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ORIGINAL ARTICLE

Alterations in brain structure in adults with anorexia nervosa and the impact of illness duration

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Background. Brain structure alterations have been reported in anorexia nervosa, but findings have been inconsistent. This may be due to inadequate sample size, sample heterogeneity or differences in methodology.

Method. High resolution magnetic resonance images were acquired of 33 adult participants with anorexia nervosa and 33 healthy participants, the largest study sample to date, in order to assess whole-brain volume, ventricular cerebrospinal fluid, white matter and grey matter volume. Voxel-based morphometry was conducted to assess regional grey matter volume. Levels of depression, anxiety, obsessionality and eating disorder-related symptoms were measured and used to explore correlations with brain structure.

Results. Participants with anorexia nervosa had smaller brain volumes as well as a global decrease in grey matter volume with ventricular enlargement. Voxel-based morphometry revealed a decrease in grey matter volume spanning across the cerebellum, temporal, frontal and occipital lobes. A correlation was found between grey matter volume loss and duration of illness in the cerebellum and mesencephalon. No correlations were found with clinical measures.

Conclusions. Findings are in accordance with several previous studies on brain structure and match functional studies that have assessed the symptomatology of anorexia nervosa, such as body image distortion and cognitive bias to food. The correlation with duration of illness supports the implication of cerebellar atrophy in the maintenance of low weight and disrupted eating behaviour and illustrates its role in the chronic phase of anorexia nervosa. The lack of other correlations suggests that these findings are not related to the presence of co-morbid disorders.

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Key words: Anorexia nervosa, brain, duration of illness, grey matter, voxel-based morphometry.

Introduction

Anorexia nervosa (AN) is an eating disorder characterized by a refusal to maintain normal body weight, an intense fear of gaining weight despite being underweight, a disturbance in the perception of one's own body weight or shape and, in postmenarcheal women, an absence of at least three consecutive menstrual cycles. AN is further divided into a binge-eating/purging (AN-BP) subtype and a restricting (AN-R) subtype. The former is defined by regular binge-eating or purging behaviour, which may include vomiting, use of diuretics, laxative and enemas, whereas the latter is defined by a lack of this behaviour (APA, 1994).

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Brain alterations have previously been associated with AN as a consequence of starvation, with underweight patients showing a reduction of grey matter volume (GMV) and an increase in cerebrospinal fluid (CSF) volume (Kerem & Katzman, 2003; Van Den Eynde *et al.* 2012). Additionally, some studies have reported a decrease in white matter volume (WMV) (Swayze *et al.* 2003; Boghi *et al.* 2011; Roberto *et al.* 2011) and a reduction of WM integrity (Kazlouski *et al.* 2011), although the literature regarding WM alterations is comparatively lacking. Some studies have suggested complete reversibility of these brain alterations with weight recovery (Swayze *et al.* 2003; Wagner *et al.* 2006; Mainz *et al.* 2012), while others have concluded that weight-recovered patients display an intermediate profile somewhere between healthy controls (HC) and currently ill patients (Katzman *et al.* 1997; Lambe *et al.* 1997; Neumärker *et al.* 2000; Joos *et al.* 2011; Roberto *et al.* 2011; Friederich *et al.* 2012).

Structural imaging studies employing a region of interest (ROI) approach have reported decreased

GMV in subcortical regions in AN. An early study by Husain *et al.* (1992) found both thalamus and midbrain volumes to be significantly smaller. Connan *et al.* (2006) reported a decrease in hippocampal volume in underweight patients with AN. Similarly, Giordano *et al.* (2001) found a significant volume reduction in the hippocampus–amygdala formation. Finally, Neumärker *et al.* (2000) reported a volume decrease in the mesencephalon and pons in patients that persisted after weight recovery.

Whilst early magnetic resonance studies relied on visual assessment and manual or semi-automated measurements of ROI, the development of automated techniques has allowed for the analysis of large samples without requiring the time-consuming measurements associated with manual quantification (Whitwell, 2009). Voxel-based morphometry (VBM) (Wright *et al.* 1995; Ashburner & Friston, 2000) is an automated technique based on voxel-wise statistical analysis of pre-processed structural magnetic resonance images (Ridgway *et al.* 2008). This technique overcomes the limitations of ROI approaches in that it does not require for *a priori* brain areas to be selected for analysis.

This approach has led to a steady increase in studies assessing brain abnormalities in AN; however, findings from these studies have been inconsistent (Van Den Eynde *et al.* 2012; Supplementary Table 1). Some studies report global GMV loss (Joos *et al.* 2010; Suchan *et al.* 2010; Gaudio *et al.* 2011; Friederich *et al.* 2012; Mainz *et al.* 2012), whereas other studies have failed to replicate this finding (Boghi *et al.* 2011; Brooks *et al.* 2011*a*). Additionally, those studies looking at focal GMV loss in AN implicate a wide range of brain regions with only a few being replicated. Castro-Fornieles *et al.* (2009) reported a decrease in GMV in the precuneus, inferior parietal lobe and superior temporal gyrus. Alongside a global decrease in GMV of 1%, Mühlau *et al.* (2007) also reported a 5% decrease in the anterior cingulate cortex (ACC). Similarly, Joos *et al.* (2010) reported GMV decreases in the ACC as well as decreases in the cerebellum, frontal operculum, precuneus, temporoparietal cortex and parietal cortex in AN-R patients. While Friederich *et al.* (2012) only found a trend for a global decrease in GMV, they did report significant regional volume decreases in the insula, hippocampus, ACC and in the amygdala. Suchan *et al.* (2010) reported significant volume loss in the extrastriate body area (EBA) and superior temporal gyrus. A recent meta-analysis of seven VBM studies in AN revealed a decrease in regional GMV in the hypothalamus, caudate nucleus, lentiform nucleus and the inferior parietal lobe (Titova *et al.* 2013). The only study reporting an increase in regional GMV was performed by Brooks *et al.* (2011*a*) who showed less age-related GM atrophy in the dorsolateral prefrontal cortex, alongside a decrease of GMV in the cerebellum, parahippocampal gyrus, fusiform gyrus, insular cortex and posterior cingulate gyrus.

Several studies have explored associations between GMV loss and clinical characteristics of AN, but again with mixed results. Both current and lowest lifetime body mass index (BMI) have been shown to correlate with global (Katzman *et al.* 1996) and regional (Mühlau *et al.* 2007; Boghi *et al.* 2011) GMV loss. A study by Brooks *et al.* (2011*a*) found that BMI and dietary restraint were correlated with preservation of dorsolateral prefrontal cortex volume with ageing compared with HC. Joos *et al.* (2010) found no correlation with BMI, but did report a positive correlation between GMV in the right inferior parietal lobe and a 'drive for thinness', as measured by the Eating Disorder Inventory (Garner *et al.* 1983). With regards to behavioural correlations, Neumärker *et al.* (2000) reported a decrease in volume in the mesencephalon that was significantly correlated with lower performance on vocabulary tests. Castro-Fornieles *et al.* (2009) reported a negative correlation between copy time on the Rey Complex Figure Test and global GMV in adolescents with AN.

The inconsistent findings in the literature could be attributed to differences in methodology and the recruitment of relatively small sample sizes and to the heterogeneity within AN (Van Den Eynde *et al.* 2012). As co-morbidity is the rule rather than the exception, brain abnormalities found in people with AN may, at least in part, reflect the influence of co-morbid disorders, such as anxiety or depression (Lorenzetti *et al.* 2009; Bellani *et al.* 2010, 2011; Martin *et al.* 2010; Radua *et al.* 2010; Bora *et al.* 2012). To date, there has not been a study that has assessed the role of co-morbidities on brain structure in AN. Onset of AN often occurs during adolescence and some studies have reported the presence of these alterations when the brain is not yet fully matured (Katzman *et al.* 1996; Castro-Fornieles *et al.* 2009; Mainz *et al.* 2012). During normal development, it is assumed that there are region-specific, age-dependent, non-linear decreases in GMV associated with increases in WMV (Zielinski *et al.* 2010; Raznahan *et al.* 2011; Franke *et al.* 2012). These changes may reflect regional alterations in synaptic density or may be due to intracortical myelination (Giorgio *et al.* 2010; Silk & Wood, 2011). However, irregular food intake leads to changes in physiology during which the body adapts to the starvation. The relatively small amount of energy that is available is conserved by, amongst other mechanisms, delaying growth and lowering metabolic rate (Hasan & Hasan, 2011). While the brain is comparatively spared, or at least catabolized more slowly than other tissues (McCue, as cited in

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Hasan & Hasan, 2011), very little is known about the effect of starvation on the brain during adolescence.

The first aim of the study was to correlate clinical measures with brain tissue volume (whole-brain volume, GMV and WMV) in AN. The second aim, as very little is known about the impact of early onset of AN or about the effects of chronic duration on brain structure, was to explore the impact of the age of onset of illness and the duration of illness on these volumetric measures.

Method

Participants

A total of 35 individuals with a current diagnosis of AN by a certified clinician, fulfilling Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) criteria, were recruited from the hospital and community services of the South London and Maudsley NHS Trust and from an online advertisement on the website of beat™ (beating eating disorders; http://www.b-eat.co.uk), the UK's largest eating disorder charity. However, three participants were excluded due to a lack of completion of all measures and one was excluded due to a BMI falling within the normal range. Out of the remaining 31, 12 (39%) reported taking antidepressant medication; 22 (71%) were diagnosed as AN-R subtype, six (19%) as AN-BP subtype and three (10%) were unspecified. A total of 37 healthy individuals with no personal or family history of eating disorders were recruited from the community and staff of the Institute of Psychiatry, King's College London. However, three healthy participants were excluded from further analysis due to currently taking antidepressant medication and three participants were excluded after optimal matching of the two groups in terms of age, group size and estimated intelligence quotient (IQ). Both groups consisted predominantly of right-handed participants (AN 25, HC 27). Age, medication and BMI scores were obtained as well as both the age of onset and the duration of illness for participants with AN. The screening module of the research version of the Structured Clinical Interview for DSM Disorders (SCID-I/P; First *et al.* 1997) was used as a screening tool for the healthy controls. The National Adult Reading Test (Nelson & Willison, 1991) was used to estimate IQ. Participants' consent was obtained according to the Declaration of Helsinki and was approved by the local research ethics committee (no. 11-LO-0952).

Clinical measures

All participants completed the questionnaires before attending the scanning session to measure their anxiety, depression, eating behaviour and obsessionality. The Hospital Anxiety and Depression Scale (HADS; Zigmond & Snaith, 1983) is a measure consisting of 14 items to assess overall severity of depression and anxiety (clinical threshold total=10). The Eating Disorders Examination Questionnaire (EDE-Q; Fairburn & Beglin, 1994) is a self-report questionnaire consisting of 36 items that look at a participant's eating behaviour over the past 28 days. The Obsessive–Compulsive Inventory – Revised (OCI-R; Foa *et al.* 2002) is an 18-item list of first-person statements describing experiences that the subject has to rate according to the level of distress they felt when they experienced those statements over the past month (clinical threshold=25).

Image acquisition

Magnetic resonance imaging (MRI) was performed using a 1.5 T GE Signa HDx TwinSpeed MRI scanner (GE-Medical Systems, USA) at the Centre for Neuroimaging Sciences, Institute of Psychiatry, King's College London. The body coil was used for radio frequency (RF) transmission, with an eight-channel head coil for RF reception. Whole-brain scans were obtained as high-resolution T1-weighted MPRAGE (magnetization prepared rapid acquisition gradient echo) acquired in the sagittal plane (repetition time=8.592 ms, echo time=3.8 ms, inversion time=1000 ms, flip angle=8°, field of view=240 mm, matrix=192×192, voxel size=1.2×1.2×1.2, 180 slices). Full brain and skull coverage was required for the MRI datasets and detailed quality control carried out on all MR images, according to previously published quality-control criteria (Simmons *et al.* 2009, 2011).

Structural image evaluation, using normalization, of atrophy (SIENAX)

Brain tissue volume, normalized for subject head size, was estimated with SIENAX (Smith, 2002), part of FSL (Smith *et al.* 2004). SIENAX starts by extracting brain and skull images from the single whole-head input data (Smith, 2002). The brain image is then affine-registered to Montreal Neurological Institute (MNI) 152 standard space (Jenkinson & Smith, 2001; Jenkinson *et al.* 2002), using the skull image to determine the registration scaling. This is primarily in order to obtain the volumetric scaling factor, to be used as a normalization for head size. Next, tissue-type segmentation with partial volume estimation was carried out (Zhang *et al.* 2001) in order to calculate total volume of brain tissue (including separate estimates of GMV, WMV and ventricular CSF volume).

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Table 1. *Demographic characteristics*

	AN (n=31)	Range	HC (n=31)	Range	Test statistic	p
Age, years[a]	23 (10.0)	18–46	25 (4.0)	22–45	U=397.5, z=−1.173	0.241
Estimated IQ[a]	110.0 (10.0)		116 (10.0)		U=319.5, z=−2.274	0.023
Years of education[b,c]	15.7 (2.5)	11–20	18.1 (2.1)	13–23	t_{59}=14.662	<0.001
BMI, kg/m^{2}[b]	15.8 (1.4)	12–18.7	21.8 (1.8)	17.9–25.5	t_{60}=4.138	<0.001
Age of onset of AN, years[a,d]	16 (4.75)	9–31				
Duration of illness, years[a,d]	7 (10)	1–35				
Medication, %	39					

AN, Anorexia nervosa; HC, healthy controls; IQ, intelligence quotient; BMI, body mass index.

[a] Scores are not normally distributed and values are given as median (interquartile range).

[b] Scores are normally distributed and values are given as mean (standard deviation).

[c] One participant did not report years of education and scores are calculated based on 61 participants.

[d] Age of onset and duration of illness were not reported by all participants and the score is calculated based on 28 participants instead of 31.

Statistical analysis

An assessment of normality was performed for all measures using the Shapiro–Wilks test in SPSS Statistics for Windows 20 (IBM Corp., USA). When the assumption of normality was violated, the non-parametric Mann–Whitney U test was used to assess differences. When scores were normally distributed, Student's t test was used. The non-parametric Spearman's rho (ρ) was used to assess correlations between volumetric measurements and clinical measures.

VBM

To assess voxel-wise changes in GMV, structural data were analysed with FSL-VBM (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLVBM) (Douaud *et al.* 2007), an optimized VBM protocol (Good *et al.* 2001) carried out with FSL tools (Smith *et al.* 2004). This approach is unbiased, in that it requires no *a priori* information about the location of possible differences in the grey matter, and is not operator-dependent. First, structural images were brain-extracted and grey matter-segmented before being registered to the MNI 152 standard space using non-linear registration (Andersson *et al.* 2007). The resulting images were averaged and flipped along the x-axis to create a left–right symmetric, study-specific grey matter template. Second, all native grey matter images were non-linearly registered to this study-specific template and modulated to correct for local expansion (or contraction) due to the non-linear component of the spatial transformation. The modulated grey matter images were then smoothed with an isotropic Gaussian kernel with a sigma of 3 mm (7 mm FWHM). Finally, a voxel-wise general linear model (GLM) was applied using permutation-based non-parametric testing, correcting for multiple comparisons across space, to search for regional differences in GMV between patients and controls. Threshold-free cluster enhancement (Smith & Nichols, 2009) was used to detect significant clusters of activation and family-wise error correction was used to correct for multiple comparisons across space.

Two separate analyses were performed to assess voxel-wise changes in GMV. In the first analysis only age, IQ and years of education were entered as confounding covariates. Because global changes in GMV have been reported in AN, the second analysis added global GMV as an additional confounding covariate to look for regional changes in GMV that cannot be explained by a global change in GMV.

Additional correlational analyses were then performed by adding a separate regressor per group to assess associations previously reported between GMV and BMI (Katzman *et al.* 1996; Mühlau *et al.* 2007; Boghi *et al.* 2011; Brooks *et al.* 2011*a*) and eating behaviour (Joos *et al.* 2010), as well as to explore possible associations with depression, anxiety and obsessive–compulsive symptoms. In the AN group correlations with duration of illness (Boghi *et al.* 2011) and age of onset were also explored. To assess any effects of medication on GMV, patients reporting antidepressant medication were compared with a matched group of patients without medication.

Results

Group characteristics

Demographic information is summarized in Table 1. Patients and controls were similar in terms of age, but there was a significant difference between groups in terms of IQ, years of education and BMI.

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Table 2. *Clinical characteristics*

	AN (n=30)	HC (n=31)	Test statistic	p
EDE-Q global[a]	4.0 (1.5)	0.6 (0.6)	U=928, z=6.680	<0.001
HADS depression[a]	11 (7)	0 (2)	U=921, z=6.673	<0.001
HADS anxiety[b]	15.3 (3.4)	4.6 (3.1)	t_{59}=−12.713	<0.001
OCI-R total[b]	25.6 (13.0)	4.5 (3.3)	$t_{32.6}$=−8.649	<0.001

AN, Anorexia nervosa; HC, healthy controls; EDE-Q, Eating Disorders Examination Questionnaire; HADS, Hospital Anxiety and Depression Scale; OCI-R, Obsessive–Compulsive Inventory-Revised.

[a] Scores are not normally distributed and values are given as median (interquartile range).

[b] Scores are normally distributed and values are given as mean (standard deviation).

Clinical measures

The screening module of the SCID-I/P revealed that there was no indication of psychiatric disorders in the HC subjects. The AN group scored significantly higher on all self-report measures compared with HC, as illustrated in Table 2. Scores on the HADS in the AN group indicate clinical levels of anxiety and depression (Bjelland *et al.* 2002). OCI-R scores illustrate a high proportion of obsessionality in AN and a likely presence of obsessive–compulsive disorder symptomatology (Foa *et al.* 2002).

Global differences in brain volume

Using SIENAX, whole-brain volume, global GMV and WMV and ventricular CSF volume were estimated and the results for both groups are summarized in Table 3. Whole-brain volume and global GMV were found to be significantly lower in AN alongside enlargement of ventricular CSF. In AN, global GMV correlated negatively with age (ρ=−0.62, p<0.001), but no other significant correlations were found. In HC, global GMV correlated with age (ρ=−0.47, p=0.008) and with the EDE-Q (ρ=0.48, p=0.006), but these correlations did not survive Bonferroni correction. No other correlations with clinical measures were found.

Focal alterations in GMV

VBM analysis revealed regional atrophy in AN compared with HC, whilst controlling for estimated IQ, age and years of education (see Fig. 1). Results are summarized in Table 4. There was no indication of regions showing greater GMV in AN compared with HC. No significant difference in GMV was found between AN taking antidepressant medication and AN not taking medication. When further controlling for differences in global GMV, no local GMV reductions were found in AN compared with HC.

Correlational analyses revealed a significant negative correlation between duration of illness and GMV (see Fig. 2 and Table 5). There was no significant correlation of GMV with age of onset. No significant correlations were found with clinical measures.

Discussion

Our findings support studies that have reported a global GMV loss (Katzman *et al.* 1996, 1997; Lambe *et al.* 1997; Joos *et al.* 2010; Suchan *et al.* 2010; Gaudio *et al.* 2011; Roberto *et al.* 2011; Friederich *et al.* 2012; Mainz *et al.* 2012; Titova *et al.* 2013), but there was no indication of differences in global WMV (Swayze *et al.* 2003; Roberto *et al.* 2011). However, the methods used in this study do not allow for a proper measurement of WM integrity or connectivity and therefore it is not possible to conclude that there are no alterations in WM in AN. Unlike previous studies, no correlations with BMI or eating disorder-related symptoms were found in AN (Katzman *et al.* 1996; Joos *et al.* 2010); however, this study does find that current age correlates negatively with global GMV. This is not uncommon, as studies have shown that in normal ageing there is a region-specific and non-linear pattern of age-related changes in GMV (Resnick *et al.* 2003; Terribilli *et al.* 2011).

Using VBM, GM atrophy was found in regions previously reported to be affected in AN using a whole-brain approach. Among these regions, the precuneus has been suggested to play a central role in a wide range of processes, including visuospatial processing, attention, episodic memory, self-consciousness and self-related mental representations (Cavanna & Trimble, 2006). Previous studies have also found GM atrophy in temporoparietal and occipital regions

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Table 3. *Whole-brain, grey matter, white matter and ventricular CSF volumes (mm^3)*

	AN (n=31)	HC (n=31)	Test statistic	p
Brain volume[b]	1517347.9 (66888.9)	1553337.1 (52290.1)	t_{60}=2.360	0.02
Grey matter volume[b]	804637.8 (47037.5)	833791.7 (38115.3)	t_{60}=2.681	0.01
White matter volume[b]	712710.2 (32691.4)	719545.3 (29275.7)	t_{60}=0.867	0.39
Ventricular CSF[a]	37956.5 (7764.1)	33639.6 (10572.3)	U=622, z=1.992	0.05

CSF, Cerebrospinal fluid; AN, anorexia nervosa; HC, healthy controls.
[a] Scores are not normally distributed and values are given as median (interquartile range).
[b] Scores are normally distributed and values are given as mean (standard deviation).

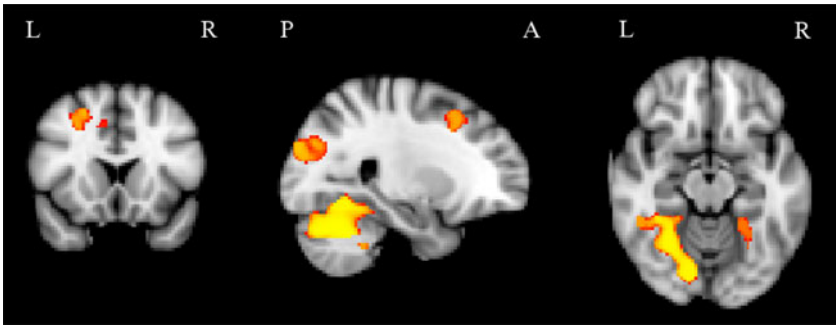

Fig. 1. Grey matter volume loss in anorexia nervosa (n=31) compared with healthy controls (n=31) using FSL-VBM (threshold-free cluster enhancement, p<0.05, family-wise error corrected), whilst controlling for age, intelligence quotient and years of education. L, Left; R, right; P, posterior; A, anterior.

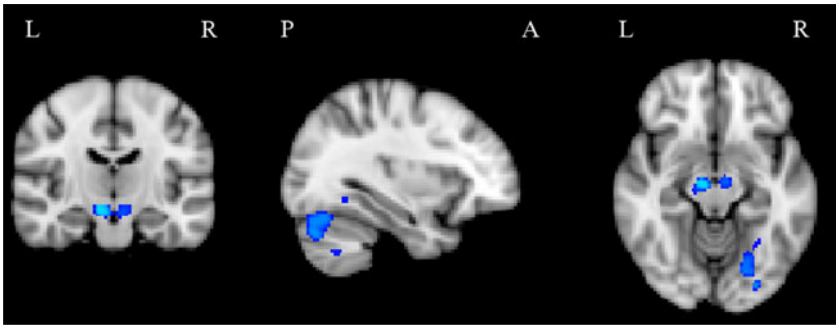

Fig. 2. Regions showing a negative correlation with duration of illness in anorexia nervosa (n=28) using FSL-VBM (threshold-free cluster enhancement, p<0.05, family-wise error corrected), whilst controlling for age, intelligence quotient, years of education and global grey matter volume. L, Left; R, right; P, posterior; A, anterior.

in combination with the precuneus (Castro-Fornieles *et al.* 2009; Joos *et al.* 2010; Favaro *et al.* 2012). Body image distortions have been postulated to reflect dysfunctional processing of information (Williamson *et al.* 2004) and both lesion and functional studies have reported alterations in regions associated with visuospatial processing (Uher & Treasure, 2005; Gaudio *et al.* 2011). More specifically, recent investigations have linked body image distortions in AN to the EBA and the posterior part of the fusiform gyrus, as both GMV of the EBA and functional connectivity from the fusiform gyrus to the EBA have been found to negatively correlated with body image distortion (Suchan *et al.* 2010, 2013). Furthermore, Vocks *et al.* (2011) reported a significant increase in neural activation in the EBA as well as frontal regions in patients with eating disorders following cognitive–behavioural body image therapy; taken together, this suggests that body image distortions are related to both functional and structural changes within these areas. The superior frontal gyrus has been suggested to play a role in introspection (Goldberg

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Table 4. *Voxel-based morphometry results showing grey matter volume loss in anorexia nervosa patients (n=31) compared with healthy controls (n=31) whilst controlling for intelligence quotient, age and years of education*

Region	Cluster size	$p_{corrected}$	*t*	MNI coordinates		
				x	y	z
Cerebellum	3229	0.001	4.613	26	−56	−34
Temporal occipital fusiform cortex				28	−50	−16
Cerebellum	1573	0.001	5.028	−28	−56	−36
Lateral occipital cortex	1144	0.01	4.258	−20	−66	34
Precuneus				−2	−54	54
Lateral occipital cortex	733	0.019	4.200	30	−90	28
Cuneus				8	−84	26
Lateral occipital cortex	238	0.022	4.109	−24	−92	20
Superior frontal gyrus	225	0.013	4.917	20	12	46
Precuneus	183	0.034	3.371	12	−68	24

MNI, Montreal Neurological Institute.

Table 5. *Regions showing a negative correlation between grey matter volume and duration of illness in anorexia nervosa patients (n=28) whilst controlling for age, intelligence quotient, years of education and global grey matter volume*

Region	Cluster size	$p_{corrected}$	*t*	MNI coordinates		
				x	y	z
Occipital fusiform gyrus	1005	0.019	4.44	−26	−82	−16
Cerebellum				−34	−74	−30
Mesencephalon	93	0.007	6.882	10	−14	−12
Mesencephalon	59	0.031	5.054	−6	−14	−14
Cerebellum	38	0.047	3.827	14	−78	−18

MNI, Montreal Neurological Institute.

et al. 2006) and reduced activation was previously reported in AN during processing of self-images (Vocks *et al.* 2010). Zhu *et al.* (2012) found reduced activation in the precuneus and parietal regions during presentation of food stimuli as well as an increase in activation in the medial frontal cortex, anterior cingulate, caudate nucleus and lentiform nucleus. It has been suggested that those with AN utilize cognitive strategies that restrict appetitive responses in the brain to reduce the strength of the saliency of food stimuli (Brooks *et al.* 2011*b*). This notion is further supported by previous studies that reported atrophy within the fusiform gyrus and posterior cingulate gyrus (Brooks *et al.* 2011*a*; Favaro *et al.* 2012), regions associated with recognition of familiar objects and spatial awareness. In addition, Brooks *et al.* (2011*a*) suggested that the cognitive bias for food stimuli in AN could, in part, be related to an imbalance in accurate object recognition due to hypoactive fusiform-driven spatial recognition systems.

As the current study demonstrated that the AN group has a smaller brain volume and less global GMV, it is possible that these effects are not region-specific but instead part of a global decrease in GMV. Indeed, when global GMV is controlled for there are no region-specific alterations on top of this global decrease. It is important to note that different adjustments for global effects in VBM can lead to different results and it is possible that employing a different approach would lead to significant findings (Peelle *et al.* 2012). Previous VBM studies in AN employing different adjustments for global effects have reported local effects in the regions we report in this study (Brooks *et al.* 2011*a*; Gaudio *et al.* 2011). Finally, a recent meta-analysis of seven studies did not find regional differences in these areas at all (Titova *et al.* 2013).

A correlation was found between cerebellar atrophy and duration of illness that could not be explained by global changes in GMV and which replicates previous findings of greater GMV loss in more chronic cases

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(Boghi *et al.* 2011). The cerebellum has been thought to play a modulatory role in higher-order neural functions and its dysfunction may be related to behaviours evident in people with AN, such as anxiety, ritualistic and stereotypical behaviours as well as dysphoria, depression, and ruminative and obsessive behaviours (Schmahmann *et al.* 2007). Animal studies have also shown disruptions in feeding behaviour and weight loss caused by cerebellar lesions (Zhu & Wang, 2008), which suggests that reductions in cerebellar volume may contribute to successfully maintaining low weight and therefore the duration of the illness.

Chronic cases also demonstrated greater GMV loss bilaterally in the mesencephalon, at the substantia nigra and ventral tegmental area. Animal studies in AN have suggested that areas such as the substantia nigra and ventral tegmental area play a prominent role in motivation and anticipation of food reward, food intake and over-exercising through projections to different cortico-limbic structures (Narayanan *et al.* 2010; Adan *et al.* 2011; Verhagen *et al.* 2011; van Zessen *et al.* 2012). Furthermore, the brainstem plays a role in autonomic and visceral control and has been implicated in abnormalities in taste and pain perception in individuals with AN (Kaye *et al.* 2009). This may suggest that the core symptoms of AN become subjectively easier to maintain in chronic cases due to subcortical changes in brain structure. Additionally, we have shown that this effect cannot be explained due to the global decrease in GMV in AN.

No correlations were found with clinical measures in AN, suggesting that the observed differences in GMV are not associated with depression, anxiety or obsessive–compulsive symptoms. Additionally, unlike previous studies there was no correlation between GMV and BMI (Katzman *et al.* 1996; Joos *et al.* 2010; Boghi *et al.* 2011; Brooks *et al.* 2011*a*). This is surprising given that those studies recruited patient cohorts with a similar average BMI to the current cohort. Samples in previous studies did all differ in terms of AN subtype ratios, presence of medication and age. One other possibility could be the low variance of BMI within the AN group in the current study. No correlations with eating disorder symptomatology were found, though the current study did not use 'drive for thinness' separately as a covariate (Joos *et al.* 2010).

Automated techniques such as VBM and SIENAX allow for relatively fast assessments of brain structure in large groups. The results presented here are consistent with the majority of the literature and indicate GM atrophy in people with AN. It is unlikely that these alterations are the cause of AN, as previous studies have shown either full recovery (Swayze *et al.* 2003; Wagner *et al.* 2006; Mainz *et al.* 2012) or an intermediate profile between controls and currently ill (Katzman *et al.* 1997; Lambe *et al.* 1997; Neumärker *et al.* 2000; Joos *et al.* 2011; Roberto *et al.* 2011; Friederich *et al.* 2012). Instead, these changes are more likely to be the result of starvation and in turn facilitate the persistence of psychopathological features found in AN. This is further supported by the finding of greater atrophy in more chronic cases. Age of onset of AN does not seem to play a role in the observed differences in brain structure, suggesting that brain maturation is not as severely hindered by starvation as the rest of the body. Despite the heterogeneity within AN and the multitude of co-morbid disorders, there was no indication of a relationship between co-morbid disorders and alterations in brain structure in this study. This would suggest that the changes are due to starvation and independent of co-morbid disorders that have been implicated in alterations in brain structure.

To date, this is the largest study that has assessed GMV alterations in AN using brain tissue volume estimation and VBM. The statistical methods used in this study avoid *a priori* assumptions by using permutation-based non-parametric testing and provide a more stringent and correct approach to neuroimaging analysis. Further studies should aim to validate the findings presented here and longitudinally assess whether full recovery occurs with weight restoration. The majority of the current sample was comprised of AN-R subtype patients; therefore it is uncertain whether there is a significant difference in GM alterations between subtypes of AN. It is important to note that there are difficulties in establishing the exact age of onset or duration of AN and more studies are required to assess these effects from early on in adolescence or those at risk of developing AN. Further longitudinal investigations of recovered AN are required to ensure that these effects are in fact state-related phenomena and not present prior to the onset of the illness. Finally, there is a lack of studies that have assessed changes in WM in AN and as of yet it is unknown if there is any change related to GM alterations.

Supplementary material

For supplementary material accompanying this paper visit http://dx.doi.org/10.1017/S0033291713002389.

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Declaration of Interest

None.

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