluded. Please see online Supplementary Information for further eligibility criteria. Participants provided written, informed consent to participate in the study. The research was approved by the University of California, San Diego (UCSD) Human Research Protections Program.

Procedure

Participants completed fMRI scans during the first 10 days (the early follicular phase) of their menstrual cycles.

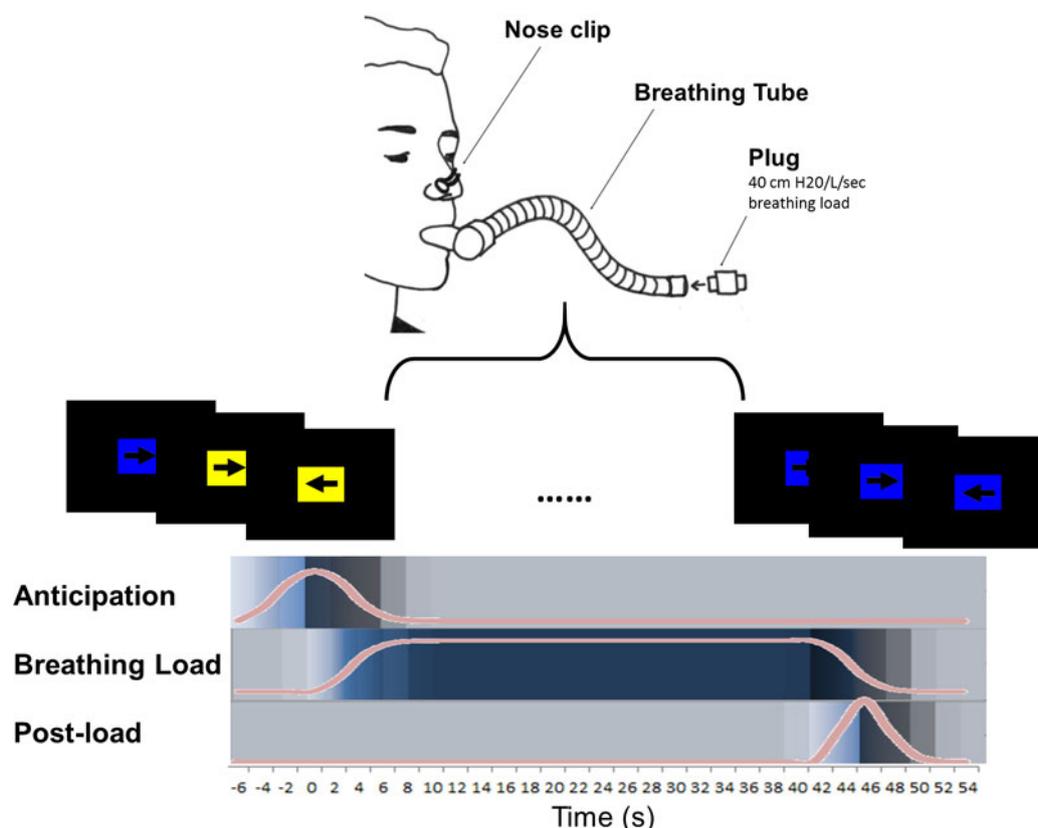


Fig. 1. Breathing load task design. Participants wore a nose clip and breathed through a hose that intermittently restricted their breathing via 40 cm H₂O/L/s loads. During scanning, participants completed a continuous performance task. Participants were instructed to press a left or right button in response to left or right pointing arrows, respectively. During ‘anticipation’ conditions, colored rectangles behind these arrows (mean duration 6 s) signaled the likelihood of an upcoming breathing load period (duration 40 s). A blue rectangle signaled no subsequent breathing load, and a yellow rectangle signaled a 25% chance of subsequent breathing load. A post-load period (mean duration 2 s) followed all breathing load conditions. The task was presented in an event-related design in two runs (total time = 17 min and 4 s; TR = 2 s). Across both runs, 34 baseline conditions and 32 anticipation conditions were jittered. Eight anticipation conditions were followed by breathing load. All conditions were jittered to optimize resolution of the hemodynamic response function.

Aversive inspiratory breathing load paradigm

The inspiratory breathing load paradigm has been used to study aversive interoception in healthy and psychiatric populations (Paulus *et al.* 2012; Galli *et al.* 2013; Haase *et al.* 2014; Stewart *et al.* 2014; Berk *et al.* 2015; Haase *et al.* 2015). During fMRI scanning, participants wore a nose clip and were instructed to breathe through a hose that intermittently restricted breathing for 40-s periods via 40 cm H₂O/L/s inspiratory loads (Fig. 1). Colored rectangles signaled the likelihood of an upcoming breathing load period (yellow = 25% chance of subsequent breathing load, blue = 0% chance). Participants were made aware of these stimulus-outcome associations via oral instructions: ‘A blue box indicates that there will be no restriction placed on your breathing. However, a yellow box means there will be a 25% chance that your breathing will become restricted for 40 s. So, blue means no restriction and yellow means there is a 1-in-4 chance of breathing restriction.’

Overlaid on each rectangle, an arrow pointing left or right cued participants to press a left button or a right button, respectively. Accuracy and reaction time (RT) on this continuous performance task measured attention. During the task, cardiac and respiratory waveforms were recorded (see online Supplementary Information for further detail).

Clinical symptoms and state measures

Participants completed the Eating Disorder Inventory-2 (Garner, 1991) and the Beck Depression Inventory (BDI-II; Beck *et al.* 1996). Temperament and Character Inventory (TCI) Harm Avoidance subscale scores (TCI-HA; Cloninger *et al.* 1994) and Spielberger State-Trait Anxiety Inventory scores (STAI; Spielberger *et al.* 1970), which measure anxious temperament and trait anxiety, respectively, were available for a subset of participants and therefore were included only in secondary exploratory analyses (see online Supplement).

After experiencing breathing load for the first time pre-scan and again immediately after scanning, participants rated the pleasantness, unpleasantness, and intensity of the experience from 'not at all' to 'extremely' using 10 cm visual analogue scales (VAS). Additional post-scan VAS ratings of feelings of faintness, choking, abdominal distress, chest pain, and heart palpitations were also collected.

Image acquisition

T2*-weighted echo-planar images were acquired on a 3T General Electric Discovery MR 750 (Milwaukee, WI). High-resolution T1-weighted anatomical images were acquired to permit activation localization and spatial normalization. EPI-based field maps corrected susceptibility-induced geometric distortions. See online Supplementary Information for additional image acquisition details.

Statistical analysis

Behavioral task performance and VAS ratings

To examine whether behavioral performance on the continuous performance task differed between groups, which could indicate differential effects of the aversive breathing load, a Group (RAN, CW) \times Condition (Anticipation, Breathing Load, Post-Breathing Load) repeated measures analysis of variance (ANOVA) was conducted on behavioral task RT and accuracy measures. Mean VAS intensity, pleasantness, and unpleasantness scores were submitted to a Group (RAN, CW) \times Dimension (pleasantness, unpleasantness, and intensity) repeated measures ANOVA. Due to significant violations of the normality assumption, independent samples Mann-Whitney *U* tests compared groups on post-scan VAS ratings of other sensations.

MRI statistical analyses

Group-level statistical analyses were conducted using AFNI, FSL, and R statistical packages (see online Supplement for additional detail).

Primary analyses examined whether groups differed in mean peak activation during breathing load anticipation, breathing load receipt, and after breathing load termination. We conducted between-group *t* tests (using AFNI's 3dttest++) within each condition. Effect sizes (Cohen's *d*) were calculated for these within-condition, between-group contrasts. We also conducted an exploratory Group (CW, RAN) \times Condition (anticipation, breathing load, post-breathing load) whole-brain linear mixed effects analysis (LME) analysis (see online Supplement).

Secondary analyses examined potential group differences in the time course of blood oxygenation level dependent (BOLD) responses across all stages of interoception (from anticipation to breathing load receipt to post-breathing load). Time course data were modeled using AFNI's 3dDeconvolve TENT function across all three conditions (see online Supplement). A Group (RAN, CW) \times Time LME using AFNI's 3dLME was used to compare time-course data from the RAN and CW groups in our *a priori* search regions of interest (ROIs).

To limit multiple comparisons, we restricted our fMRI analyses *a priori* to six bilateral regions involved in non-painful aversive interoception (Hayes & Northoff, 2012) and implicated in aversive interoceptive prediction error (Barrett & Simmons, 2015): (1) the insula in its entirety; (2) an anterior/mid-cingulate (ACC) region, including subgenual, rostral, and dorsal components of the anterior cingulate gyrus traditionally associated with affective and cognitive processing, respectively; (3) the posterior cingulate (PCC); (4) a lateral PFC region, including ventrolateral and dorsolateral PFC; (5) a striatal region, including the caudate, putamen, and ventral striatum; and (6) the amygdalae (see online Supplementary Fig. S1). Region selection was also informed by healthy control activation documented in the breathing load task (Paulus *et al.* 2012; Galli *et al.* 2013; Haase *et al.* 2014; Stewart *et al.* 2014; Berk *et al.* 2015) and locations of altered anticipatory and processing-related activation in women RAN during aversive taste and pain tasks (Cowdrey *et al.* 2011; Strigo *et al.* 2013). Regions were derived from the Harvard-Oxford atlas (Desikan *et al.* 2006).

Small-volume correction was applied as follows: Intrinsic smoothness was estimated using the spatial autocorrelation function (ACF) option in AFNI's 3dFWHMx. Results in each region were then corrected for multiple comparisons using Monte Carlo simulations via AFNI's 3dClustSim, including ACF estimates. This robust approach employs non-Gaussian models and spatial ACFs (Eklund *et al.* 2016). First-nearest neighbor clustering and a per-voxel threshold of $p < 0.01$ (two-tailed) was set for each of the six search regions with a cluster-wise two-tailed alpha threshold of 0.05.

Exploratory associations with clinical variables and potential confounds

Mean percent signal change was extracted from significant clusters resulting from within-condition *t* tests. Huber robust regressions (Huber, 1964), were conducted in R to examine associations of percent signal change with illness duration, lowest post-pubertal body mass index (BMI), months since last AN

symptoms, VAS ratings, and psychological measures. To control for family-wise error, these exploratory analyses were Bonferroni-corrected for the number of significant clusters within each breathing task condition and the number of clinical assessments tested (corrected alphas = 0.0033, 0.0002, and 0.00013 for anticipation, breathing load, and post-breathing load analyses, respectively). Additional exploratory analyses examined the potentially confounding impact of past comorbidities and AN subtype.

Results

Participant characteristics

One RAN and one CW did not complete the second run of the task, and two RAN participants were excluded because of excessive head movement, yielding a final sample of 17 RAN (11 restricting subtype, six binge-eating/purging subtype) and 25 CW who completed two full runs of the task and were included in analyses. The RAN and CW groups did not differ in age (RAN mean = 26.5; CW mean = 28.3; $p = 0.449$), BMI (RAN mean = 21.6; CW mean = 22.0; $p = 0.568$), or years of education (RAN mean = 15.9; CW mean = 15.3; $p = 0.294$; see online Supplementary Table S1). Consistent with prior findings (e.g. Wierenga *et al.* 2015), past mood and anxiety disorders were more common in the RAN group, and available data suggested that compared with controls, the RAN group reported higher, but non-clinically significant, levels of depressive symptoms, trait anxiety, and harm avoidance (see online Supplementary Table S2).

Task performance, VAS ratings, and physiology

There was no statistically significant Group \times Condition interaction on accuracy or RT on the task, and there were no main effects of group on task performance (see online Supplementary Table S2). RAN participants reported stronger post-scan choking sensations relative to CW, but groups did not differ on ratings of pleasantness, unpleasantness, or intensity of the breathing load experience, nor did they differ on reported feelings of faintness, heart palpitations, chest pain, or abdominal distress after the scan (see online Supplementary Table S3).

Groups did not differ in average (RAN: 0.59 ± 0.39 mm; CW: 0.67 ± 0.44 mm; $p = 0.501$) or maximum (RAN: 1.80 ± 0.84 mm; CW: 1.74 ± 0.86 mm; $p = 0.832$) motion during the task. Because of hardware malfunction, physiological data could be analyzed for only a subset of participants; however, analyses of available data provide some indication that groups did not differ in heart rate, breathing rate, or breathing rate variability (see online Supplement).

Group differences in peak activation within conditions

Results of between-group t tests within each task condition are presented in Table 1 and Fig. 2. Effect size maps of group effects are shown in online Supplementary Fig. S2. Results of exploratory Group \times Condition whole-brain voxel-wise analyses are in online Supplementary Table S4.

Breathing load anticipation

The RAN group showed reduced activation compared with CW in right mid-insula during breathing load anticipation (Fig. 2a).

Breathing load

During breathing load, the RAN group compared with CW showed increased activation in right dorsal ACC (dACC), bilateral PCC, bilateral inferior frontal and left middle frontal gyri (IFG and MFG), right caudate, and left putamen (Fig. 2b).

Post-breathing load

After breathing load, the RAN group showed increased activation compared with CW in clusters within all ROIs: bilateral anterior, mid-, and posterior insula, bilateral dACC, right PCC, bilateral IFG and MFG, bilateral caudate and putamen, and bilateral amygdala (Fig. 2c).

Group differences in the time course of activation

Statistically significant Group \times Time interactions were detected in clusters in mid and posterior insula, subcallosal and dACC, PCC, MFG, IFG, and left amygdala, indicating that the time course of the BOLD response, from aversive stimulus anticipation to termination, differed in RAN and CW in all of these regions (Table 2; Fig. 3; and see online Supplementary Fig. S3).

In general, CW activation these areas plateaued or gradually increased over the course of the task. In contrast, RAN participants showed steadily increasing activation during the breathing load that peaked after the offset of the aversive stimulus (Fig. 3 and see online Supplementary Fig. S3).

Exploratory associations with clinical variables

Exploratory Huber robust regression analyses revealed that RAN participants with the longest durations of illness showed the greatest hyperactivation of right MFG ($p < 0.0001$) and left caudate ($p < 0.0001$) after breathing load termination (see online Supplementary Fig. S4a). In addition, RAN participants with the greatest hyperactivation of right posterior insula after breathing load

Table 1. Between-group differences in activation during breathing load anticipation, breathing load, and post-breathing load

Hem	Region	BA	Voxels	CThr	Center of mass MNI coordinates				Cluster Cohen's <i>d</i>	% Overlap with time course cluster
					<i>x</i>	<i>y</i>	<i>z</i>	<i>t</i>		
Anticipation: RAN < CW										
R	Mid-Insula	13	9	9	43	-8	3	-3.18	1.03	
Breathing load: RAN > CW										
R	Cingulate Gyrus	24	29	8	10	5	40	3.08	0.99	38%
L	Posterior Cingulate Gyrus	31	20	12	-5	-26	44	3.03	0.98	
R	Posterior Cingulate Gyrus	23	13	12	6	-28	30	3.03	0.97	77%
L	Inferior Frontal Gyrus	47	106	13	-41	28	-11	3.20	1.03	30%
L	Middle Frontal Gyrus	9	81	13	-41	12	32	3.27	1.05	27%
L	Inferior Frontal Gyrus	44	48	13	-54	14	14	3.10	1.00	
R	Inferior Frontal Gyrus	9	36	13	53	19	25	3.09	0.99	50%
L	Middle Frontal Gyrus	45	32	13	-50	26	27	2.94	0.95	22%
L	Middle Frontal Gyrus	8	22	13	-42	12	46	3.10	1.00	36%
R	Inferior Frontal Gyrus	47	21	13	43	29	-8	3.07	0.99	14%
L	Middle Frontal Gyrus	9	17	13	-41	33	33	3.13	1.01	35%
R	Caudate		16	11	12	16	4	2.94	0.95	
R	Caudate		13	11	14	9	14	3.01	0.97	
L	Putamen		11	11	-22	6	-2	2.85	0.92	
Post-load: RAN > CW										
R	Posterior Insula	13	150	9	36	-13	7	3.37	1.08	12%
L	Posterior Insula	13	107	9	-36	-11	3	3.12	1.00	7%
R	Anterior Insula	47	25	9	30	16	7	2.95	0.95	
L	Mid/Anterior Insula	13	20	9	-30	5	11	3.32	1.07	
L	Anterior Insula	47	13	9	-38	18	-6	3.09	0.99	
R	Mid-Insula	13	10	9	38	2	5	2.99	0.96	
R	Mid-Cingulate	24	159	8	7	8	33	3.23	1.04	31%
L	Anterior Cingulate	24	18	8	-8	33	20	3.06	0.99	
R	Posterior Cingulate Gyrus	31	144	12	1	-40	28	3.28	1.06	29%
R	Posterior Cingulate Gyrus	31	36	12	11	-23	40	3.20	1.03	19%
L	Posterior Cingulate Gyrus	31	31	12	-6	-28	43	3.23	1.04	
L	Middle Frontal Gyrus	8	92	13	-32	24	46	3.11	1.00	28%
L	Inferior Frontal Gyrus	47	48	13	-39	25	-12	3.06	0.98	27%
L	Middle Frontal Gyrus	46	41	13	-50	24	29	3.03	0.98	51%
R	Middle Frontal Gyrus	8	36	13	37	22	45	3.04	0.98	53%
R	Middle Frontal Gyrus	6	32	13	32	3	51	3.14	1.01	50%
L	Inferior Frontal Gyrus	13	24	13	-38	24	20	2.99	0.96	25%
L	Inferior Frontal Gyrus	44	23	13	-48	10	12	3.13	1.01	
R	Inferior Frontal Gyrus	9	21	13	54	20	25	3.14	1.01	67%
L	Putamen		121	11	-26	-1	3	3.10	1.00	
R	Caudate		75	11	13	7	13	3.17	1.02	
L	Caudate		55	11	-15	8	17	3.31	1.07	
R	Putamen		52	11	28	-6	0	3.21	1.03	
R	Putamen		34	11	24	6	6	2.99	0.96	
L	Amygdala	34	28	4	-22	-2	-17	3.27	1.05	4%
R	Amygdala	34	5	4	27	-8	-18	3.49	1.12	

Coordinates are presented in LPI format. The percentage overlap between clusters from the Group \times Time analysis (see Table 2) and between-group, within-condition *t*-tests is noted in the last column. Hem, hemisphere; L, left; R, right; BA, Brodmann area; CThr, cluster threshold [$p < 0.05$, small-volume corrected (voxel-wise $p < 0.01$) with Monte Carlo simulations (via Analysis of Functional NeuroImages 3dClustSim) to guard against false-positive results].

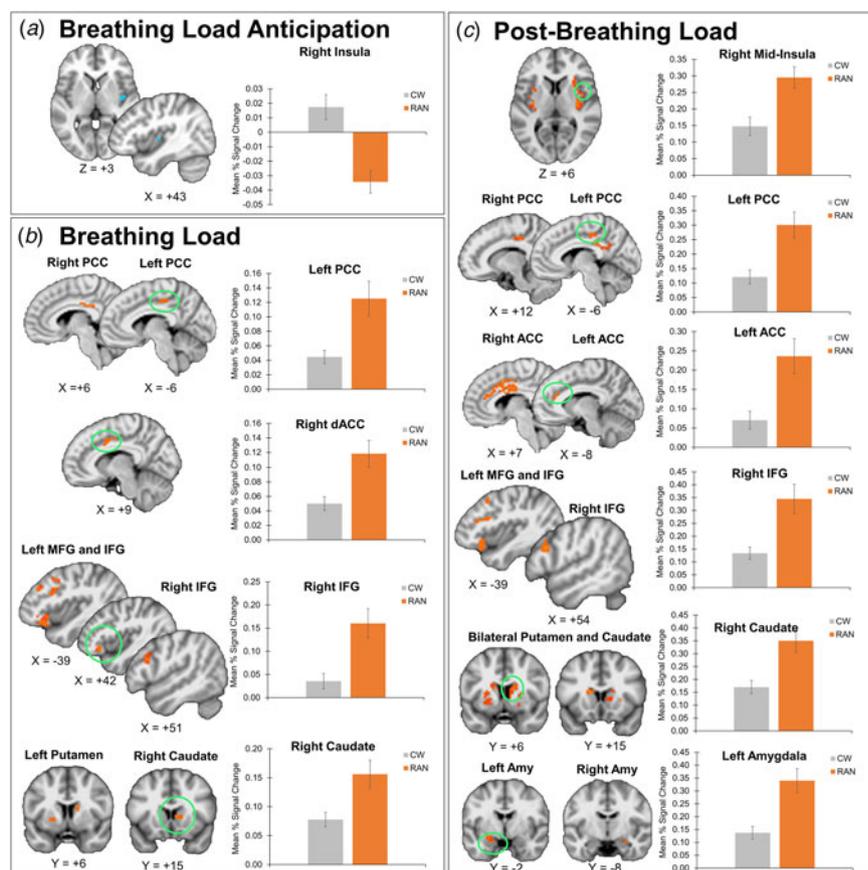


Fig. 2. Within-condition group differences in peak activation. (a) During breathing load anticipation, the RAN group showed reduced activation in the right mid-insula compared with controls. RAN participants deactivated right mid-insula during aversive interoceptive stimulus anticipation, whereas healthy controls activated this region. (b) During breathing load, the RAN group showed increased activation compared with controls in bilateral PCC, right dACC, bilateral IFG and left MFG, left putamen, and right caudate. (c) After breathing load, the RAN group showed increased activation compared with controls in bilateral anterior insula, mid- and posterior insula (graph shown for right mid-insula), bilateral caudate and putamen (graph shown for right putamen), left amygdala, bilateral PCC, bilateral ACC, bilateral IFG and left MFG, bilateral putamen and caudate, and bilateral amygdala. Error bars represent standard error of the mean. When multiple clusters are shown, the graphs correspond to circled clusters. RAN, women remitted from anorexia nervosa; CW, healthy control women; PCC, posterior cingulate cortex; IFG, inferior frontal gyrus; MFG, middle frontal gyrus; dACC, dorsal anterior cingulate cortex; amy, amygdala.

termination reported the most pronounced feelings of faintness after the scan ($p < 0.0001$; see online Supplementary Fig. S4b). RAN participants with the longest illness durations also tended to show the greatest hyperactivation of right IFG during breathing load ($p = 0.0004$), and RAN participants with the lowest lifetime post-pubertal BMIs trended toward more pronounced hyperactivation of right IFG after breathing load termination ($p = 0.001$), but these findings did not survive correction for multiple comparisons and should not be interpreted without replication. Results of additional regression analyses are reported in the online Supplement.

AN subtype and history of major depression or an anxiety disorder did not have an appreciable effect on RAN activation (see online Supplement).

Discussion

This investigation tested the hypothesis that AN is associated with altered processing of non-painful aversive interoceptive afferents. Several results support this hypothesis. First, although RAN and CW rated the experience similarly, RAN showed altered activation in aversive interoception circuitry before, during, and after breathing load. Results of within-condition analyses revealed reduced RAN anticipatory activation in right posterior mid-insula, a region implicated in the computation of the difference between anticipated and experienced stimuli. In contrast, RAN showed increased activation during and after aversive stimulus processing in frontal, striatal, and limbic regions involved in the evaluation of stimulus salience and

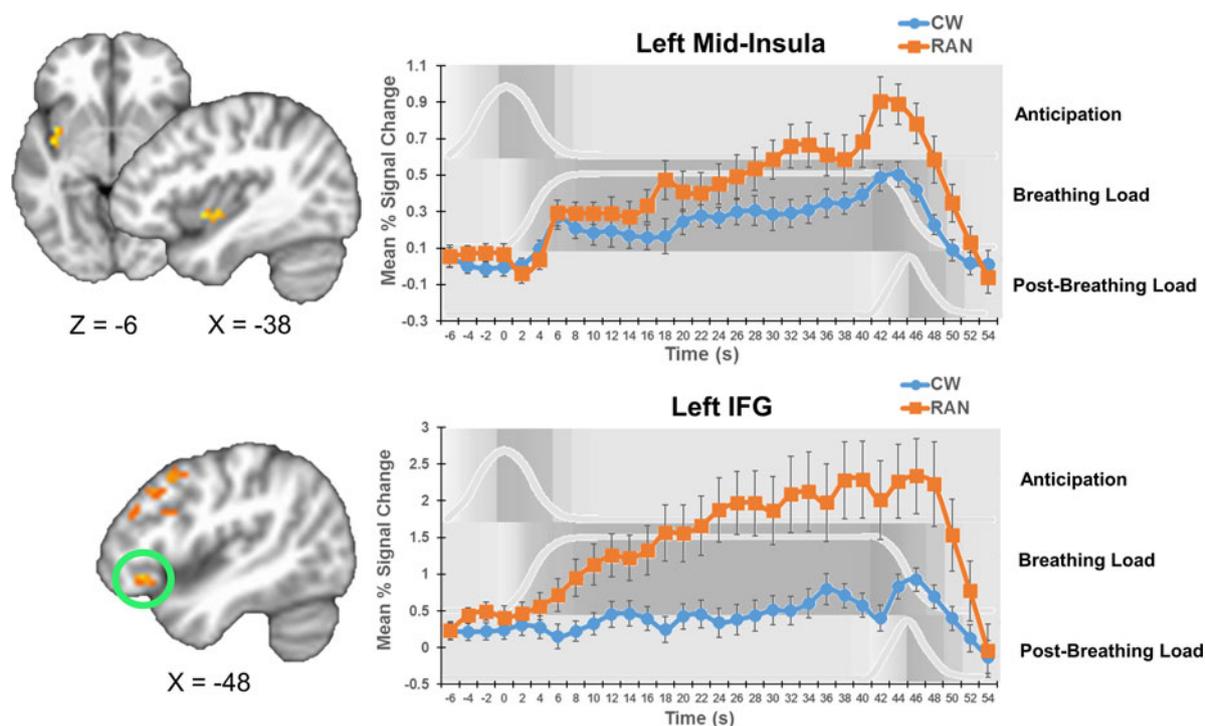


Fig. 3. Group differences in activation time course. Group \times Time interaction results suggest that the time course of activation in RAN participants statistically significantly differed from those of controls in left mid-insula and left IFG. Additional clusters in which time course differed between groups are shown in online Supplementary Fig. S3. Time course graphs for anticipation, breathing load, and post-breathing load conditions are shown in light gray and with gray shading the background. Error bars represent standard error of the mean. RAN, women remitted from anorexia nervosa; CW, healthy control women; IFG, inferior frontal gyrus.

modulation of cognitive reactions to stimuli. These between-group differences were consistently associated with large effect sizes. Second, RAN women showed elevated and steeply increasing activation during the receipt of an aversive stimulus, whereas in CW, activation remained fairly steady or more gradually increased during the sustained aversive experience. Taken together, these findings are consistent with altered anticipatory signaling and interoceptive network hyper-responsivity in RAN. Consequently, RAN may be more sensitive to and have more difficulty adjusting to changes in homeostatic state. Our exploratory results preliminarily indicate that altered processing of interoceptive state changes in RAN may contribute to or result from increased sensitivity to interoceptive sensations, prolonged periods of starvation, and ultimately more severe AN.

Anticipation phase: interoceptive prediction signaling

In contrast to prior findings of increased RAN activation in the insula during the anticipation of acute pain (Strigo *et al.* 2013) and food image exposure (Oberndorfer *et al.* 2013a), anticipation of breathing

load was associated with decreased insular activation in RAN compared with CW. This was one of the largest between-group differences observed ($d = 1.03$). Whereas in these prior pain and food tasks, interoceptive stimuli are rapidly presented (1–16 s), aversive breathing load in the current study is chronically applied for 40-s intervals. Thus, unlike anticipation periods in rapid tasks, the breathing task's anticipation period precedes a prolonged interoceptive state change. RAN deactivation of the mid-insula during anticipation of an aversive experience may therefore constitute a faulty prediction or preparatory signal for aversive state change. Unlike RAN, highly resilient elite athletes show an increased mid-insula anticipatory response and decreased receipt response to breathing load (Paulus *et al.* 2012). Thus, a decreased RAN response during anticipation may ultimately interfere with adaptive processing of the protracted aversive experience. Our results add to a growing literature suggesting anticipatory neural signals in RAN are altered.

Receipt phase: interoceptive processing

The integration and processing of interoceptive stimuli also involves mid- and posterior insula (Craig, 2002,

Table 2. Group \times time interactions: mixed effects time series analysis

Hem	Region	BA	Voxels	CThr	Center of mass MNI coordinates			
					<i>x</i>	<i>y</i>	<i>z</i>	<i>t</i>
R	Posterior Insula	13	18	9	33	-21	16	1.984
L	Mid-insula	13	16	9	-38	-3	-6	1.958
R	Cingulate Gyrus	32	21	8	7	9	40	2.072
R	Cingulate Gyrus	24	11	8	10	-11	34	1.920
R	Cingulate Gyrus	32	11	8	9	20	31	2.106
R	Subcallosal Gyrus	25	10	8	2	8	-13	2.092
L	Anterior Cingulate	32	10	8	-9	23	-6	1.900
R	Cingulate Gyrus	24	10	8	5	1	25	1.974
R	Cingulate Gyrus	24	9	8	3	1	45	2.100
R	Posterior Cingulate Gyrus	31	64	12	5	-32	31	2.194
R	Middle Frontal Gyrus	9	121	13	38	19	43	2.089
L	Middle Frontal Gyrus	46	51	13	-41	32	29	1.979
L	Middle Frontal Gyrus	8	39	13	-48	15	44	2.086
R	Middle Frontal Gyrus	6	39	13	30	3	55	2.211
L	Middle Frontal Gyrus	6	36	13	-30	7	55	2.068
R	Inferior Frontal Gyrus	9	31	13	51	17	25	1.948
L	Inferior Frontal Gyrus	47	27	13	-36	28	-9	1.961
L	Inferior Frontal Gyrus	47	25	13	-48	28	-9	2.148
L	Middle Frontal Gyrus	46	18	13	-40	15	26	1.962
R	Inferior Frontal Gyrus	45	15	13	47	28	1	2.006
L	Amygdala	34	5	4	-28	-5	-21	2.111

Coordinates are presented in LPI format. Hem, hemisphere; L, left; R, right; BA, Brodmann area; CThr, cluster threshold [voxel-wise $p < 0.01$, cluster-wise $p < 0.05$, small-volume corrected with Monte Carlo simulations (via Analysis of Functional NeuroImages 3dClustSim) to guard against false-positive results].

2003), and mid-insula may be essential to online monitoring of interoceptive state change (Klein *et al.* 2013). Again, in contrast to brief pain paradigm results, we did not find reduced insula activation in RAN during stimulus receipt. Breathing load tasks have not been used to study individuals with eating, anxiety, mood, or obsessive compulsive-related disorders, but findings in participants with substance use disorders and individuals high and low in resilience provide interesting comparisons with our RAN results. The time course of the mid-insula and posterior insula BOLD response in RAN increased more steeply during breathing load than that of CW. The pattern of reduced activation during anticipation and steeply increasing insular activation during breathing load is similar to prior findings of decreased anticipatory insular activation and increased activation during breathing load in adolescents with substance use disorders compared with controls (Berk *et al.* 2015). The response to the aversive stimulus observed in RAN is also consistent with that seen in individuals low in resilience (Paulus *et al.* 2012). Thus, the observed RAN pattern of escalating mid-insular activation during aversive stimulation

may represent a transdiagnostic neural signature of difficulty adapting to interoceptive change.

The mid-insular location of altered RAN response time course and reduced activation during anticipation is very near clusters of aberrant activation previously observed in RAN women during stomach sensation processing (Kerr *et al.* 2016), food picture viewing (Oberndorfer *et al.* 2013a) and unexpected sucrose taste omission (Frank *et al.* 2016). Our findings are also consistent with insular anatomical abnormalities documented in both ill adult and adolescent AN and remitted adults (Frank, 2015; Shott *et al.* 2015).

RAN women also showed aberrant neural responses during breathing load within the larger aversive interoceptive circuit, including the striatum, lateral PFC, and PCC. Similarly increased activation in PFC has been observed in RAN during receipt of pain (Strigo *et al.* 2013) and during breathing load in adolescents with active substance use disorders (Berk *et al.* 2015). Increased RAN activation in prefrontal top-down modulatory regions may represent an effort to downregulate 'noisy interoceptive afferents' (Paulus & Stein, 2010). Increased activation in RAN during

breathing load in caudate, putamen and MFG and IFG is consistent with findings of increased RAN response to aversive taste in these visceral and sensory information processing and integration regions (Cowdrey *et al.* 2011), and further suggests a generalized AN hypersensitivity to aversive interoceptive events.

Increased and steeply rising prefrontal, insular, and cingulate activation during breathing load in RAN may also represent an error signal or an effort to adjust to a faulty prediction signal. In healthy individuals, the processing of unexpected compared with expected pain engages greater activation in similar regions to those hyperactivated or more steeply increasing in activation in RAN compared with CW during breathing load – bilateral PFC and insula (Seidel *et al.* 2015). Similarly, receipt of an unexpected sucrose taste is associated with increased activation in right cingulate and right medial frontal cortex in ill AN compared with CW (Frank *et al.* 2012). Overall, interoceptive circuit-wide RAN hyper-responsivity may suggest difficulty adapting to and regulating responses to aversive interoceptive change.

Return to homeostasis phase: post-aversive interoceptive experience

RAN women also showed pronounced increases in activation and, in the case of right posterior insula, delayed decreases in activation after breathing load termination. One hypothetical explanation is a delayed ability to return to baseline in RAN. Prolonged hyperactivation in RAN could add ‘noise’ to aversive interoceptive processing signals and disrupt learning from experience. This could interfere with response to eating exposures during treatment and contribute to high relapse rates. Despite this potentially clinically-relevant and very preliminary hypothesis, increased post-load activation in RAN may instead reflect carry-over of breathing-load-related (rather than post-load) hyperactivation. Further investigation is needed to understand the neural response to interoceptive negative reinforcement in RAN.

Limitations and directions for future study

Several study limitations are important to acknowledge. First, although group differences in carbon dioxide levels have not been documented in prior breathing load task studies, and analysis of available data suggests our groups did not differ in cardiac or respiratory rate, we did not collect carbon dioxide levels from participants. Future studies would benefit from systematically examining the relationship between breathing rate, expired carbon dioxide, and neural activation in AN. Second, differences in fitness levels between groups could explain our

findings. Although RAN and CW groups did not differ on hours of exercise per week ($p=0.147$), the relationship between aversive interoceptive state change processing and fitness should be examined in future studies of AN. Third, we were unable to use computational modeling to quantify prediction error as this approach requires a rapid, event-related design that would not have been appropriate for the breathing paradigm. Fourth, our sample size was modest, and physiological and self-report data were missing from some participants. Despite statistical investigation of the effects of past comorbidities in the RAN group, their potential impact cannot be ruled out completely. Future studies with larger sample sizes are necessary to thoroughly examine the potential influence of diagnostic subtype, anxiety symptoms, and past comorbidities on altered interoceptive processing associated with AN. Inclusion of clinical control groups (e.g. women with a history of panic disorder or panic attacks) may help to further characterize these influences. Further, since we included only remitted AN, our results should be compared with those from an ill sample. Additional research is needed to better understand why altered interoceptive network activation in RAN was not associated with subjective intensity or unpleasantness of the breathing load.

Clinical implications

Our results have important implications for understanding AN pathogenesis and improving AN treatment. Neuropsychological task data suggest impairments or inefficiencies in cognitive flexibility in AN, some of which persist after recovery (Tchanturia *et al.* 2012), but no study to date has focused on the ability to adapt to a change in interoceptive state. Our data suggest a brain-based difficulty predicting and adapting to internal milieu state shifts that may contribute to the severity and persistence of AN. Theories linking interoceptive prediction error to anxiety (Paulus & Stein, 2010; Barrett & Simmons, 2015), associations between perceived sensory sensitivity and emotion dysregulation in AN (Merwin *et al.* 2013), and our observed relationships among markers of AN severity and prefrontal and striatal hyperactivation after aversive interoception all support this notion. In addition, more pronounced insular hyperactivation after aversive interoception in RAN participants was associated with increased feelings of faintness after scanning. In the context of unreliable and unhelpful interoceptive signals and neural hypersensitivity to non-painful aversive interoceptive stimuli, individuals with AN may rely on external feedback to guide their behavior and pursue a state of interoceptive ‘sameness.’ Because eating changes our homeostatic state, starvation may be one

method of avoiding aversive, unpredictable internal change in AN.

No empirically-supported psychotherapies exist for the treatment of adults with AN, and our results preliminarily suggest that this may be because existing interventions rely on new learning from experience. Integration of stimulus anticipation and receipt is critical for learning about and responding to salient stimuli (Schultz *et al.* 1997), and our findings add to a growing body of evidence that this integration is altered in AN (Frank *et al.* 2012). Determining next whether these alterations are plastic may help direct future treatment efforts for AN. Future studies should incorporate measures of cognitive flexibility, examine the rate and magnitude of habituation, and apply computational modeling to aversive interoceptive tasks to clarify how aversive learning may contribute to AN treatment resistance. Additionally, future research should further investigate the role of anticipation, processing, and recovery from interoceptive state changes in AN as they directly relate to eating behavior.

Supplementary material

The supplementary material for this article can be found at <https://doi.org/10.1017/S0033291717001635>.

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