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Abstract

Neurobiological findings in anorexia nervosa (AN) are inconsistent, including differences in regional grey matter volumes. Methodological limitations often contribute to the inconsistencies reported. The aim of this study was to improve on these methodologies by utilising voxel based morphometry (VBM) analysis with the use of diffeomorphic anatomic registration through an exponentiated lie algebra algorithm (DARTEL), in a relatively large group of individuals with AN. Twenty-six individuals with AN and 27 healthy controls underwent a T1-weighted magnetic resonance imaging (MRI) scan. AN participants were found to have reduced grey matter volumes in a number of areas including regions of the basal ganglia (including the ventral striatum), and parietal and temporal cortices. Body mass index (BMI) and global scores on the Eating Disorder Examination Questionnaire (EDE-Q) were also found to correlate grey matter volumes in a region of the brainstem (including the substantia nigra and ventral tegmental area) in AN, and predicted 56% of the variance in grey matter volumes in this area. The brain regions associated with grey matter reductions in AN are consistent with regions responsible for cognitive deficits associated with the illness including anhedonia, deficits in affect perception and saccadic eye movement abnormalities. Overall, the findings suggest reduced grey matter volumes in AN that are associated with eating disorder symptomatology.

Introduction

Anorexia nervosa (AN) is a complex psychiatric illness characterised by significantly low body weight, an intense fear of weight gain, and a disturbance in the experience of one's own body weight or shape (American Psychiatric Association, 2013). In addition to these diagnostic criteria, a large body of evidence suggests that AN patients exhibit anhedonia and alexithymia (Deborde *et al.*, 2005; Tchanturia *et al.*, 2012; Phillipou *et al.*, 2015a), and perform poorly on neurocognitive tasks that probe reward and cognitive flexibility (Tchanturia *et al.*, 2004a; Tchanturia *et al.*, 2004b; Wagner *et al.*, 2007). These neurocognitive functions are underpinned by distributed limbic and associative cortico-basal ganglia brain networks (Kehagia *et al.*, 2010; Reker *et al.*, 2010). Correspondingly, functional magnetic resonance imaging (fMRI) studies have reported altered functioning of the striatum in reward processing (Wagner *et al.*, 2007; Fladung *et al.*, 2010; Murao *et al.*, 2017), and disturbed functioning of the ventrolateral prefrontal cortex and parahippocampal gyrus, and fronto-parietal regions during tasks of cognitive flexibility in people with AN (Sato *et al.*, 2013; Lao-Kaim *et al.*, 2015).

Structural MRI studies of grey matter broadly corroborate the findings of cortico-basal ganglia deficits in AN. Research utilising voxel based morphometry (VBM) in the acute phase (i.e. the ill state) of AN have reported decreased grey matter volumes in a number of regions including the anterior cingulate cortex and regions of the parietal cortex (Joos *et al.*, 2010; Friederich *et al.*, 2012), the lateral occipital cortex and superior temporal gyrus (Suchan *et al.*, 2010), as well as the posterior cingulate and parahippocampal gyri (Brooks *et al.*, 2011). In addition, reduced grey matter volumes have been reported in a number of regions across the brain including the hippocampus, amygdala, precuneus, and superior/middle temporal gyrus (e.g. Gaudio *et al.*, 2011; Friederich *et al.*, 2012; Amianto *et al.*, 2013; Kohmura *et al.*, 2017; Martin Monzon *et al.*, 2017) (see Eynde *et al.*, 2012; Phillipou *et al.*, 2014a for reviews). While there are broad commonalities across this body of work, the findings are rather inconsistent; indeed, at least one group has reported increased grey matter volumes in AN patients (Frank *et al.*, 2013). There are a number of factors that may contribute to such inconsistencies, including the use of relatively small sample sizes (typically <20 AN patients) and the disparate analytic techniques used across studies. For example, early studies utilised manual tracing procedures which can be compromised by variable inter- and intra-rater reliability. The subsequent development of automated computer algorithmic VBM approaches circumvent these potential biases, particularly regarding inter-subject registration (i.e. reducing anatomical variability between individuals by spatially aligning brain images to an anatomical reference). Additionally, other techniques do not control for differences in brain size between individuals. This step is critical in VBM analyses as any observed effects may be due to global differences rather than regional differences (Mechelli *et al.*, 2005). Among the most robust current approaches to VBM analysis is the use of diffeomorphic anatomic registration through an exponentiated lie algebra algorithm (DARTEL: Ashburner, 2007), a high-dimensional inverse-consistent diffeomorphic image registration method, which has improved between-subject registration accuracy. This results in greater sensitivity compared to earlier approaches to VBM pre-processing (Klein *et al.*, 2009).

In the current study, we utilised DARTEL procedures to compare regional grey matter volumes between acutely ill AN patients and healthy controls, accounting for differences in whole-brain volume. Additionally, we followed the accepted guidelines for reporting VBM studies to facilitate comparison with other studies (Ridgway *et al.*, 2008). It was hypothesised that the AN group would exhibit reduced grey matter volumes in the basal ganglia, and fronto-striatal and temporal regions. The AN group were not expected to exhibit increased grey matter in any region.

Materials & Methods

Participants

Twenty-six females with AN and 27 female healthy control (HC) participants underwent a magnetic resonance imaging (MRI) scan. Groups were matched on age and premorbid intelligence as determined by the Wechsler Test of Adult Reading (WTAR) (Wechsler, 2001). HCs were recruited through public advertisements, whereas AN participants were recruited through public advertisements; the Body Image and Eating Disorders Treatment and Recovery Service at the Austin and St Vincent's Hospitals; and The Melbourne Clinic; all in Melbourne, Australia. All participants were English speaking and had no history of significant brain injury or neurological condition (self-report). Controls were required to have no history of an eating disorder or other mental illness (self-report in combination with the below).

The Mini International Neuropsychiatric Interview, 5.0.0 (MINI; Sheehan *et al.*, 1998) was used to screen all participants for Axis I psychiatric disorders (including recent substance abuse) according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV). The MINI was also used to confirm diagnoses of AN, with the exception of the amenorrhea criterion which is not included in DSM-5. AN was required to be the primary diagnosis of the AN group. AN participants with comorbid psychiatric conditions, other than psychotic conditions, were not excluded as this would not have represented a typical AN sample. The following comorbid conditions were identified in the AN group: current depressive episode (n= 18), dysthymia (n= 15), current manic/hypomanic episode (n= 1), current panic disorder (n= 4), social anxiety disorder (n= 10), obsessive compulsive disorder (n= 16), posttraumatic stress disorder (n= 4), alcohol dependence (n= 2), substance dependence (n=1), bulimia nervosa (n= 4), generalised anxiety disorder (n= 18). Psychiatric medications included: selective serotonin reuptake inhibitors (n=11), atypical antipsychotics (n=12), benzodiazepines (n=6), serotonin-noradrenaline reuptake inhibitors (n=3), melatonergic antidepressants (n=3), noradrenergic and specific serotonergic antidepressant (n=1) and cyclopyrrolones (n=1). Eating disorder symptomatology was interrogated with the Eating Disorder Examination Questionnaire (EDE-Q) (Fairburn, 2008) (Table 1).

[TABLE 1]

Institutional review board statement

The study was granted independent ethics approval by the Human Research Ethics committees at St Vincent's Hospital, Austin Health and The Melbourne Clinic. In addition, the study received expedited ethics approval from Swinburne's Human Research Ethics Committee and was registered with The University of Melbourne Health Sciences Human Ethics Sub-Committee, on the basis of the prior St Vincent's Hospital review. Written informed consent was obtained from all participants.

MRI image acquisition parameters

A T1-weighted spoiled MPRAGE image was acquired sagitally (bandwidth= 170 Hz/Px, TR= 1900ms, TE= 2.52ms, echo spacing= 7.5ms, flip angle= 9°, field-of-view= 350x263x350mm, voxel resolution= 1x1x1mm, slice thickness= 1mm), on a Siemens Tim Trio 3 Tesla system with a 32 channel head coil at Swinburne University of Technology (Melbourne, Australia).

MRI image pre-processing and statistical analysis

All aspects of pre-processing and statistical analyses were performed using SPM12 (Wellcome Department of Imaging Neuroscience, UK), running on Matlab 2014a (Mathworks, Natick, MA, USA) as detailed in Ashburner (2010). Images were first manually realigned to Montreal Neurological Institute (MNI) space. Initially, MRI images were segmented into grey matter, white matter, and cerebrospinal fluid using the unified segmentation model (Ashburner & Friston, 2005). Subsequently, a grey matter template was created using DARTEL procedures that determines the deformations that best align the images via iterative non-linear registration (Ashburner, 2007). This template was warped (normalised) into standard stereotactic space (i.e, MNI space), and the parameters of this transformation were applied to individual grey matter images (normalisation), which were subsequently modulated to preserve the intensities of the original images. The voxel-size of the normalised images was 1.5 x 1.5 x 1.5 mm^3. These normalised images were spatially smoothed with an 8mm FWHM Gaussian filter, and submitted to statistical analysis using the general linear model approach in SPM12.

The smoothed grey matter images were corrected for total intracranial volume (sum of grey matter, white matter and cerebrospinal fluid; no difference between groups, $p>.05$) using proportional scaling, then submitted to two-sample t-tests, from which the contrasts of AN > HC and HC > AN were computed. Standard thresholding of t-maps was used to make statistical inferences; that is, by applying an uncorrected voxel-wise threshold (see results for more details) and correcting for multiple comparisons at the cluster level using family-wise error (FWE, $p < .05$) with an absolute threshold mask of 0.2. Regions occupied by significant clusters were determined via visual inspection and the use of xjView.

Clusters of significant group difference in grey matter volume were correlated with body mass index (BMI) and illness duration as potential indicators of malnutrition, and with EDE-Q scores for each group to examine relationship between volumetric differences and eating disorder symptomatology with exploratory Pearson's correlations. Given these analyses involved exploratory correlations, a stringent alpha of 0.01 was selected as it is acknowledged that Bonferroni corrections in such circumstances are too stringent. Trends were identified with an alpha >0.01 and <0.05. A forced entry linear regression analysis was performed ('enter' method) for significant correlations, with EDE-Q and BMI as predictor variables of grey matter volume of specific clusters.

Results

Primary analyses

The AN group showed significantly reduced global grey matter volumes (F(1,52)=4.921, p=.031), but no difference in global white matter volumes (F(1,52)=1.732, p>.05), relative to HC. No significant clusters of increased grey matter volumes were found in the AN participants compared to HC. In contrast, widespread reductions in grey matter volumes were found in AN compared to HC, and are detailed below. Since the regional differences 'bled' into multiple brain regions in a cluster, we employed a graded voxel-wise thresholding ($p<.0001$, $p<.0005$, and $p<.001$) approach in conjunction with the above stated cluster thresholding (FWE $p<.05$) to facilitate exploration of regional grey matter differences between the groups.

Significantly reduced grey matter volumes were found in AN in five clusters at the strictest voxelwise threshold of $p<.0001$ (clusters A-E), and a further five clusters with more standard thresholds (clusters F-J) (see Table 2 and Figure 1). Cluster A included anterior portions of the left middle and superior temporal gyri (Brodmann Area (BA) 21), whereas cluster B comprised homologous regions to cluster A in the right hemisphere, but included more posterior regions involved in auditory processing (BA 21, 22). Despite the stricter thresholding, Cluster C overlapped the left inferior frontal and superior temporal gyri near the rolandic operculum, while Cluster D mapped onto an area of the left parietal lobe (BA 5), and Cluster E was consistent with the right middle frontal gyrus.

At the voxelwise threshold of p<.0005, three clusters of reduced grey matter volume in AN were identified. Cluster F included a region covering the right middle frontal and precentral gyri, supplementary motor area and posterior cingulate gyrus. Cluster G included a region of the left inferior parietal lobe near the supramarginal gyrus, whereas cluster H covered part of the brainstem including part of the substantia nigra and ventral tegmental area. At the standard voxelwise threshold of p<.001, Cluster I showed reduced volumes in bilateral ventral striatal nuclei including the nucleus accumbens, and Cluster J showed reduced volume in the right posterior cerebellar cortex and cerebellar tonsil.

[FIGURE 1]

[TABLE 2]

Correlational analyses

Significant correlations and trends are presented in Table 3 and Figure 2a-b. No significant correlations were found for the AN group between any of the clusters and illness duration. Correlations with Cluster H (brainstem) resulted in a number of significant correlations, for the AN group including negative correlations with each subscale of the EDE-Q. A trend for a positive correlation of moderate strength was found between Cluster H and BMI for both groups, and a trend for a weak positive correlation for Cluster I (bilateral ventral striatum) and BMI for the AN group only.

A forced entry linear regression analysis was performed on the AN group with global EDE-Q scores and BMI as predictor variables of Cluster H grey matter volume (Table 4). Global scores on the EDE-Q explained 42% of the variance in grey matter volumes of Cluster H in AN patients. Together with BMI, 56% of the variance was explained.

[FIGURE 2]

[TABLE 3]

[TABLE 4]

Discussion

The current study compared grey matter volumes between a group of individuals with AN to a group of HCs utilising VBM analysis. As hypothesised, the AN group were not found to display increased grey matter volumes of any brain region in comparison to the HC group. Reduced grey matter volumes were found in the basal ganglia, ventral striatum and temporal cortices, in addition to widespread reductions throughout a number brain regions in AN. Reduced grey matter volumes in AN patients at the strictest voxelwise threshold (p<.0001) were observed in widespread cortical brain regions, including within bilateral portions of the middle and superior temporal gyri, the left inferior frontal and superior temporal gyri, and regions of the left parietal lobe and the right middle frontal gyrus. At more standard thresholds (p<.0005 and p<.001), the supplementary motor area and cingulate gyrus, the left inferior parietal lobe, the brainstem including part of the substantia nigra and ventral tegmental area, the ventral striatum, and the right cerebellum also showed reduced grey matter volumes in AN.

Deficits in these brain regions are consistent with cognitive deficits associated with the illness. Reduced volumes of the temporal cortex are consistent with deficits in affect perception often reported in AN (Jänsch *et al.*, 2009); whereas reduced volumes of the inferior parietal lobe may contribute to the visuo-spatial processing deficits reported in the disorder (Stedal *et al.*, 2012). Further, deficits in the superior temporal gyrus are associated with difficulties in social cognition (Pelphrey *et al.*, 2004), and reduced grey matter volumes in this region potentially contribute to the social functioning impairments often observed in AN (Russell *et al.*, 2009). In addition, the ventral striatum is heavily involved in responses to reward and deficits in this area support findings of altered reward processing in AN (Wagner *et al.*, 2007; Fladung *et al.*, 2010).

This study also revealed a number of significant correlations between regions of reduced grey matter and symptomatology in AN. Specifically, grey matter volumes of the brainstem cluster (including the substantia nigra and ventral tegmental area) was negatively correlated with EDE-Q global scores and each of the EDE-Q subscales, and positively correlated (trend only) with BMI in AN. Similarly, ventral striatal volumes were also positively correlated with BMI in AN; again, only at a trend level. These findings suggest increased eating disorder symptomatology with decreased brainstem volumes, and a trend for reduced BMI with decreased volume of both the brainstem and ventral striatum. Importantly, eating disorder symptomatology (i.e. EDE-Q global scores) explained 42% of the variance in grey matter volume of the brainstem cluster, with BMI contributing to a further 14% of this variance, suggesting a strong association of reduced BMI to reduced grey matter volumes in this region.

Reductions in ventral striatal volumes with a trend for reduced BMI suggests that deficits in reward processing often reported in AN may be influenced by decreased body weight, and that increasing an individual's BMI may consequently improve symptoms of anhedonia. Further, the brainstem cluster, which included part of the basal ganglia, i.e. the substantia nigra and ventral tegmental area which are strongly linked both to reward processing and the production of saccadic eye movements (Hikosaka & Wurtz, 1985; Zaghloul *et al.*, 2009), was found to correlate with AN symptomatology (EDE-Q scores, and a trend for BMI). Further, BMI & global EDE-Q scores were found to be significant predictors of grey matter volume in this area, explaining 56% of the variance in grey matter volume in this cluster. This finding suggests that eating disorder symptomatology may contribute to reductions of grey matter in the substantia nigra and ventral tegmental area. Grey matter reductions within this region may also underpin the anhedonia experienced in AN and the saccadic eye movement abnormalities reported in the illness (Phillipou *et al.*, 2014b; Phillipou *et al.*, 2015a; Phillipou *et al.*, 2015b; Phillipou *et al.*, 2016). Therefore, improvements in eating disorder symptomatology may influence the restoration of brainstem grey matter volumes and potentially result in improvements in neurocognitive deficits associated with the illness (i.e. reward processing and saccadic eye movement function).

Previous studies have reported that many grey matter volume changes observed in acutely ill patients normalise in recovery from AN (e.g. Lázaro *et al.*, 2013; Cowdrey *et al.*, 2014; Bang *et al.*, 2016), though others have reported that some deficits persist (e.g. Katzman *et al.*, 1996; Frank *et al.*, 2013; Martin Monzon *et al.*, 2017). Nonetheless, the volumetric changes observed in the acute state may have a role in the maintenance of AN. For example, grey matter deficits in cingulate regions may be involved in motivational aspects of recovery (Holroyd & Yeung, 2012); reduced volumes in parietal regions may contribute to deficits in the perception of one's body through difficulties in spatial and visual integration (Andersen, 2011); and atrophy in superior temporal regions may be related to problems in grasping the intention of others (Pelphrey *et al.*, 2004).

A number of potential limitations related to this study must be noted. Firstly, the correlations reported are exploratory in nature; thus, the interpretation of the relationship between grey matter volumes, and eating disorder symptomatology and BMI must be interpreted with caution. Secondly, the AN group had a number of psychiatric comorbidities and were on a variety of medications which have the potential to influence the findings. Thirdly, it is not possible to differentiate whether grey matter volumes are the result of AN itself or the consequences of malnutrition and starvation. Investigating individuals who are weight-restored from AN may be beneficial for this purpose, however, the long-term effects of starvation remain unclear.

A major strength of this study is the sample assessed, i.e. a large sample of individuals in the acute phase of the illness. It is now essential to extend these findings, utilising the methodological advantages of this study, to explore structural changes in those recovered from the illness. The findings of the current study do, however, shed light onto the brain regions of structural deficit in acute AN, which may be involved in the maintenance of the illness and possibly even present prior to illness onset. However, this remains unknown as it is incredibly difficult to determine differences that are present prior to illness onset and are not related to the effects of starvation. The brain regions of deficit found in this study are, however, also consistent with replicated cognitive deficits reported in the illness, therefore potentially contributing to AN symptomatology. Further, the relationship between EDE-Q scores and BMI, and regions of reduced grey matter heavily involve in reward processing, suggest that improvements in eating disorder symptomatology may result in restoration of these brain regions, and consequently result in improved responses to reward in individuals with AN. In addition, the findings of this study suggest that therapies targeting cognitive processes such as reward processing, and novel eye movement based tasks which utilise overlapping brain areas, may promote improvements in brain regions underpinning AN, and require further investigation. In addition, a further translational implication may be to include the use of brain stimulation techniques targeting these neural regions to improve symptoms in AN.

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Conflict of Interest Statement

Prof. Castle reports grants and personal fees from Eli Lilly, janssen-Cilag, Roche, Allergen, Bristol-Myer Squibb, Pfizer, Lundbeck, AstraZeneca and Hospira, during the conduct of the study; and personal fees from Eli Lilly, Bristol-Myer Squibb, Lundbeck, Janssen Cilag, Pfizer, Organon, Sanofi-Aventis, Wyeth, Hospira and Servier, outside the submitted work. A/Prof Abel reports personal fees from Actelion Pharmaceuticals, Switzerland, outside the submitted work. Dr Phillipou, Prof Rossell, Dr Gurvich, Mr Nibbs and Dr Hughes report no conflicts of interest.

Author Contributions

AP and RN collected the data. AP and MH analysed the data. All authors designed the study, interpreted the findings and contributed to the manuscript.

Data Accessibility Statement

Data are not available on a public access repository, but may be requested from the corresponding author.

Abbreviations

AN= anorexia nervosa

BA= Brodmann Area

BMI = body mass index

DARTEL= diffeomorphic anatomic registration through an exponentiated lie algebra algorithm

DSM= Diagnostic and Statistical Manual of Mental Disorders

EDE-Q= Eating Disorders Examination Questionnaire

FWE= family wise error

HC = healthy control

MINI= Mini International Neuropsychiatric Interview

MNI= Montreal Neurological Institute

MRI= magnetic resonance imaging

VBM= voxel based morphometry

WTAR= Wechsler Test of Adult Reading

References

American Psychiatric Association (2013) *Diagnostic and Statistical Manual of Mental Disorders 5*. American Psychiatric Association, Washington, DC.

Amianto, F., D'Agata, F., Lavagnino, L., Caroppo, P., Abbate-Daga, G., Righi, D., Scarone, S., Bergui, M., Mortara, P. & Fassino, S. (2013) Intrinsic connectivity networks within cerebellum and beyond in eating disorders. *The Cerebellum*, **12**, 623-631.

Andersen, R.A. (2011) Inferior parietal lobule function in spatial perception and visuomotor integration. *Comprehensive Physiology*.

Ashburner, J. (2007) A fast diffeomorphic image registration algorithm. *Neuroimage*, **38**, 95-113.

Ashburner, J. (2010) VBM tutorial.

Ashburner, J. & Friston, K.J. (2005) Unified segmentation. *Neuroimage*, **26**, 839-851.

Bang, L., Rø, Ø. & Endestad, T. (2016) Normal gray matter volumes in women recovered from anorexia nervosa: a voxel-based morphometry study. *BMC psychiatry*, **16**, 144.

Brooks, S.J., Barker, G.J., O'Daly, O.G., Brammer, M., Williams, S.C., Benedict, C., Schiöth, H.B., Treasure, J. & Campbell, I.C. (2011) Restraint of appetite and reduced regional brain volumes in anorexia nervosa: a voxel-based morphometric study. *BMC psychiatry*, **11**, 179.

Cowdrey, F.A., Filippini, N., Park, R.J., Smith, S.M. & McCabe, C. (2014) Increased resting state functional connectivity in the default mode network in recovered anorexia nervosa. *Human brain mapping*, **35**, 483-491.

Deborde, A., Berthoz, S., Godart, N., Perdereau, F., Corcos, M. & Jeammet, P. (2005) Relations between alexithymia and anhedonia: a study in eating disordered and control subjects. *L'Encephale*, **32**, 83-91.

Eynde, F., Suda, M., Broadbent, H., Guillaume, S., Eynde, M., Steiger, H., Israel, M., Berlim, M., Giampietro, V. & Simmons, A. (2012) Structural magnetic resonance imaging in eating disorders: a systematic review of voxel-based morphometry studies. *European Eating Disorders Review*, **20**, 94-105.

Fairburn, C.G. (2008) *Cognitive behavior therapy and eating disorders*. Guilford Press.

Fladung, A.-K., Grön, G., Grammer, K., Herrnberger, B., Schilly, E., Grasteit, S., Wolf, R.C., Walter, H. & von Wietersheim, J. (2010) A neural signature of anorexia nervosa in the ventral striatal reward system. *American Journal of Psychiatry*, **167**, 206-212.

Frank, G.K., Shott, M.E., Hagman, J.O. & Mittal, V.A. (2013) Alterations in brain structures related to taste reward circuitry in ill and recovered anorexia nervosa and in bulimia nervosa. *American Journal of Psychiatry*, **170**, 1152-1160.

Friederich, H.-C., Walther, S., Bendszus, M., Biller, A., Thomann, P., Zeigermann, S., Katus, T., Brunner, R., Zastrow, A. & Herzog, W. (2012) Grey matter abnormalities within cortico-limbic-striatal circuits in acute and weight-restored anorexia nervosa patients. *Neuroimage*, **59**, 1106-1113.

Gaudio, S., Nocchi, F., Franchin, T., Genovese, E., Cannatà, V., Longo, D. & Fariello, G. (2011) Gray matter decrease distribution in the early stages of Anorexia Nervosa restrictive type in adolescents. *Psychiatry Research: Neuroimaging*, **191**, 24-30.

Hikosaka, O. & Wurtz, R.H. (1985) Modification of saccadic eye movements by GABA-related substances. II. Effects of muscimol in monkey substantia nigra pars reticulata. *Journal of Neurophysiology*, **53**, 292-308.

Holroyd, C.B. & Yeung, N. (2012) Motivation of extended behaviors by anterior cingulate cortex. *Trends in cognitive sciences*, **16**, 122-128.

Jänsch, C., Harmer, C. & Cooper, M.J. (2009) Emotional processing in women with anorexia nervosa and in healthy volunteers. *Eating Behaviors*, **10**, 184-191.

Joos, A., Klöppel, S., Hartmann, A., Glauche, V., Tüscher, O., Perlov, E., Saum, B., Freyer, T., Zeeck, A. & van Elst, L.T. (2010) Voxel-based morphometry in eating disorders: correlation of psychopathology with grey matter volume. *Psychiatry Research: Neuroimaging*, **182**, 146-151.

Katzman, D.K., Lambe, E.K., Mikulis, D.J., Ridgley, J.N., Goldbloom, D.S. & Zipursky, R.B. (1996) Cerebral gray matter and white matter volume deficits in adolescent girls with anorexia nervosa. *The Journal of pediatrics*, **129**, 794-803.

Kehagia, A.A., Murray, G.K. & Robbins, T.W. (2010) Learning and cognitive flexibility: frontostriatal function and monoaminergic modulation. *Current opinion in neurobiology*, **20**, 199-204.

Klein, A., Andersson, J., Ardekani, B.A., Ashburner, J., Avants, B., Chiang, M.-C., Christensen, G.E., Collins, D.L., Gee, J. & Hellier, P. (2009) Evaluation of 14 nonlinear deformation algorithms applied to human brain MRI registration. *Neuroimage*, **46**, 786-802.

Kohmura, K., Adachi, Y., Tanaka, S., Katayama, H., Imaeda, M., Kawano, N., Nishioka, K., Ando, M., Iidaka, T. & Ozaki, N. (2017) Regional decrease in gray matter volume is related to body dissatisfaction in anorexia nervosa. *Psychiatry Research: Neuroimaging*, **267**, 51-58.

Lao-Kaim, N.P., Fonville, L., Giampietro, V.P., Williams, S.C., Simmons, A. & Tchanturia, K. (2015) Aberrant function of learning and cognitive control networks underlie inefficient cognitive flexibility in anorexia nervosa: a cross-sectional fMRI study. *PloS one*, **10**, e0124027.

Lázaro, L., Andrés, S., Calvo, A., Cullell, C., Moreno, E., Plana, M.T., Falcón, C., Bargalló, N. & Castro-Fornieles, J. (2013) Normal gray and white matter volume after weight restoration in adolescents with anorexia nervosa. *International Journal of Eating Disorders*, **46**, 841-848.

Martin Monzon, B., Henderson, L.A., Madden, S., Macefield, V.G., Touyz, S., Kohn, M.R., Clarke, S., Foroughi, N. & Hay, P. (2017) Grey matter volume in adolescents with anorexia nervosa and associated eating disorder symptoms. *European Journal of Neuroscience*.

Mechelli, A., Price, C.J., Friston, K.J. & Ashburner, J. (2005) Voxel-based morphometry of the human brain: methods and applications. *Current medical Imaging reviews*, **1**, 105-113.

Murao, E., Sugihara, G., Isobe, M., Noda, T., Kawabata, M., Matsukawa, N., Takahashi, H., Murai, T. & Noma, S.i. (2017) Differences in Neural Responses to Reward and Punishment Processing between Anorexia Nervosa Subtypes: An fMRI Study. *Psychiatry and Clinical Neurosciences*.

Pelphrey, K.A., Morris, J.P. & Mccarthy, G. (2004) Grasping the intentions of others: the perceived intentionality of an action influences activity in the superior temporal sulcus during social perception. *Journal of cognitive neuroscience*, **16**, 1706-1716.

Phillipou, A., Abel, L.A., Castle, D.J., Hughes, M.E., Gurvich, C., Nibbs, R.G. & Rossell, S.L. (2015a) Self perception and facial emotion perception of others in anorexia nervosa. *Frontiers in psychology*, **6**.

Phillipou, A., Rossell, S.L. & Castle, D.J. (2014a) The neurobiology of anorexia nervosa: A systematic review. *Australian and New Zealand Journal of Psychiatry*, **48**, 128-152.

Phillipou, A., Rossell, S.L., Castle, D.J., Gurvich, C. & Abel, L.A. (2014b) Square Wave Jerks and Anxiety as Distinctive Biomarkers for Anorexia Nervosa. *Investigative ophthalmology & visual science*, **55**, 8366-8370.

Phillipou, A., Rossell, S.L., Gurvich, C., Castle, D.J., Troje, N.F. & Abel, L.A. (2015b) Body Image in Anorexia Nervosa: Body Size Estimation Utilising a Biological Motion Task and Eyetracking. *European Eating Disorders Review*.

Phillipou, A., Rossell, S.L., Gurvich, C., Hughes, M.E., Castle, D.J., Nibbs, R.G. & Abel, L.A. (2016) Saccadic Eye Movements in Anorexia Nervosa. *PloS one*, **11**, e0152338.

Reker, M., Ohrmann, P., Rauch, A.V., Kugel, H., Bauer, J., Dannlowski, U., Arolt, V., Heindel, W. & Suslow, T. (2010) Individual differences in alexithymia and brain response to masked emotion faces. *Cortex*, **46**, 658-667.

Ridgway, G.R., Henley, S.M., Rohrer, J.D., Scahill, R.I., Warren, J.D. & Fox, N.C. (2008) Ten simple rules for reporting voxel-based morphometry studies. *Neuroimage*, **40**, 1429-1435.

Russell, T.A., Schmidt, U., Doherty, L., Young, V. & Tchanturia, K. (2009) Aspects of social cognition in anorexia nervosa: affective and cognitive theory of mind. *Psychiatry research*, **168**, 181-185.

Sato, Y., Saito, N., Utsumi, A., Aizawa, E., Shoji, T., Izumiyama, M., Mushiake, H., Hongo, M. & Fukudo, S. (2013) Neural basis of impaired cognitive flexibility in patients with anorexia nervosa. *PloS one*, **8**, e61108.

Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R. & Dunbar, G.C. (1998) The Mini-International Neuropsychiatric Interview (MINI): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *Journal of clinical psychiatry*.

Stedal, K., Rose, M., Frampton, I., Landrø, N.I. & Lask, B. (2012) The neuropsychological profile of children, adolescents, and young adults with anorexia nervosa. *Archives of Clinical Neuropsychology*, **27**, 329-337.

Suchan, B., Busch, M., Schulte, D., Grönermeyer, D., Herpertz, S. & Vocks, S. (2010) Reduction of gray matter density in the extrastriate body area in women with anorexia nervosa. *Behavioural brain research*, **206**, 63-67.

Tchanturia, K., Anderluh, M.B., Morris, R.G., Rabe-Hesketh, S., Collier, D.A., Sanchez, P. & Treasure, J.L. (2004a) Cognitive flexibility in anorexia nervosa and bulimia nervosa. *Journal of the International Neuropsychological Society*, **10**, 513-520.

Tchanturia, K., Davies, H., Harrison, A., Fox, J.R., Treasure, J. & Schmidt, U. (2012) Altered social hedonic processing in eating disorders. *International Journal of Eating Disorders*, **45**, 962-969.

Tchanturia, K., Morris, R., Anderluh, M.B., Collier, D., Nikolaou, V. & Treasure, J. (2004b) Set shifting in anorexia nervosa: an examination before and after weight gain, in full recovery and relationship to childhood and adult OCPD traits. *Journal of psychiatric research*, **38**, 545-552.

Wagner, A., Aizenstein, H., Venkatraman, V.K., Fudge, J., J Christopher May, B., Mazurkewicz, L., Frank, G.K., Bailer, U.F., Fischer, L. & Van Nguyen, M. (2007) Altered reward processing in women recovered from anorexia nervosa. *American Journal of Psychiatry*, **164**, 1842-1849.

Wechsler, D. (2001) *Wechsler Test of Adult Reading: WTAR*. Psychological Corporation.

Zaghloul, K.A., Blanco, J.A., Weidemann, C.T., McGill, K., Jaggi, J.L., Baltuch, G.H. & Kahana, M.J. (2009) Human substantia nigra neurons encode unexpected financial rewards. *Science*, **323**, 1496-1499.

Table 1: Clinical characteristics

	AN	HC		
	M (SD)	M (SD)	F	P
Age	22.81 (6.67)	22.46 (3.16)	0.06	0.81
WTAR	104.77 (8.11)	106.19 (7.11)	0.46	0.50
BMI	16.63 (1.19)	22.60 (3.53)	67.08	< 0.01
Illness Duration	6.42 (7.43)	-	-	-
Age of Illness Onset	16.04 (3.40)	-	-	-
EDE-Q Restraint	3.93 (1.42)	0.58 (0.63)	116.84	< 0.01
EDE-Q Eating Concern	3.78 (1.24)	0.25 (0.31)	188.56	< 0.01
EDE-Q Shape Concern	5.01 (0.90)	1.17 (0.84)	236.44	< 0.01
EDE-Q Weight Concern	4.50 (1.41)	0.66 (0.82)	136.11	< 0.01
EDE-Q Global Score	4.30 (1.12)	0.67 (0.54)	211.44	< 0.01

AN: Anorexia nervosa; HC: Healthy control; WTAR: Wechsler Test of Adult Reading; BMI: Body mass index; Age, Illness duration and age of illness onset reported in years.

Table 2: Significant differences in grey matter volume, healthy controls > anorexia nervosa

Cluster	No. of Voxels	Peak t	Peak MNI coordinates			Voxel Level Threshold
			x	y	z	
Cluster A	1352	6.03	-54	0	-20	p<0.0001
Cluster B	2166	6.02	57	-21	-14	p<0.0001
Cluster C	1251	5.90	-46	6	28	p<0.0001
Cluster D	294	5.15	-14	-40	48	p<0.0001
Cluster E	350	5.04	24	-8	52	p<0.0001
Cluster F	1064	4.49	23	-42	51	p<0.0005
Cluster G	573	4.41	-47	-45	42	p<0.0005
Cluster H	735	4.24	-5	-24	-9	p<0.0005
Cluster I	1174	4.58	-3	11	-15	p<0.001
Cluster J	985	4.37	21	-63	-41	p<0.001

Note: MNI= Montreal Neuroimaging Institute; cluster level threshold = p<05, family-wise error corrected; main regions included in the clusters: A= left middle & superior temporal gyri, B= right middle & superior temporal gyri, C= left inferior frontal & superior temporal gyri. D= left parietal lobe, E= right middle frontal gyrus, F= right middle frontal & precentral gyri, G= left inferior parietal lobe, H= brainstem, I= bilateral ventral striatal nuclei, J= right posterior cerebellar cortex & cerebellar tonsil.

Table 3: Significant correlations between clusters and other variables

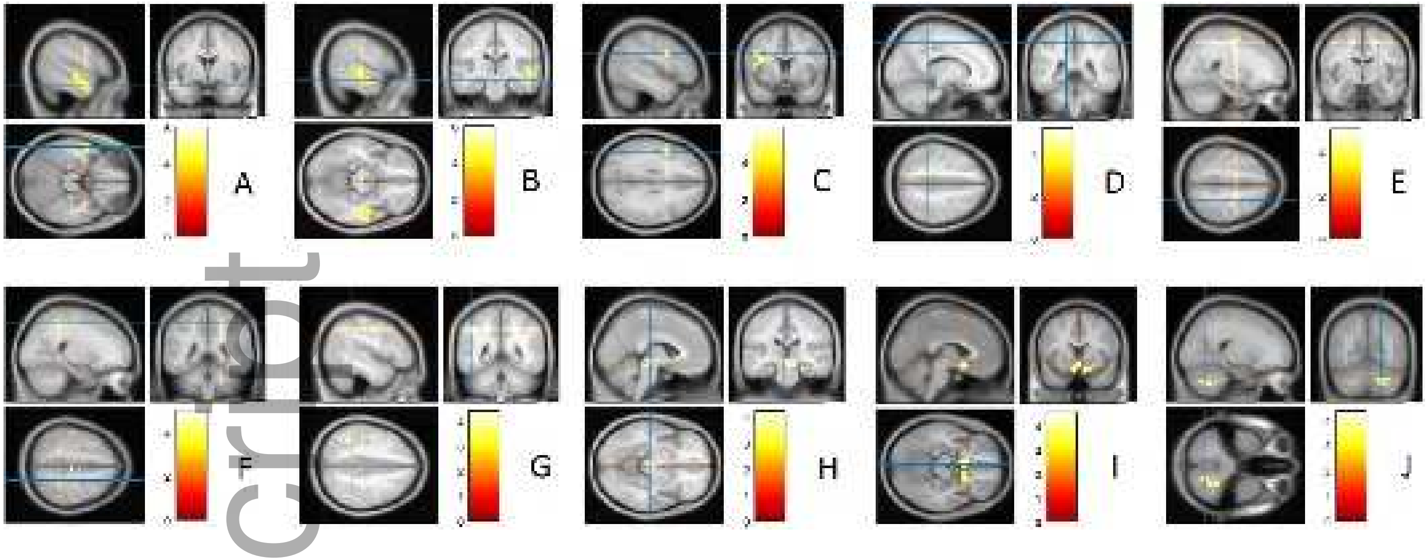
Cluster	Variable	AN		HC	
		r	p	r	p
H	EDE-Q Restraint	-0.57	0.004	0.03	0.870
H	EDE-Q Eating Concern	-0.59	0.002	0.39	0.060
H	EDE-Q Shape Concern	-0.57	0.004	0.23	0.259
H	EDE-Q Weight Concern	-0.61	<0.001	0.33	0.103
H	EDE-Q Global	-0.65	<0.001	0.29	0.167
H	BMI	0.46	0.017	0.60	<.001
I	BMI	0.39	0.044	0.32	0.134

Note: AN= anorexia nervosa; HC= healthy control; BMI= body mass index; EDE-Q= Eating Disorder Examination Questionnaire

Table 4: Linear model of predictors of Cluster H grey matter volume in anorexia nervosa participants

		B	SE b	β
Step 1				
	Constant	0.18	0.01	
	EDE-Q Global	-0.01	0.00	-0.65***
Step 2				
	Constant	0.09	0.03	
	EDE-Q Global	-0.01	0.00	-0.62***
	BMI	0.01	0.00	0.38*

Note: $R^2 = 0.42$ for Step 1; $\Delta R^2 = 0.14$ for Step 2 ($p<0.001$); EDE-Q Global = Eating Disorder Examination Questionnaire Global Score; BMI = body mass index



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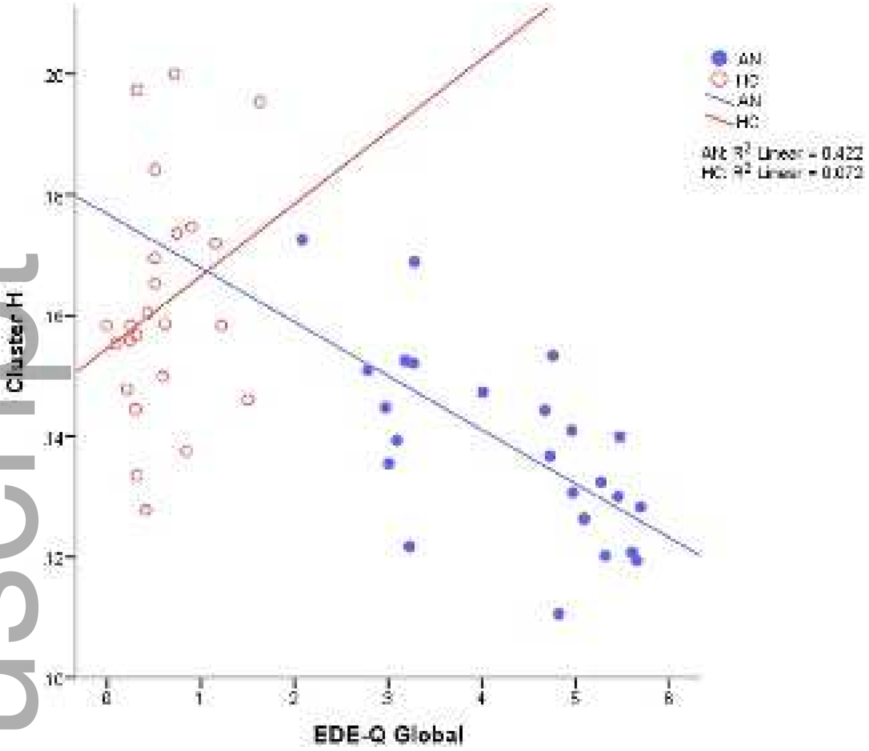

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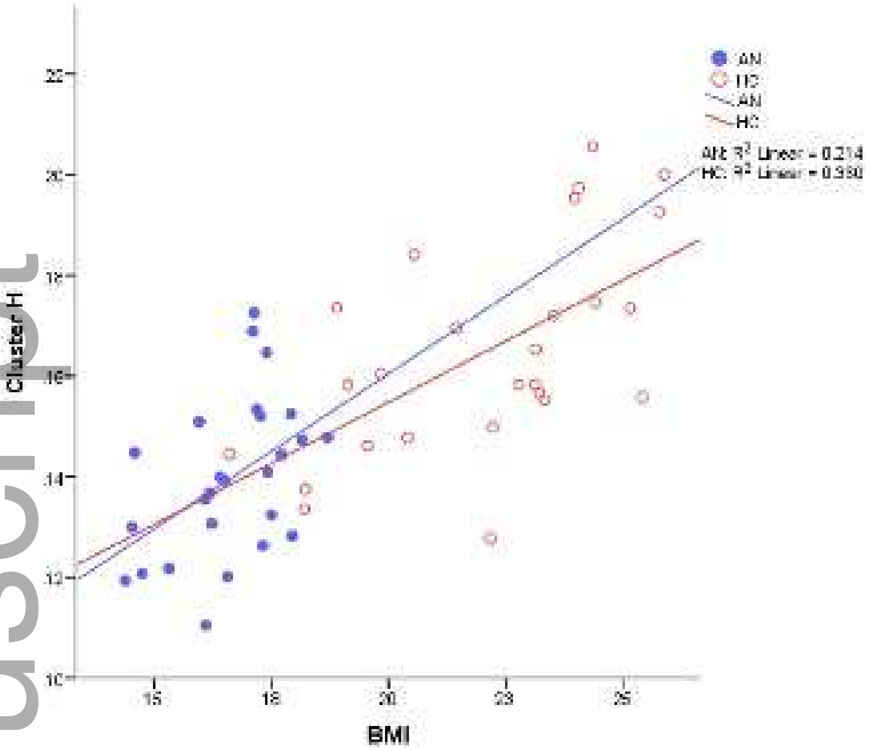

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