NEUROANATOMICAL CORRELATES OF STATE OF MIND WITH RESPECT TO ATTACHMENT IN PATIENTS WITH ANOREXIA NERVOSA

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Abstract

Objective: Anorexia Nervosa (AN) is a multi-factorial disorder (sociological, psychological and biological), but we can use two important tools to better understand its psychopathology: attachment theory and modern neuroimaging techniques. In this study, we looked for the anatomical and functional correlates of the attachment system in both young women with AN and healthy controls.

Method: We enrolled 18 participants - 10 patients with AN and 8 controls. The Adult Attachment Interview (AAI) was administered to all participants to assess their state of mind with respect to attachment. All participants also underwent an MRI scan (T1 weighted high resolution anatomical images and BOLD resting state fMRI). We analyzed MRI data using Voxel Based Morphometry and voxel-wise resting state measures (standard deviation and amplitude of low-frequency fluctuations of the timecourse).

Results: In our study, secure attachment was more common in controls than in AN patients. Some subscales of AAI were found to be significantly different between the groups: pressure to achieve exerted by the mother was higher in AN than in controls while the scores in both Coherence of Mind (CoM) and Coherence of Transcript (CoT) scales were significantly lower. CoM and CoT positively correlated with the volume of grey matter (GM) in a network of brain areas comprising the temporal poles, both amygdalae, the midbrain, the thalamus and the anterior and middle sections of cingulate cortex. The scale measuring the love received by the mother correlated with a network largely overlapping with CoM/CoT, while anger anticorrelated with parts of the same network (for instance, the precuneus and part of the limbic system). Higher passivity correlated with lower GM volumes in a network comprising mostly mesial areas such as the precuneus.

Conclusions: It seems therefore that the experience of love during childhood and the feeling of anger towards the caregivers expressed during the interview have opposite effects on brain areas: while the experience of love seems to be a protective factor, anger is correlated with lower GM volume. We conclude that non-secure attachment is a core feature of anorexia nervosa both at psychopathological and neurobiological levels.

Key words: anorexia nervosa, attachment, Voxel-Based Morphometry (VBM), resting state, Adult Attachment Interview

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Introduction

Attachment theory represents one of the most important frameworks for understanding affect regulation and human relationships (Mikulincer and Shaver 2010). John Bowlby (1969) described the attachment behavior system as a motivational inborn system, biologically evolved, that is activated by perceived threats and dangers. When the individual feels threatened he/she seeks safety and closeness from the specific person who represents the attachment figure. The repeated interpersonal relationships between infant and caregivers become encoded in the implicit memory system as internal working models of attachments that act as schemata for future relationships (Siegel 1999). Bowlby also claimed that attachment patterns are generally constant from

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infancy to adulthood and that individual differences in organization of attachment behavior are hypothesized to be related in large part to behavior of the attachment figure.

Attachment theory can be used as a tool to better understand psychopathological processes. For instance, Dozier et al. (2008) showed a strong link between the quality of attachment and psychiatric disorders, included anorexia and bulimia nervosa. In particular, these authors found a strong relationship between attachment style and risk of developing eating disorders. In the present study, attachment theory is used as the reference framework for investigating anorexia nervosa (AN), as defined by the DSM-IV TR criteria. AN is characterized by a body weight below 85% of that expected for age and height, an intense fear of gaining weight, body image distortion, and amenorrhea for at least 3 months (DSM-IV TR). The etiology of AN is considered to be multi-factorial: genetic, biological, psychological and social factors and the interactions between them all seem to play a relevant role in the onset and maintenance of this disorder (Connan et al. 2003, Jacobi et al. 2004, Adenzato et al. 2012).

The increased availability of modern neuroimaging techniques has brought new informations and insight into the pathophysiology of eating disorders. The onset of AN is often during adolescence (Currin et al. 2005), at a time when brain maturation is still incomplete (Raznahan et al. 2011) and hence may be particularly vulnerable to induced alterations. Observations of global cerebral and cerebellar atrophy in grey matter (GM) and white matter (WM) as well as ventricular enlargement in AN have led some authors to interpret these findings as representing a widespread cerebral vulnerability of this disorder (Swayze et al. 2003, Chui et al. 2008). Previous studies found that patients with AN have functional (Cowdrey et al. 2012, Amianto et al. 2013b) and structural (Boghi et al. 2011, Titova et al. 2013) alterations in a wide network of brain areas, including the precuneus, dorsolateral prefrontal cortex, cingulate cortex, insulae, temporal poles, thalamus, midbrain (Husain et al. 1992), paracentral lobule (Inui et al. 2002), hippocampus-amygdala complex (Giordano et al. 2001, Connan et al. 2006) and anterior cingulate cortex (ACC; McCormick et al. 2008).

To investigate the anatomical differences between AN patients and healthy controls, recent studies adopted voxel-based morphometry (VBM) as a tool. VBM is an unbiased automated technique based on high-resolution brain MRI sequences, widely used to measure structural differences across groups of subjects (Ashburner and Friston 2000). A recent VBM study on AN patients found a significant reduction of total WM volume and focal GM atrophy in the cerebellum, hypothalamus, caudate nucleus, frontal, parietal and temporal areas (Boghi et al. 2010). In another study, Gaudio et al. (2011) found atrophy in the middle cingulate cortex, precuneus, inferior and superior parietal lobules in AN patients at an early stage of the disease, supporting the hypothesis of a regionally specific vulnerability in the areas that are involved in mental representation of self and body imagery.

If neuroimaging techniques are important to the understanding of AN and if attachment plays a crucial role in the onset and in the development of this eating disorder, it is important to focus on the integration of these aspects. Buchheim and colleagues (2006) were the first that examined the neural correlates of attachment status in adults by assessing attachment status (organized versus disorganized) with respect to attachment trauma, administering the Adult Attachment Projective (AAP) during an fMRI scan. Only the participants with unresolved attachment showed increasing activation of medial temporal regions during the administration of AAP. This phenomenon was observed, amongst others, in regions such as the amygdala and the hippocampus, both part of the limbic system. The role of amygdala is also examined by Lemche et al. (2006), who found that levels of activity within the amygdalae were strongly correlated with attachment insecurity. Another study (Benetti et al. 2010) used VBM to examine the correlation between attachment style, affective loss (for example, death of a loved one) and GM volume in a healthy sample of adults. Attachment style was assessed on two dimensions (anxious and avoidant) using the ECR-Revised questionnaire, and the study found that high attachment-related anxiety was associated with decreased GM in the anterior temporal pole and increased GM in the left lateral orbital gyrus.

In the present study we investigated the attachment status of the participants using the Berkeley Adult Attachment Interview (AAI; George et al. 1985), widely recognized as the gold standard for investigating this motivational system. AAI is a clinical, semi-structured interview focusing upon early attachment experiences and their effects. With AAI, the interviewer aims to get a clear picture of the adult mental representations of childhood attachment experiences, the influence and possible impacts on personality and behavior of these experiences and the present relationship with parents. The participant is also asked about loss of loved ones and about other traumatic experiences. To answer the questions the interviewee uses two processes: the internal search for his own memories and the external interview with the interviewer. There is an important distinction between attachment experiences (primarily with parents) that have taken place in the past and the way in which these experiences are represented (the state of mind with respect to attachment). The AAI coding system provides measures for both aspects. When attachment is the primary area of investigation the AAI remains the most established instrument, with excellent psychometric properties and predictive validity in both clinical and nonclinical populations (Ravitz et al. 2010). However, it requires significant resources, time, and training for administration, transcription, and coding, thus limiting the feasibility of use in many settings. Therefore, it does not come as a surprise that only one study (Riem et al. 2012) validates the AAI administered during an fMRI session. This study, in 21 women without children, found that individuals with insecure attachment representations showed heightened activation in the amygdalae when compared to individuals with secure attachment representations.

The aim of the present study was to explore the functional and anatomical substrates of attachment in healthy young women and in patients with AN, paying special attention to the components of the current mental state as measured by the AAI. We expected to find involvement of regions such as the limbic system, especially temporal and mesial areas, the frontal cortex and the insulae. We also aimed to compare the networks involved in attachment with the areas thought to be more severely damaged by AN.

Materials and methods

Participants

18 subjects participated in the study: 10 young women diagnosed with AN and 8 healthy controls matched for age, sex and education. All control subjects were of normal weight (BMI comprised between 18 and 25). The clinical group was recruited from the Centre for Eating disorders of the University

of Turin. All patients were underweight (BMI < 18) at the time of the experiment and were diagnosed with AN by expert clinicians according to DSM-IV TR criteria. Patients took part in the experiment (MRI scans and AAI) before the beginning of psychotherapy. Exclusion criteria were life-time history of psychosis, schizophrenia, schizoaffective disorder, delusional (paranoid) disorder, bipolar I or II disorders, psychotic depression, organic mood disorder, severe medical illness, severe underweight that could not be managed in outpatient treatment, use of psychotropic medication and neurological diseases.

Procedure

AAI was administered to all subjects at the beginning of the experiment. AAI was recorded, transcribed verbatim and scored according to the standard AAI classification system (Main et al. 2002). The interviews were then coded by an accredited rater (R.B.A., trained by M. Main and E. Hesse in 2005) who was naïve about the identity, clinical conditions and diagnosis of the interviewees. The MRI session began for all participants about two hours after the administration of the AAI.

MRI scan

MRI study was performed at the Neuroscience Department AOU San Giovanni Battista, Turin, Italy on a Philips Achieva 1.5 T (Erlangen, Holland) equipped with dual gradient system (Quasar, Philips) of 40 mT/m and a Sense high-field, high-resolution eight-channel head coil optimized for functional imaging.

Resting state T2*-weighted echo planar images were acquired with an echo time of 50 ms and a 90° flip angle. The acquisition matrix was 128×128 and the field of view 250 mm. For each subject, 200 volumes were acquired. Each volume was acquired in 2200 ms and consisted of 22 axial 5-mm slices with a 0.5-mm gap parallel to the anterior-posterior commissural line, covering the whole brain. Three dummy volumes were added at the beginning of scanning to reach a steady-state magnetization before acquisition of the experimental data. The total acquisition time was about 7 min for each subject. None of the participants fell asleep or reported anxiety or other particular emotion during the examination.

T1-Weighted 3D Turbo Gradient-Echo sequences (matrix = 256×256; voxel size = 1×1×1 mm^3; number of slices = 190; TR = 7 ms; TE = 3 ms; TFE shots = 89) for VBM analysis were obtained with full brain coverage and isotropic voxels. Acquisition time was about 5 min for each subject.

Adult Attachment Interview

Exploring an adult's mental representations of attachment while discussing childhood experiences, the Adult Attachment Interview is designed and structured to bring into relief individual differences in deeply internalized strategies for regulating emotion and attention in response to the discussion of attachment (Main et al. 2002). Classification system is based on a number of scales, each with a score of 1-9, organized into two sections: the scales for attachment experiences or "parental behavior" (loving, rejecting, neglecting, involving or role reversing and pressure to achieve) and the scales for state of mind with respect to attachment (idealization, insistence on lack of recall, anger, derogation of parents or of attachment, fear of loss, meta-cognitive monitoring, coherence, passivity of speech and some scales for unresolved loss or trauma). Subjects are classified in three main organized attachment classifications, secure/autonomous (F), dismissing (DS), preoccupied (E). An other possible classification is "Unresolved" (U) assigned when individuals can be classified as unresolved with respect to loss, trauma, or abuse and linked to a second best-fitting classification.

VBM analysis

VBM was performed using the FSL-VBM 4.1 tool, part of the FSL software (FMRIB's Software Library, the University of Oxford; http://fsl.fmrib.ox.ac.uk). For the VBM analysis, the steps below were followed (Good et al. 2001):

1. Preparation of T1-weighted images in the correct format (compressed NIFTI);
2. Performing brain extraction using the FSL brain extraction tool (BET) on T1 images;
3. Creation of the study-specific GM template at 2×2×2 mm^3 resolution in standard MNI space,
4. Non-linear registration of all the GM images on the template. The images were then modulated and smoothed with anisotropic Gaussian kernel of 7 mm FWHM (Full Width Half Maximum).

One subject was not included in the VBM analysis due to technical issues during the acquisition of the anatomical images and the analysis was therefore conducted on 17 participants.

Resting state analysis

The data were motion corrected using an approach which minimizes the impact of the local signal variations, implemented by the McFLIRT tool (included in the FSL package), spatially normalized into the MNI space using an echo-planar imaging template, slightly resampled (using tri-linear interpolation) to a voxel size = 2 mm × 2 mm × 2 mm resulting and spatially smoothed using an 8 mm × 8 mm × 8 mm FWHM Gaussian kernel.

We applied a high-pass filter to resting state data to remove the noise from physiological sources (cardiac and respiratory), then regressed out the movement parameters (3 rotation and 3 translation, estimated by McFLIRT during motion correction) and the average WM and CSF signals. Using the denoised data we computed the amplitude of low-frequency fluctuations (ALFF, Zhang 2007) and standard deviation of the resting state time series to have a voxel-wise measure of spontaneous BOLD signal characteristics that can act as a marker of group differences.

Voxel-wise GLM analysis was carried out using permutation testing. The test was ran using FSL-Randomise version 2.8, with 5000 permutations and the Threshold-Free Cluster Enhancement (TFCE) option.

We compared patients with AN and control group in respect to GM and resting state. We also correlated GM volume, ALFF and standard deviation in each group with all the scales for state of mind and attachment experiences.

Significant results ($p < 0.005$ uncorrected for multiple comparisons, with a cluster extent > 60), were reported. We also looked at clusters surviving after correction for multiple comparisons TFCE, $p_{corr} < 0.05$. To obtain the anatomical localization of significant areas we used the FSLview atlas tool. In the figures the result maps were reported in accordance with radiogical convention (left is right). The SPSS17™ software package (SPSS Inc., Chicago, IL, USA; www.spss.com) was used to perform statistical tests (t-test for comparisons and Spearman's rho for correlations, threshold for significant results was $p < 0.05$).

Results

Participants were matched for age and education; BMI was significantly lower in AN (see **table 1**).

the pressure to achieve exerted by the mother (see **table 2**), which was, on average, higher in the AN group.

Table 3 shows that AN and controls were significantly different in the scores measuring the coherence of mind (CoM) and of transcript (CoT), which were higher for the control group.

An additional scale measuring overall anger was obtained by taking the maximum score between Anger (Father) and Anger (Mother). No statistically significant difference was found for this scale (Anger, hereafter) between the two groups (AN = 2.7, Controls = 2.6; t = 0.112, df = 16, p = N.S.).

	Controls	*Anorexics*	*t*	*df*	*Sig. (2-tails)*
Age (years)	24 (2)	22 (4)	1.872	16	N.S.
BMI (kg/m^2)	21.1 (1.7)	15.9 (1.0)	7.971	16	< 0.01
Education (years)	16 (1)	15 (2)	2.054	16	N.S.
Disease duration (months)	-	16 (9)	-	-	-
Age of onset (years)	-	20 (4)	-	-	-

Table 1. *Demographic and clinical data. Uncorrected p values*

Distribution of States of Mind with respect to attachment

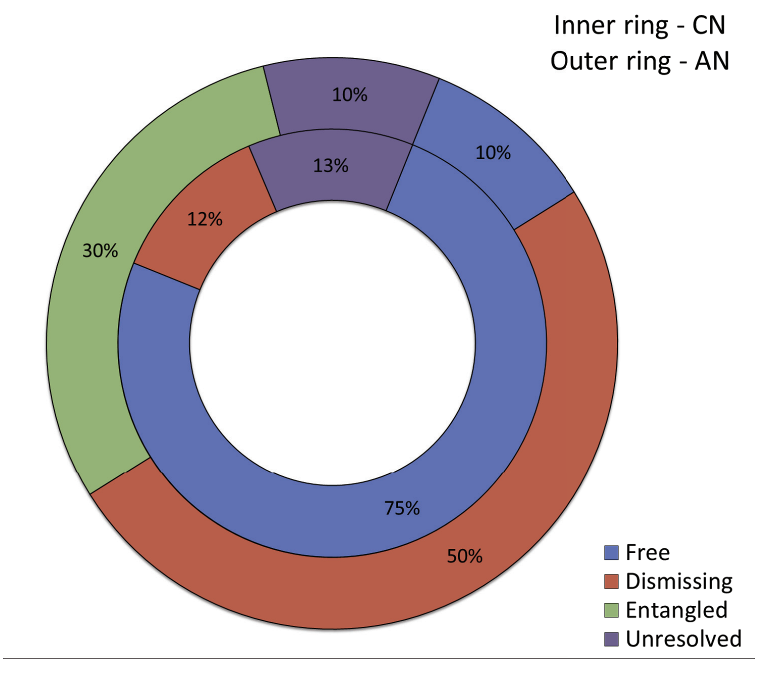
The distribution of AAI classifications is shown in **figure 1**. There was a significant difference in the AAI classifications between the clinical and non-clinical group ($p < 0.01$), and in healthy controls the Free state of mind was more frequent than in AN patients ($p < 0.01$).

AAI scales

The only significant difference between the groups (AN and controls) was in the AAI subscale measuring

Some subscales of AAI were found to be significantly correlated for our sample: in particular, we found that more loving mothers (LM) were perceived as lesser rejecting (RejM, ρ = -0.58, p = 0.02), less neglecting (ρ = -0.50, p = 0.03), exerted less pressure to achieve (PtAM, ρ = -0.73, p = 0.001) and were correlated with higher coherences of mind and of transcript (CoM: ρ = 0.64, p=0.004; CoT: ρ = 0.62, p = 0.006). Participants with an higher level of anger towards the mother also reported higher level of anger towards the father (ρ = 0.58, p = 0.01), higher passivity (Pas: ρ = 0.82, p < 0.001) and lower coherencies of mind and of transcript (CoM: ρ = -0.66, p = 0.003; CoT: ρ = -0.60, p = 0.004).

Figure 1. *Adult attachment classifications distribution in the clinical and in the control group*



Scales for attachment experiences	*Controls (N=8)*	*Anorexics (N=10)*	*t*	*df*	*Sig. (2 tailed)*
Loving (Father)	3.8 (1.0)	3.8 (1.2)	-0.066	16	N.S.
Loving (Mother)	3.7 (1.1)	2.9 (0.7)	1.856	16	*0.08*
Rejecting (Father)	3.8 (1.9)	4.0 (1.7)	-0.228	14	N.S.
Rejecting (Mother)	3.7 (2.1)	5.1 (1.7)	-1.433	14	N.S.
Involving/Reversing (Father)	1.9 (1.4)	3.0 (2.2)	-1.288	16	N.S.
Involving/Reversing (Mother)	3.4 (1.5)	3.3 (2.2)	0.080	16	N.S.
Pressure to Achieve (Father)	2.9 (2.4)	2.2 (2.2)	0.590	14	N.S.
Pressure to Achieve (Mother)	2.3 (1.5)	4.2 (2.1)	-2.090	15	**0.05**
Neglecting (Father)	5.0 (2.0)	3.3 (1.8)	1.803	16	*0.09*
Neglecting (Mother)	4.3 (1.7)	4.3 (2.5)	0.011	16	N.S.

Table 2. *AAI scales for attachment experiences. In bold significant p values, in italic p values < 0.1. Uncorrected p values*

AAI scales for States of Mind respecting the parents	*Controls (N=8)*	*Anorexics (N=10)*	*t*	*df*	*Sig. (2 tailed)*
Idealizing (Father)	2.2 (1.1)	3.0 (2.0)	-0.917	16	N.S.
Idealizing (Mother)	3.2 (0.7)	3.7 (2.0)	-0.818	11.98	N.S.
Anger (Father)	1.8 (1.1)	2.5 (1.5)	-1.025	16	N.S.
Anger (Mother)	2.6 (1.1)	2.1 (1.4)	0.758	16	N.S.
Derogation (Father)	1.0 (0.0)	1.1 (0.3)	-0.888	16	N.S.
Derogation (Mother)	1.0 (0.0)	1.1 (0.3)	-0.888	16	N.S.
AAI scales for Overall States of Mind					
Overall Derogation	1.0 (0.0)	1.3 (0.6)	-1.405	9	N.S.
Insistence on Lack of Recall	2.8 (0.7)	3.2 (2.3)	-0.545	11.14	N.S.
Metacognitive Processes	1.2 (0.3)	1.0 (0.1)	0.977	9.02	N.S.
Passivity	2.8 (1.4)	3.2 (2.0)	-0.442	16	N.S.
Unresolved Loss	3.6 (1.2)	2.8 (1.3)	1.113	14	N.S.
Unresolved Trauma	2.4 (1.1)	2.2 (1.6)	0.223	8	N.S.
Coherence of Mind	4.7 (1.9)	2.8 (1.1)	2.411	11.01	**0.03**
Coherence of Transcript	4.5 (1.8)	2.7 (1.2)	2.357	11.45	**0.04**

Table 3. *AAI scales for states of mind. In bold significant p values. Uncorrected p values*

Scores in the CoM and CoT scales were strongly correlated ($\rho = 0.97$, $p<0.001$), negatively correlated with Passivity ($\rho = -0.59$, $p = 0.01$ for both CoM and CoT) and (only as trend) correlated with Anger ($\rho = -0.45$, $p = 0.06$; for both CoM and CoT).

VBM analysis

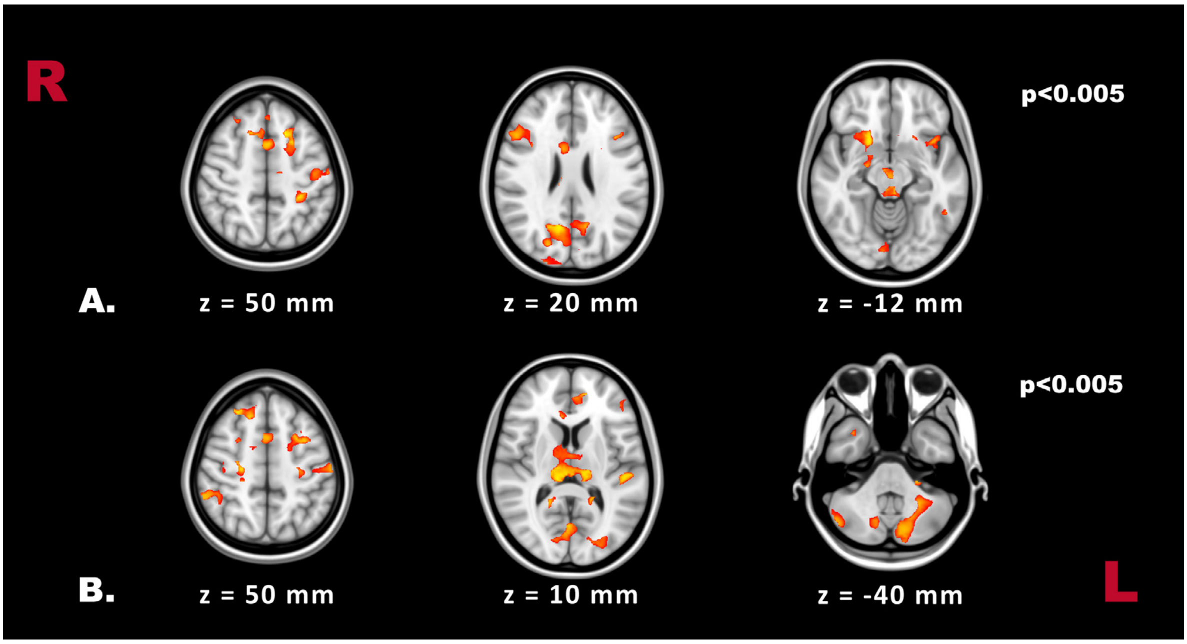
There was no difference between AN and controls. We analyzed the correlations and anticorrelation of all scales for GM volume; only correlation with '*positive*' value scales (LM, CoM and CoT) and the inverse correlation of the scales with '*negative*' value (Anger, Passivity) was found significant.

LM correlated positively (**figure 2A**) with a widespread network comprising: the bilateral insulae, the temporal poles, the amigdala, the right fusiform gyrus, the anterior and middle section of the cingulus, the thalamus, the precuneus, the precentral gyrus, the superior frontal gyrus, the middle frontal gyrus and the lingual gyrus. CoM and CoT correlated positively (**figure 2B**) with the temporal poles, the bilateral temporal gyrus, the right orbitofrontal cortex, the thalamus, the amygdala, the left fusiform gyrus, the left lingual gyrus, the precuneus, the cingulated cortex, the bilater supramarginal gyrus, the left middle frontal gyrus, the bilateral frontal inferior gyrus, the bilateral precentral gyrus, the left postcentral gyrus, the right frontal superior gyrus and the bilateral cerebellum.

Anger correlated negatively (**figure 3A**) with the bilateral basal ganglia, the right amygdala, the right middle temporal gyrus, the right inferior temporal gyrus, the bilateral lingual gyrus, the precuneus, the cingulate cortex, the left supramarginal gyrus and the bilateral somatosensory cortex.

Passivity correlated negatively (**figure 3B**) with the precuneus, the bilateral somatosensory cortex, the cingulate cortex, the bilateral lingual gyrus, the basal ganglia and the cerebellum.

Figure 2A. *Correlation between GM volume (AN = 10, controls = 7) and loving mother scale (LM)*
Figure 2B. *Correlation between GM volume (AN = 10, controls = 7) and coherence of mind scale (CoM)*



Threshold TFCE p < 0.005 uncorrected, cluster extent > 60. Radiological convention (left is right). L = left, R = right

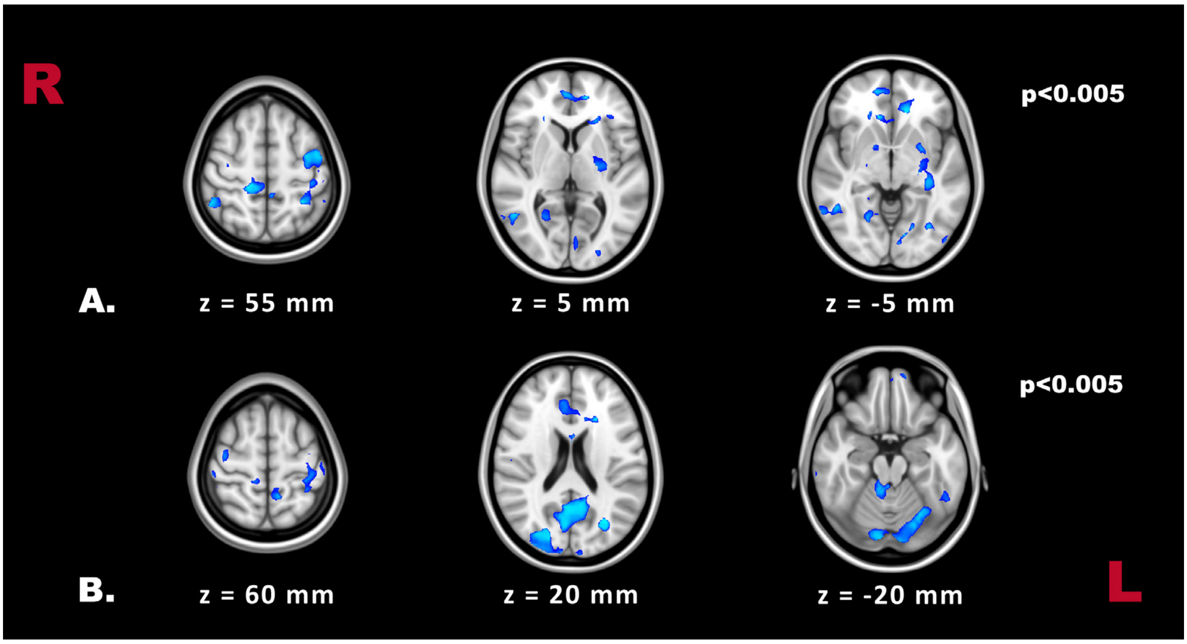
Resting state analysis

There was no difference between AN and controls and no correlation with the AAI scales, for both ALFF and standard deviation.

Discussion

The relationships between eating disorder and insecure attachment, between eating disorders and brain functionality and anatomy, as well as between insecure attachment and its possible neural substrates have been investigated singularly by a number of previous studies (i.e. Ward et al 2001, Lemche et al. 2006, Riem et al. 2012, Boghi et al. 2011). In this study we attempted to analyze in an unified perspective the relationships between all these three components of the picture, using behavioural (AAI) and neuroimaging (VBM and resting state analysis) tools. In particular, we used the AAI to assess mental representations of attachment in patients with AN and, in agreement with previous studies (Ward et al. 2001, Illing et al. 2010), we found that non-secure attachments are prevalent in this pathology, with a

Figure 3A. *Anticorrelation between GM volume (AN = 10, controls = 7) and Anger*
Figure 3B. *Anticorrelation between GM volume (AN = 10, controls = 7) and Passivity scale*



Threshold TFCE p < 0.005 uncorrected, cluster extent > 60. Radiological convention (left is right). L = left, R = right distribution of attachment patterns in our AN group comparable to what was previously reported in the literature (Ward et al. 2001).

The role of the mother in the onset and in the development of AN has been controversial, but has been recognized by previous studies and is further (Amianto et al. 2013a, Fassino et al. 2009, 2010) confirmed by our results, as the difference between AN and controls in the experienced *pressure to achieve* exerted by the mother and in the *love* received from the mother confirmed that there might be a link between mother-daughter relation during childhood and occurrence on AN later in life, an hypothesis already discussed by Ogden and Steward (2000). Our results are also in line with the findings of one recent study (Witkowska 2013) pointing out that young women with AN perceive their mothers as more demanding and rejecting and, more in general, with clinical experience and theories about eating disorders, postulating that whole family systems and their rules may have a great effect on the creation and maintenance of eating disorders (Gillet et al. 2009).

In the AAI coding system, the most accurate and final indicators of the speaker's state of mind with respect to the attachment are Coherence of Mind and Coherence of Transcript (Main et al. 2002). Poor CoM in patients with AN might represent a difficulty in creating narrative processes about their own psychological development, about self-awareness and the pathology in their life, and often some incoherencies on the report of their own experiences can be evidenced in the transcript of the interview (Tasca et al. 2011): it is therefore not surprising that scores in both CoM and CoT scales were significantly lower in AN than in healthy controls.

To the best of our knowledge, no previous study analyzed the relationship between attachment and GM volume. Indeed, the few studies that investigated the neural correlates of attachment (Buchheim et al. 2006, Lemche et al. 2006, Riem et al. 2012) used fMRI. Furthermore, while previous studies investigated the neural substrates of eating disorders or the link between attachment and anorexia (Ward et al. 2001, Zachrisson and Skårderud 2010), to the best of our knowledge, no previous study attempted to analyze together anorexia, attachment and brain anatomy and functionality.

In this study, we looked for the anatomical (VBM) and functional (resting state) correlates of attachment in young women with AN and of healthy controls. In contrast with previous works funding a decrease in GM volume in different cortical areas and in the cerebellum in anorexic patients (see Titova et al. 2013 for a review), we did not find any significant difference in brain volume between AN and controls. We believe that this result might be due to the small size of our sample and thus to a lack of sufficient statistical power.

To better identify the neural substrates of attachment and of anorexia in this study we also measured resting state brain activity using indexes such as ALFF and temporal standard deviation, testing the differences between AN and controls and the correlation with the scales of AAI. Partially in contrast with previous studies (Cowdrey et al. 2012, Amianto et al. 2013b) that found evidence of modifications of connectivity as determined by resting state measures, the present study found no difference between AN and controls, nor correlations with the AAI scales. As discussed before, the small size of the sample should kept into account and could be responsible for the lack of significant effects. Anyway, the lack of significant group effects means that the correlations between GM volumes and AAI subscales computed on the entire experimental population are not invalidated. As an additional explanation, we propose that measures such as ALFF are not indicated for correlation with behavioral variables, at least when investigating traits such as attachment organization. Future studies could confirm our hypothesis by recording during the same fMRI session both resting state activity and activation during an attachment-related test such as the AAP (Buchheim et al. 2006), testing participants with both secure or insecure attachment patterns and/or in groups composed of healthy controls and patients with eating disorders.

Some studies (such as Buchheim et al. 2006, Riem et al. 2012) indicated a possible network of cortical and subcortical areas involved in the attachment system. Among the proposed areas there are the amygdalae, the thalamus, the frontal cortex. In this study we found that GM volume of different brain areas correlate with the score of several subscales of the AAI. As mentioned before, the classification of attachment pattern is dependent on the scales assessing the overall present state of mind (CoM and CoT). In the present study we found that CoM and CoT positively correlate with the volume of GM in a network of brain areas comprising the temporal poles, both amygdalae, the midbrain, the thalamus and the anterior and middle sections of cingulate cortex, agreeing with the literature (Buchheim et al. 2006, Lemche et al. 2006, Benetti et al. 2010).

Non-secure attachments can be further classified in Dismissing (characterized by high levels of Idealization), Entangled-Preoccupied (characterized by higher Passivity and higher Anger than Dismissing) and Unresolved. In this study we found two different brain networks, linked to passivity and to anger. In particular, higher passivity correlated with lower GM volumes in mesial areas, such as the precuneus, that have been shown to be involved in autobiographical memory and auto-referential processes (Cavanna and Trimble 2006). Therefore, we propose that a lowered functionality can explain the behavioral aspects typical of passivity as the difficulty to have access to clear and coherent autobiographical memories often observed in people with Entangled attachment organization.

Finally, we found that in our sample two scales pertaining to the experienced relationship with the caregivers during childhood have a strong relation with the overall state of mind as assessed by the AAI. In particular, we found that the Loving Mother scale positively correlates with a network of brain areas very similar to the one linked to CoM and CoT, while Anger was found to be anticorrelated with the volume of parts of the same network (for instance, the precuneus and part of the limbic system). It seems therefore that the experience of love during childhood and the feeling of anger towards the caregivers expressed during the interview have opposite effects on these brain areas: while the experience of love seems to be a protective factor and almost trophic, anger is correlated with lower GM volume. However, even if the scores in the scale measuring the pressure to achieve exerted by the mother were significantly higher for patients with AN, we found no correlation between GM volume in any brain area and this scale. This could be due to the fact that in our study we found no difference in GM volumes between AN patients and controls.

While it can be argued that the evidenced network is not genuinely involved in the processes we hypothesized, and that the correlation between AAI scales and GM volume is merely due to the significant correlations between the AAI scales themselves, we can reject this hypothesis as we found no significant link between other scales (Anger Mother, Rejecting Mother) that correlated with CoM and CoT and the network we identified. However, it must be kept in mind that a single aspect of the attachment experience (such as a rejecting or non-loving mother) does not necessarily cause a non-secure attachment, as is for instance shown by the so-called "earned secure" attachments, that is the attachment of individuals who had negative early experiences that are more consistent with insecure attachment and yet have secure attachment orientation in adulthood (Grich 2002, Ardito et al. 2004, Adenzato et al. 2006).

We found that there is substantial overlapping between the areas we found to be linked to attachment and what has been reported in literature as areas with marked atrophy in anorexia nervosa (Boghi et al. 2011, Titova et al. 2013), such as the thalamus, the cingulate cortex and the amygdalae. Two hypothesis explaining the link between AN and reduced GM volume have been proposed: (i) that areas that are atrophic in AN are especially vulnerable to caloric restriction or (ii) that those areas were already hypofunctional before the onset of AN and therefore play a role in the pathogenesis of AN. Furthermore, the malfunctioning of these areas might in turn be connected to development and in particular to attachment experiences. While supporting the former or the latter hypothesis is outside of the scope of the present correlational study, we can stress that non-secure attachment is a core feature of anorexia nervosa both at behavioural/psychopatological and neurobiological levels and we believe that future studies should be aimed at further investigating this relationship using both behavioural and neuroimaging techniques.

Among the possible limitations of this study were the small sample size and the fact that in our sample all but one patient with AN had a non-secure attachment. Even if there is a strong link between AN and non-secure attachment pattern, future studies should try to avoid this possible confound by using a factorial design (2x2: secure/non-secure x control/AN) with a larger sample and therefore test the effect of the interaction between AN and attachment patterns.

To conclude, we argue that, as it has been shown in rodents and other mammals (Weaver et al. 2004, Kolb et al. 2012), receiving parental cares during the development is crucial for the healthy development of human brain. In our study, the love received from the mother has been shown to be a good indicator of secure attachment and an important factor in anorexia nervosa, and we propose that this link is mediated by the positive effects of growing up in a caring environment, which in turn can have a protective and trophic effect on brain areas shown to be linked to AN.

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