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The Clinical Significance of Posterior Insular Volume in Adolescent Anorexia Nervosa

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Abstract

Objective—The diagnostic criterion *disturbance in the experience of the body* remains a poorly understood and persistent feature of anorexia nervosa (AN). Increased sophistication in understanding the structure of the insular cortex - a neural structure that receives and integrates visceral sensations with action and meaning - may elucidate the nature of this disturbance. We explored age, weight-status, illness severity, and self-reported body dissatisfaction associations with insular cortex volume.

Methods—Structural MRI data were collected from 21 adolescents with a history of AN and 20 age, sex, and BMI-matched controls. Insular cortical volumes (bilateral anterior and posterior regions) were identified using manual tracing.

Results—Volumes of the right posterior insula demonstrated: 1) a significant age by clinical status interaction ($\beta = -0.018 \pm 0.008$; $t = 2.32$, $p = 0.02$) and 2) larger volumes were associated with longer duration of illness ($r = .48$, $p < .04$). In contrast, smaller volumes of the right anterior insula were associated with longer duration of illness ($r = -.50$, $p < .03$). The associations of insular volume with body dissatisfaction were of moderate effect size, also of opposite direction, but a statistical trend in right posterior ($r = .40$, $p < .10$ in right posterior; $r = -.49$, $p < .04$ in right anterior).

Conclusions—In this exploratory study, findings of atypical structure of the right posterior insular cortex point to the importance of future work investigating the role of visceral afferent signaling in understanding disturbance in body experience in AN.

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interoception; anorexia nervosa; insula; body image disturbance; visceral hypersensitivity

Introduction

Part of the core phenomenology of anorexia nervosa (AN) is that of having one's attention intrusively captured by the experience of one's body, combined with the presence of dangerous weight loss behaviors that result in the suppression of or alteration in somatic experiences (e.g., bradycardia, bradygastria, reduced hormonal surges with menstruation) (1, 2). The elevated mortality associated with AN is well-documented: AN remains one of the leading causes of premature mortality due to psychiatric causes with a Standardized Mortality Ratio of 5.9 (4.2–8.3, 95th%CI) and a Standardized Mortality Ratio attributable to suicide of 31 (21–44, 95th%CI) relative to other forms of mental illness or population controls (3). While there have been impressive advances in the treatment of AN, particularly for adolescents (4–6), treatments for both adults and adolescents have had limited effectiveness in improving disturbance in the experience of the body (often referred to as body image disturbance) (1, 7, 8). The degree of body image disturbance predicts poor treatment response (9) and has been reported to motivate hazardous weight loss behaviors (1, 2). Elucidation of the biological substrate of body image disturbance is thus considered essential to understanding the pathophysiology of AN.

Body image disturbance is a complex construct comprising cognitive (e.g., preoccupation with appearance), attitudinal (e.g., evaluative judgments about weight), perceptual (e.g., ability to perceive body size) and experiential components (e.g., irregularities in interoceptive sensitivity). While the cognitive, evaluative, and, increasingly, perceptual aspects of body image disturbance are well-characterized (e.g., (10, 11), there has been far less study on the experiential components of body image disturbance (12). Accumulating evidence suggests that aberrations in body experience in AN extend well beyond the restricted domain of weight and shape (as specified in the Diagnostic and Statistical Manual, 5th edition (13), and may include aberrations in basic somatosensory and interoceptive experiences (e.g., of heartbeat detection, tactile discrimination) as indexed by objective laboratory tasks (see Review by Gaudio) (14–17). Individuals with AN also report more intrusive sensory and interoceptive symptoms whether currently ill or weight-restored relative to healthy controls, intimating that they experience such signals as aberrant (18). This somatic sensitivity was positively associated with an evaluative measure of body dissatisfaction, suggesting that interoceptive signals and more top-down evaluative judgments of the body might be inter-related and further, that dietary restriction was, in part, motivated by a desire to suppress sensory sensitivity (18, 19).

Why interoceptive signals and negative attitudes about the body may be associated is unclear, but some common features of AN, such as "feeling fat," offer a hypothetical bridge as to how interoceptive experience, interpretation of that experience, and actions motivated by that experience may link these concepts. To clarify, there is evidence that those who later develop AN have increased experiences of early life events that may increase sensitivity to

and preoccupation with somatic experience. For example, there is evidence of an excess of early gastrointestinal events, eating difficulties, and somatic symptoms prior to illness onset in AN with sufficient evidence supporting digestive problems as a risk factor for AN (20–23). While these early events may increase sensitization of (and resulting intrusive visceral experience) of afferent visceral pathways independently (24), such sensitivity may be combined with a learning history in which those with AN value the suppression of bodily signals or disregard the informative value of somatic signals rather than use these somatic markers to guide adaptive decision-making (25), (for review see (26)). In the aforementioned instance, extreme sensitivity to afferent input of the gut may differentially direct attention to the body contributing to preoccupation (18), while learning and other environmentally influenced processes shape interpretation and meaning- including a generalized distrust or shame of bodily sensations that intrude upon awareness. In fact, Herbert, Blechert, Hautzinger, Matthias, and Herbert (27) demonstrated this dissociation in a study of intuitive eating, finding that interoceptive sensitivity and the appraisal of interoceptive signals were independently associated with the ability to eat in response to interoceptive signaling and body mass (27). Thus, one hypothesis about the persistence of body image disturbance in AN may be that the components of body image that are influenced by learning and environmental factors (e.g., interpretations and attitudes) can be addressed via treatment while basic aberrations in afferent signaling from the periphery are trait features that individuals must learn to manage. However, research to date cannot distinguish whether it is something about the nature of the afferent input that is aberrant (i.e., a “bottom-up” process), a “top-down” error related to the interpretation or contextualization of somatic signals, or both.

Insular Cortex Organization

The potential involvement of visceral sensory aberrations in AN suggests the insular cortex is a candidate for understanding the biological basis of AN (28). The insular cortex has been described as a “center of awareness” (29) and is situated as a relay station between afferent inputs from the viscera to neural structures that help code the salience and meaning of visceral inputs (29). There have been several proposed functional mappings of the insula cortex based on differences in structural architecture: a grandular insula located in the posterior region, an agranular insula in the anterior region, and a dysgrandular band in the middle insula (e.g.,(29)); or based on meta-analytic (e.g., (30)); or the synthesis of resting-state connectivity with meta-analytic findings from human neuroimaging studies (31). Consistent across these models is the delineation of a posterior insular cortex structure that is functionally connected to the somatosensory and supplementary motor areas and is associated with activities in somatosensory, sensorimotor, and pain domains (31). These inputs are then relayed forward to the anterior regions of the insular cortex in which a ventral anterior region, primarily functionally connected with limbic areas (e.g., amygdala, posterolateral orbitofrontal cortex, ventral tegmental area) is broadly implicated in the experience and contextualization of emotional, autonomic, and chemosensory experiences (24, 32). The dorsal anterior region is functionally connected to the anterior cingulate cortex and dorsolateral prefrontal cortex and is implicated in executive control and higher cognition (31). Notably, the insular cortex is one of the most widely activated neural regions across studies of awareness, emotional experience, craving/urges, and more recently, decision-

making (for an elegant review see (33)). Despite this diversity in function, Craig (29) suggests that this is due to the influence of the core function of the insular cortex as an integrative site for autonomic, cognitive, and affective processing.

From a developmental standpoint, interoceptive signals initially reaching the posterior insular cortex become more elaborately deciphered and contextualized throughout maturity to increasingly guide complex decisions in both adaptive and maladaptive contexts. According to this functional neuroanatomical conceptualization, aberrant somatic experience in AN at the level of afferent input should be reflected in atypical morphology in posterior regions of the insular cortex, while aberrant somatic experiences at the level of interpretation and meaning would reflect atypical morphology in more anterior regions.

Based on available data, it is therefore reasonable to hypothesize that individuals with AN would present with structural abnormalities in both the posterior and anterior insula. In regard to the directionality of hypothesized structural abnormalities, several processes may contribute to structural differences. First, early life events and premorbid activity patterns putatively support the hypothesis of increased posterior volume in AN. Early gastrointestinal pain experiences and the performance of excessive exercise or activities more generally have been documented premorbidly in AN, two classes of experience that have been associated with modulation of brain-derived neurotrophic factor (BDNF), a growth factor implicated in neuronal growth, development and survival (34) (35). Speculatively, these experiences may contribute to increase neuronal growth in regions that receive somatic motor information such as the posterior insular cortex. Second, AN emerges during a peak period of brain development in which synaptic pruning contributes to the efficiency of neural networks (36). The onset of starvation during this vulnerable period may modulate normative process with increased volume being a consequence of reduced pruning (37). Notably, a study of age-dependent changes in subcortical structure across adolescence revealed that structures such as the thalamus, implicated in somatosensory experience, exhibited an elongated developmental course relative to the cortical plate (38). Ancient cortical structures such as the insula may likewise be vulnerable to starvation if the development of the structure is elongated, particularly as the posterior portion receives somatic inputs from the thalamus and is implicated in somatosensory function (39). Thus, this region may have more gray matter (be more developed) than anterior regions in typically developing adolescents. In a related vein, Dennis et al. reported that functional connectivity of the posterior insula increased across adolescence with temporal regions, but decreased in anterior regions (40). Then, arguably, increased volumes in posterior insula might reflect less pruning in ‘early’ sensory pathways, delays in neurodevelopment, or some other developmental disruption.

Given the covariation of structure and function, atypical structure at the level of the posterior insula could impact anterior insula function in that the “feed forward” nature of visceral information conveyed from the posterior to the anterior could support that misinterpretation of somatic signals in AN is based in part on the utilization of aberrant sensory data in guiding behavior (41). This finding would be the first step towards advancing our understanding the nature of body experience in AN: rather than a reflection of cognitive biases or extreme negative evaluations, body image disturbance may be a complex interplay of aberrant sensory information that influence cognitive/attitudinal factors.

Insular Structure in Anorexia Nervosa

To date, study of insular structure in adolescent AN has been limited. In a whole-brain analysis comparing 19 adolescents with a diagnosis of AN (mean age of 15.4) to healthy controls, Frank et al.,(42) reported increased volume in the right insular region of cases relative to controls, although insular subregions were not specified. Several recent studies that investigated structural abnormalities not restricted to the insula have been inconclusive; while focal reductions in gray matter volume (43–47) and more recently global cortical thinning (48), have been reported, other data suggests that volume reductions are secondary to starvation and remit with weight restoration (46, 48). The latter findings would seem to emphasize the importance of the findings by Frank et al.(42), suggesting that increased insular volume in AN may be a vulnerability factor for the disorder.

To date, no neurobiological markers of illness severity in AN have been established, and though markers of body image disturbance are accumulating, these too remain limited (43, 49). Thus, we undertook the current research as an exploratory study to further probe the clinical significance of insular structure in relationship to clinical parameters of illness severity and to conduct exploratory analyses of insular structure in association with subjective measures of body image disturbance. We studied all adolescents with AN who presented to a specialized outpatient center for the treatment of eating disorders who either currently or previously met criteria for AN. Each individual was characterized in terms of length of their illness and current severity of weight and attitudinal symptoms (all described below). With this sample, we addressed the following questions: 1) *Do age-related differences in the size of insular cortex regions differ in subjects with a diagnosis of AN relative to sex and age-matched controls?* Our interest in age related changes was based on the following. First, AN has a rather unique illness course, with incidence rare before pubertal onset and in adulthood. Thus, age-specific differences were of interest to inform illness pathophysiology. Second, given developmental alterations in brain structure (for example,(36, 38, 50, 51)), controlling for age is necessary to avoid study confounds. While there is no evidence based on clinical presentation, structural, or functional data to suspect that aberrations in body image experience would be uni-lateral, existing findings on insular function in AN have demonstrated right-sided aberrations. Consistent with this, we hypothesized that anatomical differences will be right sided, and will occur in both the anterior and posterior regions relative to healthy controls. Such findings would suggest differences at the level of encoding basic somatosensory input as putatively contributing to disturbances in body experience. 2) *Is volume in particular insular regions associated with duration of illness (independent of weight status or lowest BMI)?* We hypothesized that volume size in both the right anterior and right posterior insula correlate with illness parameters independent of BMI. Associations between insular volume and illness parameters (controlling for the effects of body weight) would provide important support for the role of structural abnormalities in the insular cortex underlying the pathology in AN or on the maintenance of the disease, respectively. 3) *Will attitudinal measures of body dissatisfaction be associated with insular volume?* We hypothesized that the volume of the right posterior insular cortex would be associated with body dissatisfaction, a finding that would support the role of visceral afferent input as contributing to body image disturbance in AN.

Method

Overview

This data is part of a larger study of interoception across typical adolescence and interoception across adolescence in AN. Adolescents between the ages of 11–19 years old were recruited for a study of "gut feelings." Recruited individuals participated in a laboratory session and completed two MRI scans tapping decision-making and emotional regulatory capacities (reported elsewhere, see (52) and for details about these MRI tasks (53). Here we focus on the clinical sample and report on the structural data from these scans.

Recruitment

We attempted to recruit all individuals with AN who presented to a specialized outpatient medical clinic for the treatment of eating disorders at a Southeastern academic medical center. The control sample was recruited from this same medical clinic. Additional recruitment of both the clinical and healthy control samples was conducted throughout the university associated with this medical center.

Healthy control recruitment—To maximize the generalizability of our control sample, population-based screening methods were employed to screen all eligible adolescents who presented for a sick or well-child visit to the pediatric primary care practices of a Southeastern academic medical center (see Franz et al (54) for general screening strategy adapted for this study). The demographic composition of the primary care practice selected mimicked that of the surrounding county, thus helping to ensure the representativeness of our control group. Healthy controls were screened on random weekdays (See Figure S1, Supplemental Digital Content 1, for Study Flow). Additional recruitment occurred via the student health center, counseling center, and university message boards.

Clinical Recruitment—Clinical participants were also recruited within that medical practice. Recruiters attended every clinic session of pediatricians who were part of a specialized outpatient eating disorder program from the period of 9/1/2009–8/31/2011 to identify and screen all eligible AN participants, whether they currently met criteria for AN or had a history of AN and were attending a medical follow-up appointment. Additional recruitment occurred via the student health center, counseling center, and university message boards.

Inclusion Criteria—Clinical participants were required to have a current or prior diagnosis of AN consistent with symptoms delineated in the Diagnostic and Statistical Manual of Mental Disorders, 5th edition(13). To maximize the generalizability of our sample, we were inclusive of psychiatric comorbidities. Medications were also permitted provided the individual was on a stable dose for a period ≥ 3 months. See Table S1, Supplemental Digital Content 1, for medication list.

Exclusion Criteria—Adolescents were excluded if either they or their mother did not have fluency in English, had an IQ < 70, failed to meet MRI safety requirements (see Supplemental Methods, Supplemental Digital Content 1), were suicidal, exhibited symptoms

of psychosis, or actively abused substances. Healthy control participants could not have a history of an eating disorder or currently meet criteria for a psychiatric diagnosis as determined by screening for current symptoms (see below), parent and participant report, and medical chart abstraction.

Procedures

Overview—Adolescents and their parents attended an initial laboratory session during which diagnostic information was obtained. The adolescent participated in a mock scanning session to familiarize herself with the scanning environment and train her in procedures that would maximize the amount of usable scanning data (e.g., teaching to minimize movement). Height and weight were obtained at the time of scanning. Individuals on medications with short half-lives (e.g., stimulant medication) were asked to refrain from taking medication on the day of scanning.

Consent—Written informed consent was obtained from parents and participants above the age of 18, assent was obtained from participants from age 11 up to 19 years. The study was approved by the Institutional Review Board at Duke University Medical Center (Pro00019295).

Assessments

Screening—Control participants were screened for the absence of mental health symptoms using questions used to predict diagnostic status from a prior population cohort study of child and adolescents psychopathology.(54) Children who scored above the screen cut-off were excluded from further participation but were given a small prize.

Determination of Diagnosis and Diagnostic History—We attempted to describe individuals with AN both categorically and dimensionally. For both, diagnosis and parameters of illness history were determined by systematically combining several sources of data: 1) maternal report of her child's illness history; 2) adolescent completion of self-report measures of current symptoms; 3) adolescent report of illness history; and 4) medical chart abstraction. This included both BMI and zBMI (i.e., age-adjusted BMI, which accounts for height, weight, and age). Categorically, groups were classified into Current AN (AN), Weight-Restored AN (AN-WR), and Healthy Controls (HC). To be classified as a HC, 1) parent report indicated no history of an eating disorder; 2) adolescent self-report of Drive for Thinness were within 1 standard deviation of normative values; and 3) the medical chart contained no reference to an eating disorder diagnosis. For an individual to be considered AN-weight-restored, 1) the z-bmi score had to within 1 standard deviation of normative values, 2) the parent report indicated that the child was without an eating disorder for 3–6 months; and 3) the medical chart review did not contain a current diagnosis of AN and 4) there was no evidence of a medical sign that weight was low (e.g., bradycardia, orthostatic hypotension). For individuals to be considered AN, 1) zBMI score was 1 std below normative values; 2) parent records indicated the child had AN within past 3–6 months; 3) medical chart had a diagnosis of AN; and 4) the adolescent had a Drive for Thinness score >1 std above normative values. To determine length of illness, mothers were asked the age at which their child first developed an eating disorder, the type of eating disorder, and whether

this diagnosis was verified by a health care professional. This information was compared and combined with the medical record and referenced against the child's weight history, current weight, and current symptom endorsement. In only one case was there a discrepancy.[1] A similar strategy was employed to determine months of weight restoration. Parents were asked the length of time the child had been at a healthy weight and this was verified relative to the child's weight history and medical record. Again, there was one discrepancy, noted below.

Self-report measures—The Eating Disorder Inventory (3rd Edition) is one of the most widely used measures of eating disorder symptomatology and associated features (55). This measure was used to characterize the sample relative to other studies and provide a continuous index of current symptoms. Three subscales that measure the core pathology of eating disorders were administered in the current sample: Drive for Thinness, Bulimia, and Body Dissatisfaction. All scales have extensive validity and reliability information as well as normative data from clinical and non-clinical samples. The Drive for Thinness Scale is a 7-item scale that assesses "an extreme desire to be thinner, preoccupation with weight, and an intense fear of weight gain" (p. 14, 55). Extensive reliability, construct, and predictive validity have been established (56, 57). The internal consistency of this scale in our scale was .95. The Bulimia subscale is an 8-item scale used to index the tendency to think about or engage in uncontrollable overeating or eating in response to emotions. Along with demonstrations of discriminant validity (55), the internal consistency in our sample was .89. The Body Dissatisfaction Scale is a 7-item scale that assesses discontentment with the size and shape of various body parts that are of particular concern to those with eating disorders (e.g., stomach). The internal consistency in our sample was .94.

Neuroimaging Data Acquisition

Image Acquisition and Preprocessing—Subjects were scanned on a 3 Tesla GE MR 750 system. T1-weighted structural scans were acquired using a 3D FSPGR BRAVO pulse sequence (repetition time, 7.58 ms; echo time, 2.936 ms; flip angle, 12°; image matrix, 256 × 256; voxel size, 1 × 1 × 1 mm; 206 contiguous axial slices). T1-weighted images were preprocessed using the VBM8 toolbox (http://dbm.neuro.uni-jena.de/vbm) as implemented in SPM8 (Wellcome Department of Cognitive Neurology) in order to estimate the volume of grey matter, white matter, and cerebral spinal fluid for computation of total intracranial volume for the purpose of correcting insular volumes.

Tracing—Insula volumes were traced from raw T1 images. The total volume of each insula sub-region was calculated for each subject by summing across all voxels (each had a volume of .001 mL). To account for changes in overall brain volume, insula volumes were scaled relative to the total intracranial volume as computed using the VBM8 software. Inferential analyses were conducted on proportionally scaled data.

[1]In this case, the parent indicated that the child still had an eating disorder but had been at a healthy weight for 3-6 months. However, the child's weight and endorsed of clinical symptoms were both above clinical cut-offs. Of interest, this child had a long duration of illness (>7 years) and a lowest BMI of 11. Her current BMI of 18, may have seemed to parent as significant progress (as it was), yet an anchor of normality had been lost.

Insular volumes were manually traced using ITK-SNAP software (http://www.itksnap.org; 58), dividing the insula into anterior and posterior regions as in (59). Tracing started by identifying the central insular sulcus on two parasagittal slices as a point of reference, and tracing posterior and anterior aspects of the insula separately on successive caudal-to-rostral coronal slices. In the sagittal plane, the superior circular sulcus of the insula (SCSI) was used to demarcate the superior boundary of the insula and the inferior circular sulcus of the insula (ICSI) determined the inferior boundary. The anterior boundary of the insula was defined using the orbitoinsular sulcus (OIS), and the fusion of the superior and inferior circular sulci was used to identify the posterior boundary. Tracing proceeded rostrally from the deepest caudal point of the SCSI until the ICSI and OIS disappeared into the most ventral lateral point at the anterior aspect of the insula. Throughout the rostrocaudal extent of the insula, the central insular sulcus was used to segment the short anterior and long posterior insular gyri. A random subset of scans from 16 subjects was scored by an additional experimenter. Consistency between volume estimates was assessed and fell into the "excellent" range (60). (See supplemental materials, Supplemental Digital Content 1, for additional validation information).

Statistical Methods

Given the exploratory nature of this study, we present plots of raw values and emphasize effect size estimates over statistical significance, though we do present both. The aim of the study was to 1) examine the association between insular volume and AN and 2) to explore differences in that association at different points over the course of the disorder. As brain structure and size change rapidly during adolescence, analyses, age and BMI (normalized: z-scores) were included as covariates. Thus, study focus was on the association between MRI-derived measures of insular volume and illness parameters (duration of illness) including subjective measures of body dissatisfaction, a central psychological indicator of anorexia. Where appropriate, bivariate associations between groups were estimated and tested using both Spearman correlations (adjusted Fisher's Z transformation) and nonparametric rank Mann-Whitney-Wilcoxon procedures; correlational studies, designed to provide concordant tests of multivariable findings (see below), were partialled for age and BMI. Although not all tested instances failed to satisfy distributional assumptions for parametric testing, numerous instances did, leading us to apply non-parametric tests in all instances to facilitate comparisons. Bivariate investigations were expanded using multivariable regression modeling to accommodate putative covariates and modifiers as well as diagnostic status (group). The latter models included both linear and non-linear (i.e., quadratic, piecemeal) regressions. In some instances, models were extended to examine putative differential effects for age by group using interaction terms; BMI was included as a covariate in all instances. Model fit was examined using standard diagnostics including examination of residuals, leverage, and outliers. Analyses were supplemented using graphical procedures including box-and-whisker plots, scatter plots, and LOESS regressions. Specifically, analyses were guided by the following hypotheses: Hypothesis1: For our investigation of age-related differences in volume between individuals with AN and HC, we hypothesized that anatomical differences will be right sided, and will occur in both the anterior and posterior regions relative to healthy controls. Tests of Hypothesis 1 were based on an ANCOVA approach using a dichotomous factor denoting diagnostic status (AN = 1; CNTL = 0) to test

for volumetric differences between groups over time. Thus, measures of insular volume were regressed on a model including age (standardized), the proxy variable denoting diagnosis, an interaction term crossing the latter two factors, and BMI (standardized); in some instances, models were re-estimated after the inclusion of a quadratic term for time. Support for the hypothesis was based on the significance of the interaction term. Hypothesis 2 examined the effects of insular volume on illness duration. We hypothesized that volume size in both the right anterior and right posterior insula would correlate with illness parameters independent of BMI. Hypothesis 3 examined the association of insular volume with subjective measures of body image evaluation. We hypothesized that the volume of the right posterior insular cortex would be associated with body dissatisfaction. Tests of Hypotheses 2 and 3 were restricted to the diagnostic cohort. In each instance, measures of insular volume were regressed on scale factors denoting, respectively, illness severity and body dissatisfaction. Support for both hypotheses were based on significance of the latter terms. Models included covariates for age and BMI. For all models, we examined measures of fit (Studentized residuals), influence (Cook's D and DF fits) and leverage (DFbetas) – all of which were found to be within acceptable limits. Models were estimated using SAS 9.3 statistical software.

Results

Demographic Features

Table 1 presents demographic characteristics of the entire sample and features of the illness course and severity of the clinical sample. Average age at the time of interview was 17 years with a range between 11 and 20 years. Groups did not differ on age, or BMI. The majority of the sample was white (83%). The average age of onset of of AN was 13.78 years, with an average time to treatment of 11.35 months, and an average length of illness of 34.64 months. Based on Mann-Whitney-Wilcoxon tests, measures of eating disorder symptoms differed significantly between groups on the Drive for Thinness and Body Dissatisfaction subscales ($z = -3.5$, $p < .001$; $z = -3.6$, $p < .001$, respectively), but not the Bulimia subscale ($z = -.81$, $p > .40$).

Age

Table S2, Supplemental Digital Content 1, presents global comparisons of white matter, gray matter, cerebrospinal fluid, and total intracranial volume (the sum of gray matter, white matter, and cerebrospinal fluid). There were no differences in these global volumes across groups. All subsequent examinations of insular volumes are the percentage of insula volumes relative to these volumes.

Results for Hypothesis 1, examining between group differences in insular volume by region and age (normalized) and controlling for z-BMI are presented in Figure 1 and Table S3, Supplemental Digital Content 1. Anterior regions in both hemispheres exhibited a gradual u-shaped curvilinear pattern over time, with model fit significantly improved in both instances following the addition of a quadratic (squared) term for age. Tests of main effects for group (see Table S3, Supplemental Digital Content 1, for all insular region model coefficients) and of interaction terms crossing the age components with group status were nonsignificant in

the anterior region. Correlations of volume measurements between left and right anterior regions for both control and case subjects were significant (Control: r >.73, p<.0002; Case: r >.80, p<.0001). In the left posterior insular region, the association between volume and age was essentially constant over time for both control and case subjects; model fit was not significantly improved by addition of either quadratic and/or interaction terms. In contrast, patterns of associations in the right posterior insular region varied differentially between diagnostic groups. The association between volume and age in the right posterior region among control subjects was essentially flat, whereas, the association in the right posterior region among diagnosed subjects increased into mid-adolescence before declining (Figure 1). The differential association between age and volume by diagnostic group in the right posterior insula region was statistically significant ($\beta = -0.018 \pm 0.008$; $t = 2.32$, $p < 0.02$). The robustness of this interaction was further confirmed by examining hemispheric associations: the correlation of volume measurements between left and right posterior insular regions for control subjects was statistically significant ($r = .75$, $p < 0.001$). In contrast, the left/right correlation of volumes in right posterior regions for clinical participants did not differ significantly from zero ($r = .04$, $p > .80$), i.e., volume from the left and right posterior cortex were unrelated in clinical participants. Associations between insular volumes and age of onset, handedness, and medication status were tested both individually and concurrently; in all instances, associations were non-significant (data not shown). Thus, in support of our initial hypothesis, volume differences were noted in the right posterior insular cortex in AN subjects relative to the control group. In contrast to initial hypotheses, differences between volumes in anterior regions did not differ by group.

Illness Severity

The tests of our second hypothesis positing associations between volumetric abnormalities in insular regions with indices of illness severity are presented in Table 2 and Figure 2 (Model Coefficients are in Table S4, Supplemental Digital Content 1). In anterior insular regions, residual volumes (adjusted for normalized measures of age and BMI) decreased with length of illness to a midpoint of approximately 40 months where after the association became constant. The magnitude of the decrease was of large effect and statistically significant in the right anterior region ($r = -.5$, $p < .03$). Although the loess fit (Figure 2) suggested a nonlinear association between length of illness and volume, adding a quadratic component to the models did not significantly improve fit (data not shown). In contrast, the association in the right posterior region exhibited a pronounced positive association - marked by an almost constant increase with increasing length of illness ($r = .5$, $p < .04$). The correlation in the left posterior region was essentially zero.

Body Dissatisfaction

The test of our third hypothesis examined associations between insular volumes and a subjective measure of body dissatisfaction (Eating Disorder Inventory Body Dissatisfaction Subscale; Table 2). Findings replicated the above pattern of results: a negative association between volume measurements in the right anterior insula and the index of body dissatisfaction ($r = -.49$, $p < .04$) and a positive association between volume measurements in the right posterior insula and the index of dissatisfaction ($r = .40$, $p < .10$; Table 2).

Discussion

The most robust and consistent finding across all research questions was related to the volume of the right posterior insula cortex. Our results show 1) age-related differences relative to healthy controls, 2) a positive correlation with duration of illness, and 3) a positive association with a subjective measure of body dissatisfaction, though this moderately sized effect only approached statistical significance. This distinct pattern contrasted with those observed in either bilateral anterior regions of the insula or in the left posterior region (Figures 1 and 2).

Our data suggest that increased right posterior insular volume may be worthy of further exploration as a putative marker of illness progression or as a vulnerability factor for severity of illness in AN. The region receives and encodes visceral interoceptive input relayed via the nuclei in the solitary tract and thalamus (61). How structural irregularities in a neural region that relays somatosensory and pain information is putatively associated with illness pathophysiology in AN is unclear: our cross-sectional data cannot adjudicate how the structure of the posterior insular cortex is associated with illness pathophysiology. Notwithstanding, consideration of various hypothetical scenarios can inform the design of future studies that can address if and how afferent visceral input relates to the core pathology of AN.

For instance, one hypothesis is that the pathophysiology of AN is partially caused by or associated with developmental alterations in afferent signaling from the viscera. Given the early maturation of this region (e.g., see Mayer for review (24)), one could postulate that early life events that sensitize pathways of the gut-brain axis may influence the development of this structure. In support of this, there is evidence of an excess of early gastrointestinal events, eating difficulties, and somatic symptoms prior to illness onset in AN (20–23). Notably, while some gastrointestinal symptoms such as abdominal pain persist beyond weight restoration, many gastrointestinal symptoms also arise as a function of eating disorder symptoms and complicate treatment (62–64). Such signaling is mediated by the vagus nerve, and atypical vagal tone has also been reported in AN (though the trajectory of development in relation to illness course is unclear) (65). Thus, one possibility is that early experiences that sensitize pain pathways may influence disorder emergence via alterations in insula morphology that may be further altered by AN.

A related hypothesis is that the context of starvation itself influences the structure of this region. Notably, AN has been consistently associated with global and focal reductions of gray matter volume with some evidence documenting a restoration and normalization of cortical mass with weight restoration (43–45, 66). Findings of *increased* volume are sparse, but noteworthy (42, 43). Frank et al. (42) reported, as we did, increased volume in the right insular cortex in adolescents with AN. Using voxel-based morphometry, peak differences were localized to a region more anterior than the current study, just anterior to the central sulcus in the mid-insula. Aminato et al.(43) reported increased volume of the somatosensory cortex in adults with AN relative to controls. While these structural abnormalities cannot point to specific pathways of disorder pathophysiology, these findings do highlight the

importance of further study of regions such as the somatosensory cortex and associated somatomotor regions to inform the nature of experience in AN.

Related to this hypothesis is that starvation, and associated disruptions in hormone signaling, may delay typical neurodevelopmental changes. Thus, current findings may be reflections of this delay. Dennis et al.(40) investigated the functional connectivity of insular networks across puberty and adulthood. Results revealed that while connections largely decreased in anterior insular regions, these connections increased in posterior insular – temporal regions (40). Further, Takahashi et al. reported that bilateral insular gray matter volume is negatively correlated with age in a sample of adults (ages ≥20)(67). Better understanding of the effects of starvation at specific developmental stages may help to elucidate neurodevelopmental factors that maintain the disorder.

Yet, to understand the role of atypical structure in body image disturbance in AN, novel designs are needed. Our data demonstrated a moderate (statistically insignificant) association between size the right posterior insular cortex and body dissatisfaction and the inverse association with the right anterior insula and body dissatisfaction. However, to elucidate the significance of brain structure, brain function, and the mediators of the structure-function relationship, there are several links in the mechanistic chain missing from this investigation. Studies are needed that characterize structure, the efficiency of functional circuits mediating afferent signaling and those circuits that mediate awareness and interpretation of that signaling, and manipulations of state conditions that can inform how features of AN may alter interoceptive experience. As described in the work by Herbert et al. (68), interoceptive signals are sensed, interpreted, and then evaluated. Each of these processes can independently contribute to eating behavior and body experience.

Prior work in typically developing controls indicates that behaviors associated with AN impact interoceptive sensitivity, and further, that sensitivity to interoceptive signals and the interpretation of those signals are independent processes that differentially impact eating behavior. For example, Herbert et al. (68) investigated the effects of an acute fast on interoceptive sensitivity, finding that sensitivity to heartbeat and experienced hunger increases following an acute fast, partially due to changes in cardiodynamic activity. Further, Herbert et al. (27) reported that interoceptive sensitivity and evaluations of the aversiveness of interoceptive experience independently contributed to eating behavior. Exercise is an additional example of a behavior associated with AN that may alter the strength of interoceptive signaling and the perception of those signals. Exercise has also been shown to increase interoceptive awareness and accuracy, highlighting a functional path that may correspond with possible changes in structure. As noted, exercise is associated with alternations in BDNF function, a mechanism whereby the symptoms of AN may alter brain structure. Combined, this pattern of findings point to how aberrations in lower level encoding, when combined with behaviors and attitudes that may influence interoceptive experience, may theoretically contribute to body image disturbance in AN. Thus, one hypothetical model of the pathophysiology of anorexia is that individuals who are premorbidly viscerally hypersensitive (as due to experiences that sensitize gut brain pathways), may be differentially or more strongly reinforced by events/behaviors that alter

interoceptive experience and this may influence the interpretation and meaning of these experiences.

Further, the behaviors that constitute the illness of AN may differentially influence these processes over time - and thus, the model of interoceptive sensitivity and accuracy may alter throughout the illness of AN. Thus, future studies need to incorporate behavioral tasks that assess interoceptive sensitivity, the interpretation and evaluation of interoceptive signals, the functional circuitry in structural architecture that support these processes. Finally, given our findings regarding a region implicated in somatomotor processing, future work that particularly emphasizes the somatosensory cortex would be of special interest.

This study had several limitations. The clinical significance of findings from our cross-sectional design requires verification with a longitudinal design. While our use of population screening methods with limited exclusion criteria strengthen generalizability, it weakens internal validity as many participants were on psychotropic medications. Inclusion of a medication proxy did not alter study findings, and given the limited empirical evidence regarding the effectiveness of a psychotropic agent on AN symptoms, the concern is lessened. Finally, our sample size, while typical of published neuroimaging studies, was small and so precluded the detection of smaller but still clinically significant effects.

In summary, this evidence highlights the need for future work to explore a new model of body image disturbance that further examines the role of sensory experience. This model may lead to novel conceptualizations of AN and avenues for intervention such as those that help those with AN to experience and contextualize volatile somatic signals to better guide adaptive behavior. Such an approach might offer an alternative or supplement to cognitively-focused interventions and enhance treatment effectiveness (69).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AN	anorexia nervosa
MRI	magnetic resonance imaging
BMI	body mass index

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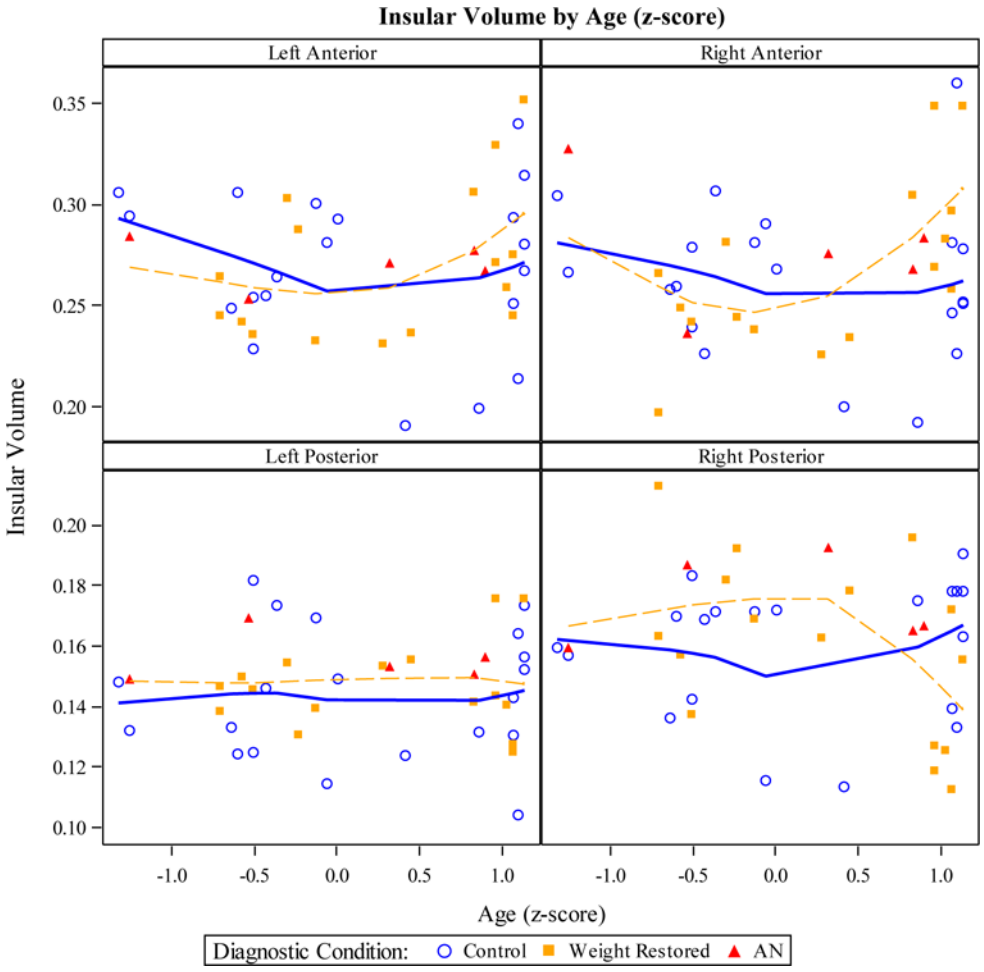

Figure 1. Age by Posterior and Anterior Insular Volume in Clinical Participants Relative to Controls

This figure depicts volume size as a function of age and group membership, controlling for body mass index. Reported volumes are the proportion of two volumes ml (insula)/ml (ICV). Age was standardized and thus the x-axis reflects z-scores that reflect relative age within the sample. The dotted yellow line depicts adolescents with a current or past diagnosis of anorexia nervosa (n=21), the blue line depicts typically developing adolescent control participants (n=20). Blue circle = healthy controls; red triangle = current diagnosis of anorexia nervosa; yellow square = history of anorexia nervosa, but weight restored. Tests of main and interaction effects in bilateral anterior regions and the left posterior was nonsignificant. The differential association between age and volume by diagnostic group in the right posterior insula region was statistically significant ($\beta = -0.018 \pm 0.008$; $t = 2.32$, $p = 0.02$).

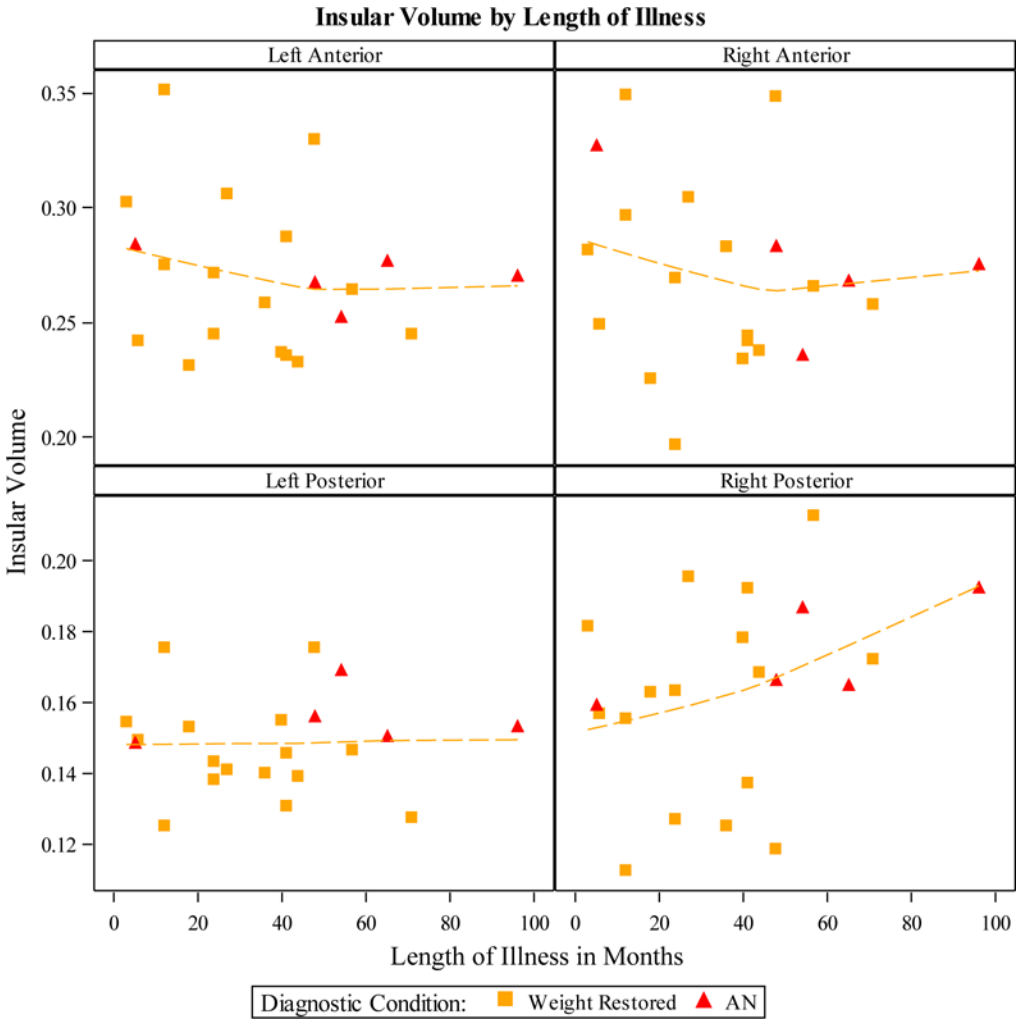

Figure 2. The Relationship Between Length of Illness and Insular Volume in Adolescents with Anorexia Nervosa

This figure depicts the relationship between length of illness in months and volume in anterior and posterior insular components in 21 adolescents with a current or prior diagnosis of anorexia nervosa. Reported volumes are the proportion of two volumes ml (insula)/ml (ICV). The magnitude of association was of large effect but in opposite direction: being negatively associated with right anterior insular volume ($r = -.5$, $p = .03$) and positively associated with right posterior volume ($r = .5$, $p = .04$). Red triangle = current diagnosis of anorexia nervosa; yellow square = history of anorexia nervosa, but weight restored.

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Table 1

Demographic and Illness Characteristics of the Sample

	Participants with A History of Anorexia Nervosa			Typically Developing Controls				
Demographic Variables	***n***	***Mean***	***SD***	***n***	***Mean***	***SD***	***|Z|***	***Pr > |Z|***
Age z-score	21	0.23	0.76	20	0.16	0.86	0.26	0.80
Age in Years	21	17.48	1.72	20	17.40	1.85	0.50	0.99
	n	***%***		***n***	***%***			
Asian	2	8.70		1	4.55			
Black	1	4.35		4	18.18			
Other	1	4.35		2	9.09			
White	19	82.61		15	68.18			

Stage/Severity of Illness	***N***	***Mean***	***SD***	***N***	***Mean***	***SD***	***|Z|***	***Pr > |Z|***
BMI z-score	21	−0.35	0.93	20	0.02	0.84	1.21	0.23
BMI (kg/m^2)	21	20.01	2.61	20	21.34	3.00	1.47	0.15
Body Dissatisfaction[a]	21	15.15	7.31	17	5.47	5.39	−3.55	0.001
Drive for Thinness[a]	21	15.40	7.85	17	4.82	6.07	−3.54	0.001
Bulimia	20	3.70	5.32	17	1.59	1.70	−0.81	0.42
Age of onset	21	13.78	1.76	n/a	n/a	n/a	n/a	n/a
Duration of Illness[b]	20	3.14	2.03	n/a	n/a	n/a	n/a	n/a
Length of Weight Restoration[b]	21	3.33	2.59	n/a	n/a	n/a	n/a	n/a

Length of Weight Restoration by Category[c] (n=21)	**n**	**%**
Unhealthy weight	5	24
Less than 3 months	1	4.8
3–6 months	6	28.6
7–9 months	1	4.8
10–12 months	0	0
Over a year	2	9.5

Length of Weight Restoration by Category[c] *(n=21)*	n	%
1–2 years	1	4.8
More than 2 years	5	23.8

Drive for Thinness Classification (weight-restored group, n = 16)	*n*	%
Clinical Range	10	63
Normative Range	5	31
Missing	1	6

	n	*Mean*	*Std*
Lowest BMI while Ill	21	15.19	2.53

Group Membership	*n*	*% of group*
Current AN	5	23.81
Weight-Restored AN	16	76.12
History of purging	3	21.74

Model Covariates	*Control (n)*	*% of group*	*AN (n)*	*% of group*
Left-handed	1	5	4	24
Psychotropic Medication	0	0	14	67

Notes:

[a]: Subscale Scores from the Eating Disorder Inventory, 3rd Edition;

[b]: Number and proportion of years;

[c]: At time of intake interview.

Table 2

Association of Insular Volume with Illness Parameters

Length of Illness[a]					
Insular region	**Pearson r**	**Fisher's z**	**−95CI**	**+95CI**	**pH0: Rho=0**
Left Anterior	−0.38	−0.40	−0.72	0.11	0.13
Right Anterior	−0.50	−0.55	−0.78	−0.05	0.03
Left Posterior	−0.05	−0.05	−0.51	0.42	0.83
Right Posterior	0.48	0.52	0.02	0.77	0.04

Body Dissatisfaction[b]					
Insular region	**Pearson r**	**Fisher's z**	**−95CI**	**+95CI**	**pH0: Rho=0**
Left Anterior	−0.38	−0.40	−0.72	0.11	0.12
Right Anterior	−0.49	−0.53	−0.78	−0.02	0.04
Left Posterior	−0.38	−0.40	−0.72	0.11	0.12
Right Posterior	0.40	0.43	−0.08	0.73	0.10

Notes:

[a] Length measures in years and proportion of years.

[b] Body dissatisfaction subscale of the Eating Disorder Order Inventory.