Psychiatry Research: Neuroimaging 191 (2011) 24–30

Contents lists available at ScienceDirect

Psychiatry Research: Neuroimaging

journal homepage: www.elsevier.com/locate/psychresns

Gray matter decrease distribution in the early stages of Anorexia Nervosa restrictive type in adolescents

Santino Gaudio[a,*], Federico Nocchi[b,c], Tiziana Franchin[b,d], Elisabetta Genovese[e], Vittorio Cannatà[e], Daniela Longo[f], Giuseppe Fariello[f]

[a]Department of Neuroscience, Bambino Gesù Children's Hospital, Piazza S. Onofrio, 4, 00165 Rome, Italy
[b]Department of Clinical Engineering, Bambino Gesù Children's Hospital, Rome, Italy
[c]Department of Applied Electronics, University Roma TRE, Rome, Italy
[d]Department of Bioengineering, IIT Unit, Politecnico di Milano, Milan, Italy
[e]Department of Occupational Health and Safety, Medical Physics, Bambino Gesù Children's Hospital, Rome, Italy
[f]Department of Radiology, Bambino Gesù Children's Hospital, Rome, Italy

ARTICLE INFO

Article history:
Received 24 February 2009
Received in revised form 29 May 2010
Accepted 10 June 2010

Keywords:
Voxel-Based Morphometry
Eating disorders
Precuneus
Cingulum
Parietal lobe

ABSTRACT

Few studies have used Voxel-Based Morphometry (VBM) to examine brain structure in Anorexia Nervosa patients. The purpose of the present study was to investigate a sample of Anorexia Nervosa restrictive type (AN-r) adolescent patients in the early stages of the illness, using VBM in order to characterize morphometric gray matter (GM) changes. Participants were 16 AN-r female patients (with no other psychiatric disorders) whose AN-r had been in progress for less than 12 months and 16 age-matched healthy female subjects. High-resolution T1-weighted magnetic resonance images were preprocessed according to the optimized VBM method, and statistically analyzed. The analyses revealed a significant global GM decrease in the AN-r patients; furthermore, a significant region-specific decrease in GM volume was found bilaterally in the middle cingulate cortex, the precuneus, and the inferior and superior parietal lobules. The significant early GM decrease in the aforementioned regions in AN-r adolescent patients suggests that there might be a region-specific GM vulnerability that could play a role in the pathophysiology of the disease. Given that these regions are also involved in the manipulation of mental images and the mental representation of the self, this might explain the presence of a distorted body image in these patients.

1. Introduction

Anorexia Nervosa (AN) is a disorder that generally begins in adolescence and primarily affects females. Its relevance is not only related to the increased incidence of AN over the last few decades (Lucas et al., 1999), but also to the high occurrence of medical complications, which can occasionally prove fatal (Harris and Barraclough, 1998).

The DSM-IV-TR classification of AN recognizes two subtypes: AN restrictive type (AN-r) and AN binge/purging type (AN-b/p).

The use of advanced brain-imaging techniques (functional magnetic resonance imaging, positron emission tomography, and single photon emission computed tomography) allowed the identification of several cerebral regions involved in AN. However, the direct involvement of these regions in the onset of AN still remains unclear and under debate (Chowdhury et al., 2003).

In previously published neuroimaging structural studies, most authors (Dolan et al., 1988; Krieg et al., 1988; Golden et al., 1996; Kohn et al., 1997) showed a global gray matter (GM) and white matter (WM) decrease with a cerebral spinal fluid (CSF) increase, while Swayze et al. (2003) demonstrated a WM decrease and a CSF increase, with no significant GM decrease. Some authors indicated a relationship between brain mass loss and severity of AN (Kohlmeyer et al., 1983; Golden et al., 1996; Katzman et al., 1996), a full reversibility of brain mass loss (Golden et al., 1996; Swayze et al., 1996; Chowdhury et al., 2003), and persistence of GM decrease (Katzman et al., 1997; Lambe et al., 1997).

A few authors used magnetic resonance imaging (MRI) and manual volume measurement in AN patients demonstrating a significant volume reduction in the hypophysis (Doraiswamy et al., 1990) and the amygdala–hippocampus complex (Giordano et al., 2001).

In recent years, the availability of the automated Voxel-Based Morphometry (VBM) technique has enabled us to make a more reliable global and regional evaluation of GM, WM and CSF volume and concentration alterations. VBM allows for a voxel-by-voxel assessment of differences across the whole brain, thus enabling us to evaluate regional volumetric alterations without *a priori* hypotheses about their localization (Ashburner and Friston, 2000). This approach is also minimally operator-dependent, in that it avoids the potential confounds and challenges associated with Regions of Interest hand-tracing methods (Gilbert et al., 2008). VBM is a group

* Corresponding author. Tel.: +39 336 872773; fax: +39 0668593824.
E-mail address: santino.gaudio@libero.it (S. Gaudio).

0925-4927/$ – see front matter © 2010 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.pscychresns.2010.06.007

analysis technique, which enables operators to reveal statistically significant local morphological differences between samples: the experimental question it seeks to answer is whether the clinical sample shows specific structural features that could be related to the pathology. Thus a reliable analysis must rely on homogeneous samples of appropriate size. In AN patients this technique produced differing results: Wagner et al. (2006) found no GM, WM and CSF differences between 40 recovered patients with eating disorders (ED) and a healthy control group; Mühlau et al. (2007) demonstrated, in a sample of 22 recovered AN patients, a global GM loss of 1% and a region-specific GM loss of 5% in the anterior cingulate cortex; Castro-Fornieles et al. (2009) in 12 AN patients (9 AN-r and 3 AN-b/p) showed a global GM decrease which normalized at follow-up (after 7 months and weight recovery) and several regions particularly affected (temporal and parietal areas), although only left and right supplementary motor areas and middle cingulate cortex remained significantly altered at follow-up.

The purpose of the present study is to perform, via VBM, a global and local GM analysis in a sample of adolescent patients whose AN-r had been in progress for less than 12 months at the time of scanning.

2. Methods

2.1. Subjects and procedures

The clinical sample was composed of 16 adolescent patients, all fulfilling the DSM-IV-TR diagnostic criteria for AN-r, under treatment at the Unit of Child Neuropsychiatry of the Bambino Gesù Children's Hospital of Rome from November 2005 to July 2008. Informed consent was obtained from all parents and the study followed the ethical guidelines of the hospital.

The inclusion criteria for the AN-r patient sample were: 12–18 years of age; duration of AN-r less than 12 months at the time of scanning; no other current or previous DSM-IV-TR disorders; right-handedness. The exclusion criteria were a previous history of other eating disorders and concomitant medical diseases.

Diagnosis of past or current EDs and diagnosis of past or current other Axis I disorders was made in accordance with DSM-IV-TR criteria by clinical interview. The Structured Clinical Interview for Axis II Disorders (SCID II) (First et al., 1997) was used to assess personality disorders in patients older than 16 years of age. Moreover, the Children's Depression Inventory (CDI) (Kovacs, 1988) and the Eating Attitudes Test (EAT 26) (Garner et al., 1982) were administered to all patients. All interviews were carried out, prior to scanning, by an experienced investigator (the first author) who was specifically trained to use the diagnostic tools applied. Data concerning age, body-mass index (BMI), AN-r age of onset, lowest BMI and current psychopharmacological treatment were also retrieved.

The same psychopathological examination was carried out on a volunteer control group prior to scanning. The group was composed of 16 adolescent right-handed females, less than 18 years of age, with neither previous nor current psychiatric disorders (DSM-IV-TR) and without any concomitant medical diseases. Informed consent was obtained from all parents.

At the time of scanning, all participants of both groups had received similar schooling and had no specific training or skills (Draganski et al., 2004).

2.2. MRI acquisition

All scans were performed on the same scanner (1.5 T Magnetom Vision, Siemens, Erlangen, Germany), equipped with a standard head coil. The imaging protocol consisted of a 3D T1-weighted sequence (Magnetization Prepared Rapid Acquisition Gradient Echo, MPRAGE) covering the entire head with the following image parameters: acquisition plane = sagittal; number of slices = 128; slice thickness = 1.25 mm; pixel size = 0.97 × 0.97 mm, matrix size = 256 × 256, field of view = 249 × 249 mm, flip angle = 15°, echo time = 4.40 ms and repetition time = 1.14 ms.

2.3. Voxel-Based Morphometry preprocessing

Morphometric changes were investigated with the optimized VBM protocol (Good et al., 2001) using the Statistical Parametric Mapping software package version 2 (SPM2; Wellcome Department of Cognitive Neurology, London, UK) running in MATLAB version 7.0 (The Mathworks, Natick, MA).

Since subjects ranged from 12 to 18 years old, study-specific *a priori* probability maps were created and a customized T1 template was used rather than the standard Montréal Neurological Institute (MNI) template as the reference stereotactic space (Mechelli et al., 2005). Data were modulated in order to account for the shrinkage or growth of brain regions following the nonlinear spatial normalization step. As a result, volume changes rather than concentration changes were investigated. A Gaussian kernel of 8 mm was used for smoothing the images.

2.4. Statistical analyses of gray matter

Two different analyses of covariance (ANCOVAs) were performed on preprocessed GM images, both including age as a nuisance variable in order to control its potential effect. In the second ANCOVA, global volume of GM was included as a further nuisance variable, so that changes which cannot be explained by global effects can be exclusively identified. According to Mühlau et al. (2007), we refer to the first analysis, including only age as a nuisance variable, as “analysis for the regional distribution of GM changes”, while we refer to the second approach as “analysis for region-specific GM changes”.

For both analyses a height (statistical) threshold of $P<0.05$ at the voxel-level, corrected with the Family-Wise Error (FWE), was applied. In all the aforementioned tests, an absolute threshold was set, so that only voxels with a probability greater than 20% of being GM were included in the analysis.

2.5. Global and local volume of gray matter

Total Intracranial Volume (TIV) was obtained from the sum of global volumes of GM, WM, and CSF. These values were derived from the non-normalized segmented images yielded by the first segmentation in optimized VBM. One-sided independent *t*-tests were then performed on TIV and global volume of GM to look for significant differences between AN-r and control groups.

For local analyses, the calculation of GM volumes was performed on the most significant regions (MSRs) of GM differences as resulting from the second ANCOVA. The GM content of those voxels belonging both to a significant cluster and to the corresponding main anatomical area as defined according to an automatic anatomical labeling (AAL) algorithm (Tzourio-Mazoyer et al., 2002) was considered. Only MSRs consisting of 1000 voxels or more were taken into account.

2.6. Correlation analyses

An analysis of the correlations between GM and clinical parameters was performed within the AN-r group. The following parameters were taken into account: TIV; global volume of GM; GM content of the MSRs of GM differences; age at onset; lowest BMI; duration of AN-r, age, and BMI at the time of scanning.