Title: A systematic review of resting-state functional-MRI studies in anorexia nervosa: evidence for functional connectivity impairment in cognitive control and visuospatial and body-signal integration.

Author: Santino Gaudio MD Lyle Wiemerslage PhD Samantha J. Brooks PhD Helgi B. Schiöth PhD

PII: S0149-7634(16)30230-5
DOI: http://dx.doi.org/doi:10.1016/j.neubiorev.2016.09.032
Reference: NBR 2618

To appear in:

Received date: 14-4-2016
Revised date: 11-8-2016
Accepted date: 30-9-2016

Please cite this article as: Gaudio, Santino, Wiemerslage, Lyle, Brooks, Samantha J., Schiöth, Helgi B., A systematic review of resting-state functional-MRI studies in anorexia nervosa: evidence for functional connectivity impairment in cognitive control and visuospatial and body-signal integration.Neuroscience and Biobehavioral Reviews http://dx.doi.org/10.1016/j.neubiorev.2016.09.032

Title: A systematic review of resting-state functional-MRI studies in anorexia nervosa: evidence for functional connectivity impairment in cognitive control and visuospatial and body-signal integration.

Authors: Santino Gaudio[1,2,*], MD, Lyle Wiemerslage[1], PhD, Samantha J. Brooks[1,3], PhD, Helgi B. Schiöth[1], PhD

[1]Department of Neuroscience, Functional Pharmacology, Uppsala University, BMC, Box 593, 751 24, Uppsala, Sweden.

[2]Centre for Integrated Research (CIR), Area of Diagnostic Imaging, Universita` ‘‘Campus Bio-Medico di Roma’’, via Alvaro del Portillo, 200 - 00133 - Rome, Italy**.**

[3]Psychiatry Neuroimaging Group (PNG), University of Cape Town, Department of Psychiatry and Mental Health, Old Groote Schuur Hospital, Anzio Road, Observatory, Cape Town. 7925, South Africa.

Corresponding author:

Santino Gaudio, MD

Address: Via Pietro Tacchini, 24

00197, Rome, Italy

Email: santino.gaudio@gmail.com, santino.gaudio@libero.it

Highlights:

- AN resting state fMRI studies used seed-based, ICA, and graph analysis approaches
- Some overlap in results between resting-state methods concerning AN symptom domains
- Equally distributed usage of methods, and differences in *a priori* assumptions
- Results suggest impaired cognitive control and visual/somatosensory integration

Abstract

This paper systematically reviews the literature pertaining to the use of resting-state functional magnetic resonance imaging (rsfMRI) in anorexia nervosa (AN), classifying studies on the basis of different analysis approaches. We followed PRISMA guidelines. Fifteen papers were included, investigating a total of 294 participants with current or past AN and 285 controls. The studies used seed-based, whole-brain independent component analysis (ICA), network-of-interest ICA based and graph analysis approaches. The studies showed relatively consistent overlap in results, yet little overlap in their analytical approach and/or *a-priori* assumptions. Functional connectivity alterations were mainly found in the corticolimbic circuitry, involved in cognitive control and visual and homeostatic integration. Some overlapping findings were found in brain areas putatively important in AN, such as the insula. These results suggest altered functional connectivity in networks/areas linked to the main symptom domains of AN, such as impaired cognitive control and body image disturbances. These preliminary evidences suggest that more targeted treatments need to be developed that focus on these two symptom domains. Further studies with multi-approach analyses and longitudinal designs are needed to better understand the complexity of AN.

Keywords: Eating disorders; Anorexia nervosa; Neuroimaging; Resting-state; Default mode network; Insula; Cognitive control; Body image disturbances.

1. Introduction

Anorexia nervosa (AN) is a severe mental disorder with the highest rate of mortality in all psychiatric disorders (Arcelus et al., 2013) and it typically affects adolescent girls and young women. AN is characterized by self-induced starvation and severe weight loss, an intense fear of weight gain or becoming fat, a distorted body image and food aversion (APA, 2013). To date, the aetiology of AN is not fully understood. However, a growing consensus suggests a multifactorial origin, in which neurobiological factors can contribute to the vulnerability, onset, maintenance and relapse of AN (Kaye et al., 2013; Treasure et al., 2015; Zipfel et al., 2015).

Modern neuroimaging techniques have provided important insight for AN pathophysiology and its neurobiological substrate. Both structural and functional neuroimaging techniques were used to explore brain abnormalities in AN patients and to try to encode the neural circuits involved in AN. Structural neuroimaging studies, via voxel based morphometry (VBM), showed that AN patients have decreases in global gray and white matter and increases in cerebrospinal fluid (Van den Eynde et al., 2012, Titova et al., 2013). Furthermore, a recent meta-analysis of VBM studies in AN patients (Titova et al., 2013) highlighted regional gray matter decreases overlapping between studies in specific areas: left hypothalamus, left inferior parietal lobe, right lentiform nucleus and right caudate – areas related to appetite and somatosensory perception. On the other side, functional neuroimaging studies have mainly used functional magnetic resonance (fMRI) utilizing specific stimuli related to main AN symptoms, such as food-related, body-related and reward-related tasks, as well as executive control-related tasks (Kaye et al., 2013, Gaudio & Quattrocchi, 2012). In particular, altered functional activations were found in cognitive control areas in response to food images (Brooks et al., 2011, Brooks et al., 2012), in the ventral anterior cingulate-striato-thalamic areas in response to cognitive-behavioural flexibility tasks (Zastrow et al., 2009), and in the posterior parietal areas and prefrontal cortex-insula network in response to tasks related to the

perceptive and the affective components of body image distortion respectively (Gaudio & Quattrocchi, 2012). On the basis of neuroimaging findings as well as psychological descriptions, a current neurobiological model of AN suggests that childhood temperament and personality traits (e.g. anxiety, cognitive inflexibility, obsessionality and perfectionism) might be a sign of neurobiological risk factors for the development of AN, and that altered eating patterns in those with AN could be a means of decreasing negative mood brought about by altered interactions between the serotoninergic system (i.e. aversive or inhibitory) and the dopaminergic (i.e. reward) system (Kaye et al., 2013; Zipfel et al., 2015; Treasure et al., 2015). Particularly considering neuroimaging findings, this neurobiological model suggests that AN patients may have an imbalance in information processing, linked to alterations of the ventral limbic circuits (which comprises amygdala, anterior insula, anterior ventral striatum, anterior cingulate cortex, and the orbito-frontal cortex), as well as the dorsal executive circuits (which particularly includes dorsal regions of the caudate, dorso-lateral prefrontal cortex, and parietal cortex). These two brain circuits are primarily implicated in inhibitory decision making processes and reward-related behaviours and their alteration might sustain AN symptomatology (Kaye et al., 2013). Other neurobiologically informed models of AN have pointed out the role of anxiety, stress, and fear, the gratifying nature of AN symptoms, and the consequent shift to habitual or compulsive behaviours as possible key factors in the persistence of AN (Zipfel et al., 2015; Treasure et al., 2015).

During the last decade, several research groups have used a stimulus-free fMRI approach, defined as resting-state fMRI (rsfMRI) (e.g. Fox and Greicius, 2010). In these studies, the participants are positioned in the scanner in an awake-state without performing any particular task. This method allows for making temporal correlations between brain areas, based on spontaneous task-independent fluctuations of the blood-oxygenation level dependent (BOLD) signal in the brain (Biswal et al., 2010; Greicius et al., 2009). RsfMRI considers the functional connectivity of brain areas, whereas traditional, task-dependent fMRI concerns activity of particular brain areas in

response to a stimulus. RsfMRI is a simple procedure, has a short scan time – on the order of minutes, and the reproducibility of results is robust within the same participant (Zuo et al., 2010). However, while rsfMRI is thought to reflect fundamental traits in personality or psychological function (Harmelech & Malach, 2013), some have criticized the ultimate usefulness of rsfMRI results (Morcom & Fletcher, 2007). Moreover, the debate on the role of unwanted thoughts in resting state functional connectivity is still open (Kuhn et al., 2013) and it could be a methodological concern in psychiatric disorders, due to the fact that several psychiatric disorders have intense forms of unwanted thoughts (e.g. obsessive compulsive disorder (Julien et al., 2007) and rumination on weight and body shape is present in AN (APA, 2013). While generalities of the basic methodology in rsfMRI would benefit from further standardization and optimization (e.g. scan while eyes closed/open, or awake/asleep), rsfMRI has regardless emerged as a staple tool in neuroimaging studies – perhaps equal in utility to task-based fMRI (Fox and Greicius, 2010).

A number of studies have used rsfMRI to investigate functional connectivity in patients with eating disorders. Several different approaches can be used in rsfMRI analysis. In particular, the most common are network-based and seed-based approaches (Fox & Raichle, 2007; van den Heuvel & Hulshoff Pol, 2010). Regarding the first approach, independent component analysis (ICA) is the most common analysis to investigate intrinsic neural networks, and it does not necessarily require an *a priori* hypothesis. ICA analyzes the entire BOLD signal and isolates different independent components putatively reflecting separate resting-state networks (RSNs) (Fox & Raichle, 2007; van den Heuvel & Hulshoff Pol, 2010). RSNs are defined as the sets of brain areas that show strong temporal coherence in the resting brain, and are thought to represent specific frameworks of brain function (Damoiseaux et al., 2006; Smith et al., 2009). A number of RSNs have been identified and investigated, and among these, the most studied network is the default-mode network (DMN) (eg. Raichle et al., 2001; Smith et al., 2009). The DMN involves areas such as the medial prefrontal cortex, the posterior cingulate/precuneus, hippocampus and the inferior parietal cortex (Raichle et

al., 2001). It is also known by alternative nomenclature, such as the task-negative network (TNN), or the hippocampal-cortex memory system (Vincent et al., 2008). The DMN is recruited during non-goal oriented cognitive activity, introspective rumination, low-level arousal, homeostatic- and self-regulation, such as during "day-dreaming," and supporting autobiographical, internally focused, declarative, episodic memory retrieval (Smith et al., 2009). The DMN is deactivated antagonistically and anti-correlated with the executive control network, which is a medial-frontal system including the anterior cingulate and para-cingulate cortex (Smith et al., 2009; Barkhof et al., 2014). The executive control network, or task-positive network, underlies executive functioning, such as working memory, goal-oriented cognition, impulse-control, and emotional processing (Smith et al., 2009; Barkhof et al., 2014). In addition to the DMN and executive control network, there are many other major canonical resting state networks that are frequently identified in the literature (for details see Smith et al., 2009; Barkhof et al., 2014). In particular, these networks include the sensorimotor network (i.e. striatal and parietal cortex), visual network (i.e. occipital cortex), cerebellum network (i.e. cerebellum), salience network (SN: frontal cortex, anterior cingulate and anterior insular cortex circuitry), and dorsal attention network (DAN: insular cortex and posterior parietal). The seed-based approach is based on an *a priori* hypothesis and it assesses functional connectivity between a preselected seed region and other brain regions (van den Heuvel & Hulshoff Pol, 2010). In brief, a functional connectivity map is extracted from the temporal correlations between the region of interest and all other brain regions (e.g. Lee at al., 2014).

Other rsfMRI approaches include graph analysis and effective connectivity. In graph analysis, the brain is considered a complex network consisting nodes (i.e. designated brain areas distributed across the whole brain) connected by edges (i.e. the functional relationship between nodes), which the underlying activity over time dividing and organizing brain networks within the field of nodes (eg. Ribinov & Sporns, 2010, Geisler et al., 2015). Graph analysis assesses the global and local properties of brain regions and analyzes a number of global and nodal metrics, such as the average

path length between all pairs of nodes, and the sum of the edges' weights that connects to a given node, called degree centrality (DC). Two other common techniques include dynamic causal modelling (DCM) and Granger causality, which both calculate effective connectivity (Friston, 2011). In brief, the effective connectivity assesses the causal and dynamic influence of one region on another, as well as the directionality of information flow between brain regions (e.g. Kullmann et al., 2014).

Currently, the literature on rsfMRI studies in AN seems to point to a lack of consistency in the analytic approaches and, subsequently, also the findings. The aim of this paper, therefore, is to systematically review studies using rsfMRI in patients suffering from AN and explore whether rsfMRI adds useful insight in understanding of AN pathophysiology. In pursuit of this aim, we also compare the results and subsequent interpretations from the different rsfMRI approaches to determine if there is consensus in the brain areas/networks affected. Lastly, we shall discuss the relevant themes and future considerations regarding the ultimate usefulness of rsfMRI in AN research and treatment.

2. Methods

We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (Moher et al., 2009). The statement consists of a checklist of recommended items to be reported (Appendix A: PRISMA check list) and a four-step flow diagram (Appendix B: PRISMA flow diagram).

2.1. Search strategy and inclusion criteria

Databases used for the search were: PubMed (from inception to May 2016) and Scopus (from inception to May 2016). We searched using the terms: ‘‘anorexia nervosa’’ or ‘‘eating disorders’’

AND “fMRI”, “resting state”, or “functional connectivity”. The reference lists of examined full-text papers were scrutinised for additional relevant publications. In addition, expert colleagues in the field were contacted for suggestion on further studies not considered in our search.

To be included in the review, studies were required to: 1) be written in English, 2) investigate a sample of participants with current or recovered from AN in cross-sectional case-control or longitudinal design, 3) investigate brain functionality at rest. Studies using tasks or stimuli (e.g. hunger state) during the MR scanning sessions were excluded. Due to the limited number of peer-reviewed rsfMRI studies on AN, we did not consider confounding factors such as sample inhomogeneity (e.g. AN subtypes), presence of psychiatric comorbidity or pharmacological history, which may limit some of the studies included. The heterogeneity of rsfMRI approaches and lack of sufficient number of papers using similar approaches or similar seed or networks of interest do not allow us to perform a meta-analysis.

2.2. Quality assessment and data abstraction

To reduce a risk of bias, PRISMA recommendations for systematic literature analysis have been strictly followed. Two authors (S.G. and L.W.) independently selected paper abstracts and titles, and analyzed the full papers that met the inclusion criteria, resolving disagreements through consensus.

Data extracted from each study were: sample type, study design, sample size, resting-state fMRI approaches, and selected findings.

3. Results

Fifteen resting-state fMRI studies of participants with current or past AN were included in the review (Appendix B: PRISMA flow diagram). Overall, the studies included a total of 294

participants with current or past AN and 285 control participants. Table 1 reports the sample characteristics of each AN study. Of the fifteen studies included in this review, four studies used a seed-based approach and eleven a data-driven approach (Table 2). Of the eleven data-driven approach studies, two studies adopted a whole brain ICA based approach, five studies used a network of interest ICA based approach, and four studies adopted graph analysis (Table 2). In the following, studies that applied seed-based, whole brain ICA based, network of interest ICA based, and graph analyses will be summarized. Fig. 1 reports the main findings of the rsfMRI studies in AN.

3.1. Seed-based approach Studies (n=4)

The seed-based approach is the most common method to examine functional connections of an *a priori* selected brain region with all other regions (van den Heuvel & Hulshoff Pol, 2010). The region of interest can be selected from previous, task-dependent fMRI results or it can be an *a priori* defined region. Four rsfMRI studies adopted a seed-based approach (Lee et al., 2014; Favaro et al., 2013; Biezonski et al., 2015; Collantoni et al., 2016) each selecting different seeds (Table 2). Lee and colleagues (2014) enrolled a sample of adult AN outpatients (18 AN, 20 BN and 20 controls) utilizing a cluster within the dorsal anterior cingulate cortex (dACC) as the seed region. This area was selected based on their previous fMRI study of AN patients showing altered functional activation in response to high-calorie food images (Kim et al., 2012). The seed analysis showed that AN patients had a stronger synchronous activity between the dACC and retrosplenial cortex and between the dACC and precuneus compared to controls. Furthermore, both groups of eating disordered patients (AN + BN) demonstrated stronger synchronous activity between the dACC and precuneus compared to controls. In a second study, Favaro and colleagues (2014) scanned an adult subsample of their participants, which comprised 33 AN patients and 30 controls. Three bilateral seeds were selected: dorsolateral prefrontal cortex (DLPFC), ventromedial PFC (vmPFC), and ventrolateral PFC (vlPFC). No significant differences were found between AN patients and

controls. However, when analyzing differences within the AN group on the basis of genotypes for functional polymorphisms of the catechol-O-methyltransferase (COMT) protein, functional connectivity differences were found in the prefrontal seeds between the Met- and Val- carrier subsamples. The third study by Biezonski and colleagues (2015) examined a clinical sample of 16 late adolescent and adult AN inpatients and 15 controls, and tested seven bilateral thalamic seeds. Each seed corresponded to a region of the thalamus with preferential connections to a specific lobe or cortical region (Johansen-Berg et al, 2005) and functional connectivity analyses were focused on thalamic connectivity with the frontal lobes. AN patients had greater connectivity between the centralmedial thalamus and the bilateral DLPFC and lower functional connectivity between the anterior thalamus and the left anterior PFC compared to controls. While the alterations in thalamo-frontal connectivity were not associated with attention and visuospatial processing, they were associated with impairments in performance on tasks assessing cognitive control and working memory. Most recently, Collantoni and colleagues (2016) examined an adult subsample that comprised 35 AN patients and 34 controls. They selected three different seeds: superior parietal lobule, right inferior frontal gyrus and pre-supplementary motor area, considering them as part of the dorsal attention, ventral attention, and pre-supplementary motor area networks respectively. The authors found that AN patients showed altered functional connectivity in the right inferior frontal gyrus compared to controls.

In sum, given that the seed-based rsfMRI studies use different brain region seeds, comparisons between the studies are limited. Nevertheless, functional connectivity alterations were found between dACC and the precuneus, in thalamus-frontal circuits, and in the right inferior frontal gyrus – regions associated with cognitive control processes and rumination on weight and body shape.

3.2. Whole brain ICA based Studies (n=2)

The approach most utilised to examine whole-brain connectivity patterns is ICA (Fox & Raichle, 2007; van den Heuvel & Hulshoff Pol, 2010). This approach does not require an *a priori* seed region, hence, is data-driven, and allows general patterns of connectivity between several brain regions to emerge based on temporal synchronicity (van den Heuvel & Hulshoff Pol, 2010). ICA then compares these patterns between groups. Since its development, rsfMRI studies via ICA have robustly described several different and widely accepted resting-state networks (Damoiseaux et al., 2006 and Smith et al., 2009), which have led to the network-of-interest based approach (see section below).

Two rsfMRI studies investigated whole brain functional connectivity via ICA, focusing on the most widely accepted resting-state networks (Cowdrey et al., 2014; Gaudio et al., 2015) (Table 2). The first study was conducted on adult participants recovered from AN (16 past AN, 15 controls) (Cowdrey et al., 2014). The AN patients had recovered by at least one year. Twelve resting-state networks were identified, corresponding to previously-described RSNs (Smith et al., 2009). Altered functional connectivity was found within the default mode network (DMN). In particular, AN recovered participants showed increased resting-state functional connectivity between the DMN and the precuneus and the DLPFC/inferior frontal gyrus compared to controls. The second study was conducted on a sample of medication-naïve adolescent outpatients at the earliest stages of AN (less than 6 months of AN duration) and with no psychiatric comorbidity (Gaudio et al., 2015). Eight resting-state networks were indentified and all of them corresponded to previously reported significant RSNs (Smith et al., 2009). The functional connectivity maps showed alterations within the executive control network. Specifically, decreased functional connectivity was found in AN patients compared to controls between the executive control network and in a region of the ACC close to the border of he paracingulate gyri.

To sum up, these two studies, while using the same approach (whole-brain ICA), investigated samples that differed in age (i.e. adults vs adolescents) and stage of AN (recovery state vs earliest

stages of AN). The findings of the two studies implicated two different networks: the executive control network and DMN. In particular, decreased functional connectivity between the executive control network and the ACC was seen in the earliest stage of AN, brain regions associated with cognitive control and emotional processing which are perhaps altered neural circuits in early stages of the disorder. Conversely, increased functional connectivity between the DMN and the precuneus and the DMN to the DLPFC/inferior frontal gyrus was seen in adults whom had recovered from AN, a possible residual neural pattern associated with altered attentional and cognitive control resources.

3.3. Network of interest ICA based approach Studies (n=5)

As mentioned above, a number of robust and widely accepted RSNs have been identified using ICA (Damoiseaux et al., 2006; Smith et al., 2009). Building on this, another rsfMRI approach uses established RSNs as *a priori* networks-of-interest similarly as in the seed-based approach (e.g. Favaro et al., 2012). Five rsfMRI studies adopted this approach investigating one or more resting-state networks of interest and focusing on AN patients (Amianto et al. 2013; Boehm et al., 2014; Phillipou et al., 2016), participants with current or past AN (Favaro et al.,2012), or only participants recovered form AN (Boehm et al., 2016) (Table 2). The first by Favaro and colleagues (2012), focused on four network of interest: visual networks (i.e. medial, lateral, and ventral) and one somatosensory network, hypothesizing an impairment of multisensory integration processing in AN. They scanned a sample that was composed of AN patients (N = 29), AN recovered participants (disease free for at least 6 months. n = 16) and controls (n = 26). The authors found that AN patients showed altered functional connectivity within the ventral visual network and the somatosensory network and that AN recovered participants only showed functional alterations within the ventral visual network. Specifically, there was decreased functional connectivity between the ventral visual network and the left occipitotemporal junction and increased functional connectivity between the somatosensory network and the left superior parietal cortex in AN patients compared to controls.

Decreased functional connectivity between the ventral visual network and the right middle frontal gyrus was also found in AN recovered participants compared to controls. In the second study, Amianto and Colleagues (2013) scanned a sample composed of 12 adult AN outpatients, 12 adult BN patients and 10 controls, investigating the cerebellar network. The authors found a number of differences between the groups. In particular, AN outpatients showed increased functional connectivity between the cerebellar network and the vermis, left insula, bilateral temporal pole, and posterior cingulate cortex and decreased functional connectivity between the cerebellar network and parietal lobe compared to controls. BN patients and the mixed group of patients (AN+ BN) showed partially similar results compared to controls. In addition, BN patients showed increased functional connectivity between the cerebellar network and lateral hemispheric areas of the cerebellum, ACC, and precuneus as well as decreased functional connectivity between the cerebellar network and the right inferior frontal gyrus. A comparison between the AN and BN groups revealed AN patients had increased functional connectivity between the cerebellar network and the bilateral anterior insula, precuneus, and right inferior frontal gyrus, as well as decreased functional connectivity in the cerebellar hemispheres and ACC. The third study by Boehm and colleagues (2014), focused on five resting state networks: the DMN, the salience network, the fronto-parietal network, the visual network, and somatosensory network, to investigate some important domains that may be of relevance to AN clinical symptoms (e.g. self-referential processing, rewarding and emotional stimuli processing, visual and somatosensory information processing). The authors studied a sample of adolescent and young- adult patients with AN (n = 35) and a control sample (N = 35). Differences were found within the DMN and the frontal-parietal network. Specifically, AN patients showed increased functional connectivity between the DMN and the left anterior insula/frontal operculum and increased functional connectivity between the frontal-parietal network and the left angular gyrus compared to controls. A later study by Boehm et al., 2016, tested 31 women recovered from AN against 31 age/sex-matched controls. Three networks (i.e. the default mode, frontal-parietal, and salience netwroks) were tested, investigating within and between resting state

functional connectivity and functional network connectivity. No differences were found in the DMN, but the frontal-parietal network had reduced connectivity with the dorsolateral prefrontal cortex in the recovered AN participants compared to control. As was also reported in patients with active AN (Bohem et al., 2014), a further hypothesis-driven analysis again found increased connectivity between the frontal-parietal network and the left angular gyrus in the participants recovered from AN. Finally, no differences were found in functional network connectivity. The fifth study by Phillipou et al., (2016) investigated the DMN, sensorimotor network, and visual network in 26 females with AN and 27 age/sex-matched controls. While this study technically was not ICA-based (but ROI-to-ROI seed-based type), we have included it here, as it limited its analysis to functional networks. No differences were found in the DMN, but reduced functional connectivity was found between the sensory-motor and visual networks in AN patients compared to controls.

In sum, and similarly as in the seed-based approach mentioned above, the network-of-interest ICA based studies did not always use the same *a priori* networks of interest, limiting an aggregation of findings, and also comparison between studies. In particular, altered functional connectivity was found in AN patients within and between the somatosensory and visual networks and within the default mode, fronto-parietal and cerebellar networks. On the other hand, altered functional connectivity was found in participants recovered from AN in the default mode, fronto-parietal and visual networks. However, these results were partially discordant between both the rsfMRI studies on AN patients and participants recovered from AN. In addition, differences between AN and bulimia nervosa patients were found in the cerebellar network functional connectivity in the only study which also recruited bulimia nervosa patients. Combined, this suggests that networks implicated in cognitive control and visual and somatosensory integration may be affected in AN and that these alterations could partially persist in participants recovered from AN. Furthermore, it can be preliminary suggested that rsfMRI may be useful for differential ED types.

3.4. Graph analysis Studies (n=4)

Graph analysis is similar to whole-brain ICA analysis in that both are emergent, data-driven methods, which do not rely on *a priori* information. Graph analysis builds a network of nodes (i.e. designated brain areas distributed across the whole brain), such as a grid of arranged nodes, and creates weighted edges between nodes to describe functional connectivity between brain regions. Here, we examine four studies utilizing such techniques (Kullmann et al., 2014; Ehrlich et al., 2015; Geisler/Borchardt et al., 2015; Lord et al., 2016) (Table 2). The method employed by Kullmann and colleagues (2014) built a network based on nodes with the most numerous, statistically-significant edges (i.e., degree centrality). These highly-connected regions were then compared between three different groups (all young adult females): 12 AN patients, 14 controls, and 12 athletes. Reduced connectivity was found within the inferior frontal gyrus (IFG) bilaterally for AN patients compared to controls. There were no differences between controls and athletes. The authors also performed a seed-based analysis using the IFG as the *a priori* region. They further found decreased connectivity between the right IFG and bilateral orbital frontal gyrus, and increased connectivity between the left IFG to the bilateral insula in AN patients compared to controls. Again, no differences were found between controls and athletes. The next study by Ehrlich and colleagues (2015) tested 35 AN patients and 35 age-matched controls (all female, ranging from early teenager to young adult). Networks were built by picking the cluster with the most numerous, statistically-significant nodes/ROI based on resampling statistics. Compared to controls, they found decreased connectivity in the AN group for a network of seven nodes with regions including the left amygdala, left thalamus, right fusiform gyrus, bilateral putamen, and bilateral posterior insula. Geisler/Borchardt and colleagues (2015) tested the same cohort using an initial graph of 160 nodes (arranged by meta-analysis from fMRI (Dosenbach et al, 2010), and searched for networks in each participant by recursively removing edges until all edges met statistical thresholds. Quantitative measurements of the networks, as well as of individual nodes, were then compared between groups. In terms of the network, the AN group had increased average path length (sum of edges) compared to the controls,

indicating an inefficient network. More specifically, at the nodal level, AN patients had increased path length in the left middle insula, right posterior insula, and bilateral thalamus. AN patients also had low local efficiency in a right posterior occipital node, and increased efficiency in a node within the right anterior prefrontal cortex, however, these differences in local efficiency seemed unstable. A final graph-based rsfMRI study again examined the same cohort from Ehrlich et al., 2015 and Geisler/Borchardt et al., 2015, comparing methodological strategies for graph analysis (Lord et al., 2016). In this most recent work, Lord et al. compared graph analysis between the AN patients and controls with two different bases for analysis: 1) an anatomical-based analysis where activity in regions was assessed based on traditional, anatomically-defined regions (e.g., insula, cingulate, etc.), and 2) a calculation-based analysis where activity was measured in the same 160 spherical ROI nodes assigned as mentioned above (and established by Dosenbach et al., 2010). The two schemes converged on only 5 out of 20 regions when detecting differences between AN patients and healthy controls, with the anatomical analysis finding differences in parietal and cingulate regions and the node-analysis finding differences in the insula and thalamus. The authors conclude that the most prominent differences in activity between AN patients and controls are robust – regardless of the scheme used for analysis. However, the authors recommend that an *a priori* selection of regions, specific for a disease, are likely more useful than arbitrary or random assignments.

In summary, partially consistent results were found for the graph analysis studies. Functional connectivity alterations mainly involved the insula and thalamus as well as the inferior frontal gyrus, amygdala, fusiform gyrus, or putamen. In particular, the last study by Lord et al., which compared two types of graph analyses based on either an anatomical or a calculated basis, generally echoes the differences found in our comparisons of the *a priori* and emergent, data-driven analyses: while each type of analysis indeed detects differences between AN and controls at the most basic level, the details can be variable. From all this, more standardization of resting-state methods,

particularly graph analysis, is required. Regardless, the areas found to be related to AN in the above studies can all reasonably be linked to the symptomology of AN regarding somatosensory/visual integration processes and cognitive control.

4. Discussion

4.1. Main findings

The present paper is the first, to our knowledge, to systematically review resting-state functional magnetic resonance imaging (rsfMRI) studies conducted with anorexia nervosa (AN) patients. Although the use of rsfMRI in AN research is rather new, the number of published studies indicates an increasing interest in this neuroimaging technique among groups of research. The growing interest among researchers is related to the fact that rsfMRI is a simple procedure that can improve the understanding of psychiatric disorders (Barkhof et al., 2014). In this systematic review, we included 15 papers using different resting-state approaches (i.e. seed-based, whole brain ICA-bases, network of interest ICA-based, and graph analysis studies), with a total of 294 participants with current or past AN and 285 control participants. Overall, studies showed relatively consistent overlap in results (see Table 2 and Figure 2), specifically involving the corticolimbic circuitry, which helps to form a clinical perspective on the underlying neurobiological links to AN symptoms. On the other hand, the studies had little overlap in their analytical approach and/or *a-priori* assumptions. The seed-based studies focused on different seeds (see table 2) and showed functional connectivity differences between AN and HC in the dACC (Lee et al., 2014), the Thalamus (Biezonski et al., 2015), and the right inferior frontal gyrus (Collantoni et al., 2016). Moreover, the network-of-interest ICA-based studies focused on different networks (e.g., visual, somatosensory, cerebellar, fronto-parietal, default mode, and salience networks) in both AN patients (Favaro et al., 2012; Amianto et al., 2013; Boehm et al., 2014) and participants recovered from AN (Favaro et al., 2012; Boehm et al., 2016); all of which found altered functional connectivity within different

networks (see Table 2). In particular, a recent study, via a ROI to ROI approach, focused on default mode, visual, somatosensory networks and found decreased functional connectivity between the sensory-motor and visual networks in AN patients compared to controls (Phillipou et al., 2016). However, results were inconsistent between studies investigating the same networks (i.e. DMN and visual networks) (Favaro et al.,2012; Boehm et al., 2014; Phillipou et al., 2016). Regarding the visual networks, while Favaro and colleagues (2012) found decreased functional connectivity within the ventral visual network in both AN patients and recovered AN participants, Boehm and colleagues (2014) did not find differences in this network and Pilippou and colleagues only found alterations between the visual and somatosensory networks in AN patients. Regarding the DMN, increased functional connectivity was found in AN patients (Bohem et al., 2014), but no differences were found in participants recovered from AN (Bohem et al., 2016) or when adopting a ROI to ROI approach in AN patients (Phillipuo et al., 2016). Furthermore, the two data-driven approaches, which do not have *a priori* hypotheses (whole-brain, ICA-based and graph analysis), also showed different results. The two studies that used a whole-brain ICA-based approach showed functional connectivity alterations in the DMN in participants recovered form AN (Cowdrey et al., 2014) and executive control network in adolescent patients with AN (Gaudio et al., 2015), respectively. And the four studies that adopted graph analysis found alterations involving either the inferior frontal gyrus (Kullmann et al., 2014), insula (Ehrlich et al., 2015; Lord et al., 2016), or thalamus (Geisler et al., 2015; Lord et al., 2016).

The discordant results between studies may partly be due to practical limitations such as differences between cohorts: e.g. age, eating disorder duration, current or past pharmacological treatment, psychiatric comorbidity, prandial state. In particular, severity and duration of AN are well-known confounding factors in AN neuroimaging studies due to the impact of malnutrition on neural processing (Kaye et al., 2013). Thus, sample heterogeneity could explain some differences in this analysis. For example, the samples in two whole-brain ICA-based studies were composed of either

adult participants recovered from AN (Cowdrey et al., 2014) or adolescent patients in the early stages of AN (Gaudio et al., 2015), while the samples of the four graph-based studies included adults and a mixed sample of adults and adolescent patients with unreported durations of AN (Kullmann et al., 2014; Ehrlich et al., 2015; Geisler et al., 2015; Lord et al., 2016). The difference in resting-state approach is another confounding factor, as even studies using the same cohort found different results using different methods (Boehm et al., 2014; Ehrlich et al., 2015; Geisler et al., 2015). This is expected to some extent, as some rsfMRI studies (such as network-of-interest, ICA-based) are limited to specific areas/networks and have very specific testing hypotheses (e.g. Amianto et al., 2013). Results of seed-based studies, as well, are limited to the *a priori* regions chosen for analysis, and different seed regions may produce better overlapping results with the emergent, whole-brain ICA and graph-based approaches. In addition, it cannot be excluded that differences may be partially due to unwanted thoughts that can occur during the scan (Kühn et al., 2014) and could be related to AN symptoms (e.g. rumination on food and body shape) (APA. 2013), that are currently unexplored in rsfMRI studies.

4.2. AN symptom domains and etiological and clinical insight: overlap in results between the rsfMRI studies

Despite the differences among the findings of the reviewed rsfMRI studies, the results show functional alterations in networks and/or areas related to the main symptom domains of AN: impaired cognitive control and flexibility, and impaired visual and somatosensory integration, which can sustain ruminations about food/eating and body image disturbances (see Table 2). In particular, the different seed regions used (i.e. dACC, inferior frontal gyrus, thalamus) are all involved in cognitive control circuits (Lee et al. 2014, Biezonsky, 2015, Collantoni, 2016), and some of these areas are also part of well-known resting state networks, such as the executive control network (i.e. ACC) and ventral attention network (i.e. inferior frontal gyrus). Taken together, seed-based findings seem to describe excessive top-down control, which has previously been implicated

in the neuropathology of AN (Kaye et al 2011; Brooks et al., 2012; Brooks, 2016). Furthermore, altered fronto-parietal network alterations, a brain network involved in cognitive control functions, were found in both AN patients (Bohem et al., 2014) and participants recovered from AN (Bohem et al., 2016). These resting-state findings may underlie the impaired cognitive control of appetitive processes as well as ruminations concerning the self and body image. Furthemore, it is of interest that altered functional connectivity of the fronto-parietal network persists in participants recovered from AN, suggesting persistent alteration of cognitive control functions (Bohem et al., 2016). This is of note for the clinician, as altered activation at rest of the executive control and/or frontro-parietal areas may be a significant biomarker for disease and recovery as well. This suggestion is in line with current neurobiological models of the development and maintenance of AN (e.g. Treasure et al. 2015; Zipfel et al., 2015), particularly implicating dysfunctional activation of cognitive control networks (Kaye et a., 2013; Brooks, 2016).

On the other hand, alterations were found between the visual and somatosensory networks (Phillipuo et al., 2016) and within the visual, somatosensory (Favaro et al., 2012) and occipital networks (Amianto et al., 2013) in three network of interest ICA-based studies. These networks are also related with integration process of visual and somatosensory signals (Smith et al., 2009; Barkhof et al., 2014) and may be involved in body image disturbances (Favaro et al., 2012, Phillipuo et al., 2016). These brain networks/areas partially also overlap with the findings of fMRI studies that used tasks related to body in AN patients (Gaudio and Quattrocchi, 2012) and seem to confirm the complexity of this puzzling symptom of AN (Zipfel et al., 2015, Treasure et al., 2015, Dakanalis et al., 2016).

Some areas overlapped between methods: insula (network-of-interest ICA, graph analysis) (Boehm et al., 2014; Ehrlich et al., 2015; Geisler et al., 2015; Lord et al., 2016), and thalamus (seed based, graph analysis) (Biezonski et al., 2015; Ehrlich et al., 2015; Geisler et al., 2015; Lord et al., 2016) (also see Figure 2). The insula showed functional connectivity alterations as part of the DMN (Boehm et al. (2014) as well as nodes in the graph analyses (Ehrlich et al., 2015; Lord et al., 2016).

The DMN is mainly involved in self-reflection (Raichle et al., 2001) (for network details also see the introduction paragraph). The insula is functionally connected with multiple areas (also see table 2) and is mainly involved in interoception of bodily feelings and sensorimotor integration (Craig et al., 2002; Simmons et al., 2013). The rsfMRI results discussed further implicate the insula in AN and its body-image disturbances via several functional connections (see Table 2, main interpretation column), which are also observed in some task-related fMRI studies (Gaudio and Quattrocchi, 2012, Frank et al., 2016). The thalamus showed altered functional connectivity both as an explored seed region (e.g. altered connectivity between thalamus and prefrontal cortex) (Biezonski et al., 2015) and as a node in graph analysis (Geisler et al., 2015; Lord et al., 2016). The thalamus is comprised of multiple distinct nuclei with many patterns of anatomical connectivity that are seldom reported (Zhang et al., 2008, 2010). In particular, the listed rsfMRI studies found altered thalamo-prefrontal cortex connectivities, mainly involved in cognitive and executive processes (Biezonski et al., 2015). Furthermore, altered functional connectivity was found in a subnetwork that also includes the posterior insula and is speculated to be a source of impaired integration between visuospatial and somatosensory signals in AN patients (Ehrlich et al., 2015; Geisler et al., 2015; Lord et al., 2016). Also considering the above reported alterations of visual and somatosensory networks, these resting-state findings suggest that several brain regions could be involved body image disturbances and may sustain an impaired integration between real and perceived internal/external state of one's own body in AN patients (Favaro et al., 2012; Gaudio & Riva, 2013; Ehrlich et al., 2015; Geisler et al., 2015; Phlippou et al., 2016; Lord et al., 2016), that may partially persist after AN recovery (Favaro etal., 2012). Further studies with multiple approaches, for example rsfMRI and diffusion tensor imaging, are needed to better clarify the role of these functional connectivity alterations and their role in AN pathogenesis and complex AN symptomatology.

While specific pathways between regions were generally not replicated between the different studies and approaches, these altered areas/networks which differ between studies are related to AN symptomatology and offer useful etiological and clinical insight. In particular, two ICA-based rsfMRI studies (whole-brain and network-of-interest analyses) found altered functional connectivity within the DMN for participants recovered from AN (Cowdrey et al., 2014) and those with a current AN diagnosis (Boehm et al., 2014), although there were differences between the affected areas (see table 2), while no differences were found in this network in two subsequent network of interest ICA-based study on and patients with AN (Philippou et al., 2016) and participants recovered from AN (Bohem et al., 2016) respectively. These findings suggest that the DMN is pertinent to consider in terms of the neuropathology of AN, because it is perhaps low-level arousal (linked to appetitive and affective processes for example) that is being excessively controlled and that also drives self- and body-focused ruminations (Bohem et al., 2014). On the other hand, only functional connectivity alterations within the executive control network (i.e. decreased functional connectivity between the executive control network and the anterior cingulate cortex) were found at the earliest stages of AN (in whole-brain, ICA-based analysis) (Gaudio et al., 2015). Interestingly, altered functional connectivity of the anterior cingulate cortex was found using this area as a seed region (Lee et al., 2014) (also see above and Table 2). The five network-of-interest ICA based studies did not investigate the executive control network (Favaro et al., 2012, Amianto et al.., 2013; Bohem et al., 2016; Phillipou et al., 2016; Bohem et al., 2016) and two of them did not investigate the DMN (Favaro et al., 2012, Amianto et al.., 2013). These results suggest that the DMN and executive control network, for example, may be differentially affected depending on the time-course of the disease state, such that networks related to cognitive control and emotional processing may be involved within the early stages of AN, while the DMN, mainly involved in self-referential processing, may display long-term dysfunction. In particular, the three previously reported ICA-based studies with participants recovered from AN [adopting a network of interest (Favaro et al., 2012; Bohem et al., 2016) or a whole brain (Cowdrey et al., 2015) ICA-based approach] showed

different results: altered functional connectivity was found within the DMN (Cowdrey et al., 2015), the visual network (Favaro et al., 2012), and the fronto-parietal network (Boehm et al., 2016). These findings suggest that functional connectivity may be differently affected after recovery from AN and that the different alterations could represent possible neurobiological traits and/or scar effects of AN. These last points speak to the need of longitudinal studies with a data-driven approach (i.e. ICA whole-brain and graph analysis) to confirm these findings and to investigate the different stages of AN and its recovery.

5. Conclusions

In conclusion, the variety of rsfMRI approaches seem to effectively describe the neural phenomena of AN, although the variation of rsfMRI approaches and the relatively low number of AN studies in comparison to other fields makes definitive assumptions untenable at present. Each of the resting-state approaches listed seemed to adequately quantify the trends of increased/decreased functional activity, showing several functional alterations in the networks and/or areas mainly involved in cognitive control and visual and homeostatic integration, with at least some overlap in brain areas/networks putatively important in AN, such as the insula.

Overall, rsfMRI studies provide preliminary evidence of brain alterations at rest in networks/areas involved with the main symptom domains of AN both in patients with current AN and in participants recovered from AN: cognitive inflexibility and altered processing/integration of body signals. The functional brain alterations related to cognitive control could sustain rumination on food and weight and the functional brain alterations related to body signal integration/processing could sustain the complex body image disturbances of AN, with a possible interconnection between the two domains. These findings suggest developing more targeted treatment strategies focused on the two symptom domains of AN.

Sample heterogeneity and methodological differences are likely the most important factors for the differences between studies. It remains crucial to improve our understanding of altered functional connectivity at rest in AN and its involvement in complex AN symptomatology. Multicentre studies with larger and homogeneous samples and with longitudinal designs are needed to better clarify the role and the nature of functional connectivity alterations in AN. Furthermore, although the seed-based and the network of interest approaches have been favoured because of the simplicity in interpreting findings, the whole-brain approaches (whole-brain ICA and graph analysis) should be encouraged with the aim to establish a comprehensive view of the functional connectivity at rest in AN. As done by a group of authors (Boehm et al., 2014; Ehrlich et al., 2015; Geisler et al., 2015; Lord et al., 2016), future studies could adopt a multi-approach analysis, integrating methods with and without *a priori* assumptions, and also exploring unwanted thoughts related to the disease during scan (Kühn et al., 2014). In addition, it would be useful to combine different methods using both diffusion tensor imaging and rsfMRI to investigate both structural and functional connectivity. Resting-state neuroimaging methods remain a means to improve our knowledge on AN pathophysiology, investigate functional differences throughout stages of disease, and help in defining possible neural markers involved in the development and maintenance of AN.

References

American Psychiatric Association, 2013. Diagnostic and Statistical Manual of Mental Disorders, 5th ed.. 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. Cerebellum 12, 623–631.

Arcelus, J., Mitchell, A.J., Wales, J., Nielsen, S., 2011. Mortality rates in patients with anorexia nervosa and other eating disorders: a meta-analysis of 36 studies. Arch. Gen. Psychiatry 68, 724-731.

Barkhof, F., Haller, S., Rombouts, S.A., 2014. Resting-state functional MR imaging: a new window to the brain. Radiology, 272, 29-49.

Beckmann, C.F., DeLuca M., Devlin, J.T., Smith, S.M., 2005. Investigations into resting-state connectivity using independent component analysis. Philos. Trans. Roy. Soc. B: Biol. Sci. 360, 1001-1013.

Biezonski, D., Cha, J., Steinglass, J., Posner, J., 2015. Evidence for Thalamocortical Circuit Abnormalities and Associated Cognitive Dysfunctions in Underweight Individuals with Anorexia Nervosa. Neuropsychopharm. doi: 10.1038/npp.2015.314.

Biswal, B.B., Mennes, M., Zuo, X.N., Gohel, S., Kelly, C., Smith, S.M., ... & Dogonowski, A.M., 2010. Toward discovery science of human brain function. Proc. Natl. Acad. Sci. U.S.A. 107, 4734–4739.

Boehm, I., Geisler, D., King, J.A., Ritschel, F., Seidel, M., Deza Araujo, Y., Petermann, J., Lohmeier, H., Weiss, J., Walter, M., Roessner, V., Ehrlich, S., 2014. Increased resting state functional connectivity in the fronto-parietal and default mode network in anorexia nervosa. Front. Behav. Neurosci. 8, 346.

Boehm, I., Geisler, D., Tam, F., King, J. A., Ritschel, F., Seidel, M., Bernardoni, F., Murr, J., Goschke, T., Calhoun, V.D., Roessner, V., Ehrlich, S., 2016. Partially restored resting-state functional connectivity in women recovered from anorexia nervosa. J Psychiatry Neurosci. 41, 150259.

Brooks, S.J. (2016). A debate on working memory and cognitive control: can we learn about the treatment of substance use disorders from the neural correlates of anorexia nervosa?. BMC psychiatry, 16, 10.

Brooks, S.J., O'Daly, O., Uher, R., Friederich, H.C., Giampietro, V., Brammer, M., Williams, S.C.R., Schiöth H.B., Treasure J., Campbell, I.C., 2012. Thinking about eating food activates visual cortex with reduced bilateral cerebellar activation in females with anorexia nervosa: an fMRI study. PLoS One, 7, e34000.

Brooks, S.J., Owen, G.O., Uher, R., Friederich, H.C., Giampietro, V., Brammer, M., Williams, S.C.R., Schiöth H.B., Treasure J., Campbell, I.C. (2011). Differential neural responses to food images in women with bulimia versus anorexia nervosa. PLoS One, 6, e22259.

Collantoni, E., Michelon, S., Tenconi, E., Degortes, D., Titton, F., Manara, R., Clementi, M., Pinato, C., Forzan, M., Cassina, M., Santonastaso, P., Favaro, A., 2016. Functional connectivity correlates of response inhibition impairment in anorexia nervosa. Psychiat. Res. Neuroim. 247, 9-16.

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. Hum. Brain. Mapp. 35, 483-491.

Craig, A.D., 2002. How do you feel? Interoception: the sense of the physiological condition of the body. Nat. Rev. Neurosci. 3, 655-66.

Dakanalis, A., Gaudio, S., Serino, S., Clerici, M., Carrà, G., & Riva, G., 2016. Body-image distortion in anorexia nervosa. Nat. Rev. Dis. Primers. 2, 16026.

Damoiseaux, J.S., Rombouts, S.A.R.B., Barkhof, F., Scheltens, P., Stam, C.J., Smith, S.M., Beckmann, C.F., 2006. Consistent resting-state networks across healthy subjects. Proc. Natl. Acad. Sci. U.S.A. 103, 13848–13853

Dosenbach, N.U., Nardos, B., Cohen, A.L., Fair, D.A., Power, J.D., Church, J.A., Nelson, S.M., Wig, G.S., Vogel, A.C., Lessov-Schlaggar, C.N., Barnes, K.A., Dubis, J.W., Feczko, E., Coalson, R.S., Pruett Jr., J.R., Barch, D.M., Petersen, S.E., Schlaggar, B.L., 2010. Prediction of individual brain maturity using fMRI. Science, 329, 1358-1361

Ehrlich, S., Lord, A.R., Geisler, D., Borchardt, V., Boehm, I., Seidel, M., Ritschel. F., Schulze. A., King. J.A., Weidner. K., Roessner, V., Walter, M., 2015. Reduced functional connectivity in the thalamo‐insular subnetwork in patients with acute anorexia nervosa. Hum. Brain Map. 36, 1772-1781.

Favaro, A., Clementi, M., Manara, R., Bosello, R., Forzan, M., Bruson, A., Tenconi, E., Degortes, D., Titton, F., Di Salle, F., Santonastaso, P., 2013. Catechol-O-methyltransferase genotype modifies executive functioning and prefrontal functional connectivity in women with anorexia nervosa. J Psychiatry Neurosci. 38, 241.

Favaro, A., Santonastaso, P., Manara, R., Bosello, R., Bommarito, G., Tenconi, E., Di Salle, F., 2012. Disruption of visuospatial and somatosensory functional connectivity in anorexia nervosa. Biol. Psychiatry 72, 864–870.

Fox, M.D, Raichle, M.E., 2007. Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. Nat. Rev. Neurosci., 8, 700-711.

Fox, M.D., Greicius, M., 2010. Clinical applications of resting state functional connectivity. Front. Syst. Neurosci. 4, 19.

Frank, G.K., Collier, S., Shott, M.E., O'Reilly, R.C., 2016. Prediction error and somatosensory insula activation in women recovered from anorexia nervosa. J. Psychiatry Neurosci. 41, 150103.

Friston, K. J., 2011. Functional and effective connectivity: a review. Brain Connect. 1, 13-36.

Gaudio, S., Piervincenzi, C., Zobel, B.B., Montecchi, F.R., Riva, G., Carducci, F., Quattrocchi, C.C., 2015. Altered resting state functional connectivity of anterior cingulate cortex in drug naïve adolescents at the earliest stages of anorexia nervosa. Sci. Rep. 5, 10818.

Gaudio, S., Quattrocchi, C.C. (2012). Neural basis of a multidimensional model of body image distortion in anorexia nervosa. Neurosci. Biobehav. Rev. 36, 1839-1847.

Gaudio, S., Riva, G. 2012. Body image in anorexia nervosa: the link between functional connectivity alterations and spatial reference frames. Biol. Psychiatry. 73, e25-e26.

Geisler, D., Borchardt, V., Lord, A. R., Boehm, I., Ritschel, F., Zwipp, J., Clas, S., King, J.A., Wolff-Stephan, S., Roessner, V., Walter, M., Ehrlich, S., 2015. Abnormal functional global and local brain connectivity in female patients with anorexia nervosa. J. Psychiatry Neurosci. 41, 6-15.

Greicius, M.D., Supekar, K., Menon, V., Dougherty, R.F., 2009. Resting-state functional connectivity reflects structural connectivity in the default mode network. Cereb. Cortex 19, 72–78.

Harmelech, T., & Malach, R. (2013). Neurocognitive biases and the patterns of spontaneous correlations in the human cortex. Trends Cogn. Sci. 17, 606-615.

Johansen-Berg, H., Behrens, T.E., Sillery, E., Ciccarelli, O., Thompson, A.J., Smith, S.M,, Matthews, P.M., 2005. Functional-anatomical validation and individual variation of diffusion tractography-based segmentation of the human thalamus. Cereb. cortex 15, 31-39.

Julien, D., O'Connor, K.P., Aarema, F., 2007. Intrusive thoughts, obsessions, and appraisals in obsessive-compulsive disorder: a critical review. Clin. Psychol. Rev. 27, 366-83.

Kaye, W.H., Wagner, A., Fudge, J.L., Paulus, M., 2011. Neurocircuity of eating disorders. Curr. Top. Behav. Neurosci. 6, 37-57.

Kaye, W.H., Wierenga, C.E., Bailer, U.F., Simmons, A.N., Bischoff-Grethe, A., 2013. Nothing tastes as good as skinny feels: the neurobiology of anorexia nervosa. Trends. Neurosci. 36, 110-120.

Kim, K.R., Ku, J., Lee, J.H., Lee, H., Jung, Y.C., 2012. Functional and effective connectivity of anterior insula in anorexia nervosa and bulimia nervosa. Neurosci. Lett. 521,152–157.

Kühn, S., Vanderhasselt, M.A., De Raedt, R., Gallinat, J., 2014. The neural basis of unwanted thoughts during resting state. Soc. Cogn. Affect. Neurosci. 9, 1320-1324.

Kullmann, S., Giel, K.E., Teufel, M., Thiel, A., Zipfel, S., Preissl, H., 2014. Aberrant network integrity of the inferior frontal cortex in women with anorexia nervosa. Neuroimage Clin. 4, 615–622.

Lee, S., Kim, K.R., Ku, J., Lee, J.H., Namkoong, K., Jung, Y.C., 2014. Resting-state synchrony between anterior cingulate cortex and precuneus relates to body shape concern in anorexia nervosa and bulimia nervosa. Psychiat. Res. 221, 43–48.

Lerman, C., Gu, H., Loughead, J., Ruparel, K., Yang, Y., Stein, E.A., 2014. Large-scale brain network coupling predicts acute nicotine abstinence effects on craving and cognitive function. JAMA psychiatry, 71, 523-530.

Lord, A., Ehrlich, S., Borchardt, V., Geisler, D., Seidel, M., Huber, S. Murr, J., Walter, M., (2016). Brain parcellation choice affects disease-related topology differences increasingly from global to local network levels. Psychiatry Research: Neuroimaging, 249, 12–19.

Moher, D., Liberati, A., Tetzlaff, J., Altman, D.G., PRISMA Group, 2009. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Ann. Intern. Med. 151, 264-269.

Morcom, A. M., & Fletcher, P. C. (2007). Does the brain have a baseline? Why we should be resisting a rest. NeuroImage, 37(4), 1073–1082. http://doi.org/10.1016/j.neuroimage.2006.09.013

Phillipou, A., Abel, L. A., Castle, D. J., Hughes, M. E., Nibbs, R. G., Gurvich, C., & Rossell, S. L. (2016). Resting state functional connectivity in anorexia nervosa. Psychiatry Res. Neuroimaging. 251, 45-52.

Raichle, M.E., MacLeod, A.M., Snyder, A.Z., Powers, W.J., Gusnard, D.A., Shulman, G.L., 2001. A default mode of brain function. Proc. Natl. Acad. Sci. 98, 676-682.

Rubinov, M., Sporns, O. (2010). Complex network measures of brain connectivity: uses and interpretations. NeuroImage, 52, 1059e1069.

Simmons, W.K., Rapuano, K.M., Kallman, S.J., Ingeholm, J E., Miller, B., Gotts, S.J., Avery, J.A., Hall, K.D., Martin, A., 2013. Category-specific integration of homeostatic signals in caudal but not rostral human insula. Nat. Neurosci. 16, 1551-1552

Smith, S.M., Fox, P.T., Miller, K.L., Glahn, D.C., Fox, P.M., Mackay, C.E., Filippini, N., Watkinsa, K.E., Torod, R., Laird, A.R., Beckmann, C.F., 2009. Correspondence of the brain's functional architecture during activation and rest. Proc. Natl. Acad. Sci. U.S.A. 106, 13040–13045

Sutherland, M. T., McHugh, M. J., Pariyadath, V., & Stein, E. A. 2012. Resting state functional connectivity in addiction: Lessons learned and a road ahead. Neuroimage, 62(4), 2281-2295.

Titova, O.E., Hjorth, O.C., Schiöth, H.B., Brooks, S.J., 2013. Anorexia nervosa is linked to reduced brain structure in reward and somatosensory regions: a meta-analysis of VBM studies. BMC Psychiatry 13, 110.

Treasure, J., Zipfel, S., Micali, N., Wade, T., Stice, E., Claudino, A., Schmidt, U., Frank, G.K., Bulik, C.M., Wentz, E. 2015. Anorexia nervosa. Nat. Rev. Dis. Primers. 1, 15074.

Van den Eynde, F., Suda, M., Broadbent, H., Guillaume, S., Eynde, M., Steiger, H., Israel, M., Berlim, M., Giampietro, V., Simmons, A., Treasure, J., Campbell, I., Schmidt, U., 2012. Structural Magnetic Resonance Imaging in Eating Disorders: A Systematic Review of Voxel‐Based Morphometry Studies. Eur. Eat. Disord. Rev. 20, 94-105.

Van Den Heuvel, M.P., Hulshoff Pol, H.E., 2010. Exploring the brain network: a review on resting-state fMRI functional connectivity. Eur. Neuropsychopharm., 20, 519-534.

Vincent, J.L., Kahn, I., Snyder, A.Z., Raichle, M.E., Buckner, R.L., 2008. Evidence for a frontoparietal control system revealed by intrinsic functional connectivity. J. neurophysiol. 100, 3328-3342.

Zastrow, A., Kaiser, S., Stippich, C., Walther, S., Herzog, W., Tchanturia, K., Belger, A., Weisbrod., M., Treasure, J., Friederich, H.C., 2009. Neural correlates of impaired cognitive-behavioral flexibility in anorexia nervosa. Am. J. Psychiatry. 166, 608–616.

Zhang, D., Snyder, A.Z., Shimony, J.S., Fox, M.D., Raichle, M.E., 2010. Noninvasive functional and structural connectivity mapping of the human thalamocortical system. Cereb. Cortex 20, 1187-1194

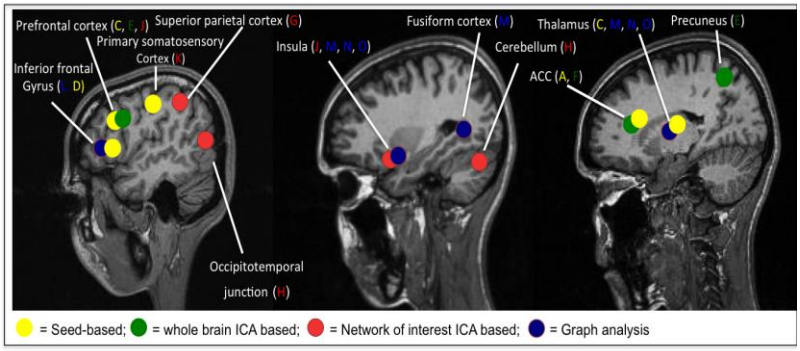
Zhang, D., Snyder. A,Z,, Fox. M,D,, Sansbury, M.W., Shimony, J.S., Raichle, M.E., 2008. Intrinsic functional relations between human cerebral cortex and thalamus. J. Neurophysiol. 100, 1740-1748.

Zipfel, S., Giel, K.E., Bulik, C.M., Hay, P., Schmidt, U., 2015. Anorexia nervosa: aetiology, assessment, and treatment. Lancet Psychiatry. 2, 1099-1111.

Zuo, X.N., Di Martino, A., Kelly, C., Shehzad, Z.E., Gee, D.G., Klein, D.F., Castellanos, F.X., Biswal, B.B., Milham, M.P., 2010. The Oscillating Brain: Complex and Reliable. NeuroImage, 49, 1432-1445.

Figure legend:

***Figure 1*. Main brain areas implicated in resting-state functional MRI**. Diagram of the main findings from each resting-state fMRI study of AN patients classified on the basis of the different resting state approaches. Each circle represent the regions where AN patients exhibited functional connectivity alterations: yellow refers to the seed based approach studies (seeds are reported); green refers to whole-brain ICA-based studies (altered areas within the networks are reported); red refers to network-of-interest ICA-based studies (altered areas within the networks are reported); blue refers to graph analysis studies (altered areas are reported). Labels are reported in Table 2. See Table 2 for specific details of the areas implicated by each method.



Appendix B. PRISMA flow diagram.

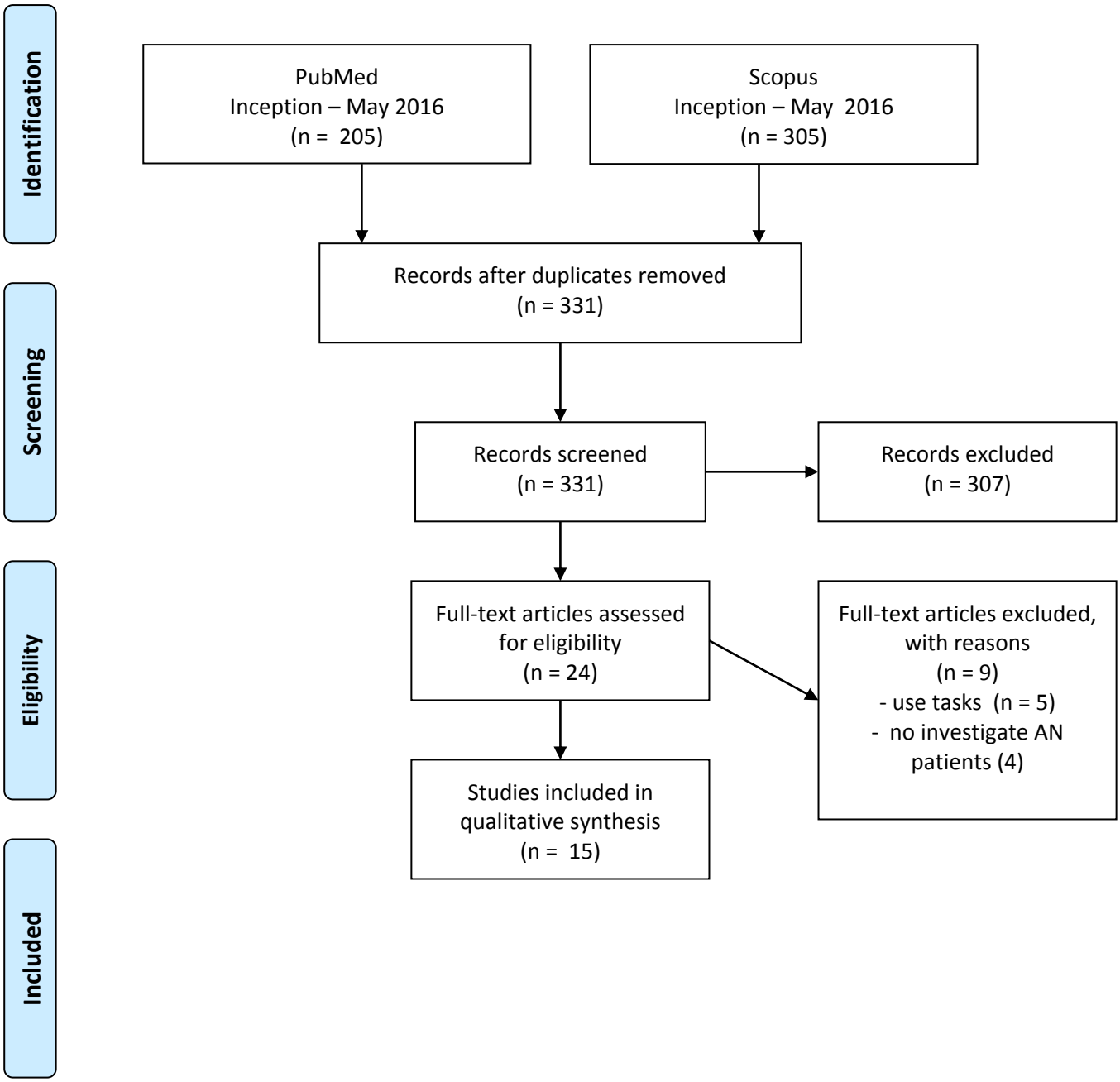


Table 1. Sample characteristics of resting-state-fMRI studies

Study	Participants	Age (years)		BMI		ED duration (years)	
		Mean	(SD)	Mean	(SD)	Mean	(SD)
Amianto et al. (2012)	AN: n = 12	20.00	(4.00)	16.27	(0.99)	0.91	0.41
	BN: n = 12	23.00	(5.00)	21.57	(2.38)	-	-
	HC: n = 10	24.00	(3.00)	21.35	(3.16)	-	-
Biezonski et al. (2015)	AN: n= 15	19.00	(0.50)	17.37	(0.29)	4.73	(0.83)
	HC: n = 16	20.31	(0.77)	21.57	(0.45)	-	-
Boehm et al. (2014) *	AN: n= 35	16.10	(2.56)	14.78	(1.26)	1.57	(2.25)
	HC: n = 35	16.16	(2.64)	20.81	(2.72)	-	-
Bohem et al. (2016)	rec-AN: n = 31	22.27	(3.08)	20.69	(1.62)	3.72	(2.66)
	HC: n = 31	21.73	(2.99)	21.37	(2.10)	-	-
Collantoni et al. (2016)	AN: n= 35	25.40	(6.90)	15.80	(1.80)	6.70	(6.90)
	HC: n = 34	25.00	(6.20)	-	-	-	-
Cowdrey et al. (2014)	rec-AN: n = 16	23.06	(3.55)	21.33	(2.17)	3.50	(2.38)
	HC: n = 14	24.11	(2.85)	21.01	(1.56)	-	-
Ehrlich et al. (2015) *	AN: n = 35	16.10	(2.56)	14.78	(1.26)	-	-
Geisler et al. (2015) * Lord et al. (2016)*	HC: n = 35	16.16	(2.64)	20.81	(2.72)	-	-
Favaro et al. (2012)	AN: n = 29	25.80	(6.90)	14.50	(2.30)	6.20	(6.90)
	rec-AN: n = 16	23.80	(4.80)	19.20	(1.00)	(2.30)	(1.70)
	HC: n = 26	26.70	(6.70)	21.80	(3.20)	-	-
Favaro et al. (2013)	AN: n = 33	26.90	(7.30)	15.80	(1.90)	-	-
	HC: n = 30	25.80	(6.70)	-	-	-	-
Gaudio et al. (2015)	AN: n= 16	15.80	(1.70)	16.20	(1.20)	0.33	(0.15)
	HC: n = 16	16.30	(1.40)	21.10	(1.90)	-	-
Kullmann et al. (2014)	AN: n= 12	23.30	(4.70)	15.50	(1.50)	-	-
	HC: n = 14	24.60	(2.90)	21.40	(1.50)	-	-
	HCA: n = 12	24.10	(3.20)	22.00	(1.90)	-	-

Lee et al. (2014)	AN: n = 18	25.20	(4.20)	16.00	(1.70)	3.80	(2.60)
	BN: n = 20	22.90	(3.90)	21.60	(2.30)	3.80	(4.70)
	HC: n = 20	23.30	(1.80)	19.90	(1.90)	-	-
Phillipuo et al. (2016)	AN: n= 26	22.81	(6.67)	16.63	(1.19)	6.42	(7.43)
	HC: n = 27	22.46	(3.16)	22.60	(3.53)	-	-

Note : BMI = Body mass index. ED = eating disorder. AN = anorexia nervosa. BN = Bulimia Nervosa. HC = healthy control. rec- AN = recovered AN. HCA = healthy control athletes. * these four samples are composed by the same participants.

Table 2. Description of resting-state fMRI studies in AN

Label	Study	Brain regions and/or networks analysed	Main findings	Main clinical interpretation
Seed-based studies				
A	Lee et al. (2014)	Seed: dorsal anterior cingulate cortex (dACC)	AN patients showed stronger synchronous activity between the dACC and retrosplenial cortex and between the dACC and precuneus. ED group demonstrated stronger synchronous activity between the dACC and precuneus.	The altered dACC-precuneus synchrony might be associated with the disorder-specific rumination on eating, weight and body shape in patients with eating disorders.
B	Favaro et al. (2013)	Seed: dorsolateral, ventrolateral and ventromedial prefrontal cortex	Analyses did not reveal any significant differences between AN patients and healthy women. Within AN sample, FC differences were detected between patients with different variants for the COMT protein.	AN patients with a particular variant for the COMT protein had increased prefrontal cortex FC compared to carriers of a different variant.
C	Biezonski et al. (2015)	Seed: seven bilateral thalamic seeds. Only connectivity with the frontal lobes was investigated	AN patients showed greater connectivity between the central medial thalamus and the bilateral dorsolateral prefrontal cortex and lower FC between the anterior thalamus and the left anterior prefrontal cortex.	Alterations in thalamo-frontal circuits may have a role in mediating aspects of cognitive dysfunction in AN patients.
D	Collantoni et al. (2016)	Seed: superior parietal lobule, right inferior frontal gyrus and pre-supplementary motor area	AN patients showed altered FC (in both the positive and negative connectivity) of the right inferior frontal gyrus, considered as part of the ventral attention network	Impaired ventral attention network connectivity (i.e. the right inferior frontal gyrus alteration) may affect cognitive control processes and exogenous stimuli filtering.
Whole brain ICA based Studies				
E	Cowdrey et al. (2014)	Twelve resting-state networks identified and investigated	Increased temporal coherence between the DMN and the precuneus and the dorsolateral prefrontal cortex/inferior frontal gyrus in subjects recovered from AN.	The findings are compatible with the core symptoms of AN including ruminative preoccupation on eating, weight and shape, excessive planning and impaired cognitive flexibility.
F	Gaudio et al. (2015)	Eight resting-state networks identified and investigated	Decreased temporal correlation between the executive control network FC maps and the anterior cingulate cortex (ACC) in AN patients.	The decreased FC between executive control network and the ACC could explain the impaired cognitive flexibility in relation to body image and appetite in AN patients.
Network of interest ICA based studies				
G	Favaro et al. (2012)	Networks of interest: medial, lateral, and ventral visual networks and somatosensory	Decreased FC in the left occipitotemporal junction within the ventral visual network and increased FC in the left superior parietal cortex FC within the somatosensory network in AN patients. Decreased FC in the right middle frontal gyrus within the ventral visual network in recovered AN subjects.	The findings may explain the failure of the integration process between visual and somatosensory perceptual information that sustains body image disturbance.
H	Amianto et al. (2013)	Networks of interest: cerebellar	Alterations within the cerebellar network between ED patients compared to controls (e.g., increased connectivity with insulae and vermis decreased connectivity with parietal lobe). Increased connectivity with the insulae in	The findings support the role of the cerebellum as a key area of integration of different functions important in ED psychopathology.

			AN compared to BN, increased connectivity with anterior cingulate cortex in BN compared to AN	
I	Boehm et al. (2014)	Networks of interest: fronto-parietal, default mode, salience, visual and sensory-motor	Increased FC between the angular gyrus and the fronto-parietal network and between the anterior insula and the default mode network in patients with AN.	Increased FC within the fronto-parietal network may be related to excessive cognitive control in AN patients. The anterior insula/DMN alterations may be related to ruminations about food and bodily appearance.
J	Bohem et al. (2016)	Networks of interest: fronto-parietal, default mode, salience	Recovered AN subjects showed reduced FC between the dorsolateral prefrontal cortex and the fronto-parietal network. Additional a priori analysis found increased FC between angular gyrus and FPN in recovered subjects. No differences were found in the DMN.	Some altered FC patterns found in AN patients are still present after long-term AN recovery. In particular, DMN alterations seem to normalize, while fronto-parietal network alterations, also related to cognitive control functions, seem to persist. The authors suggested this interpretations on the basis of their previous study [i.e. Bohem et al (2014) (label I)].
K	Phillipou et al. (2016)	Networks of interest: ROIs of default mode, sensory-motor and visual networks. a ROI to ROI approach was used. (see the paper for details)	Decreased FC were found between the sensory-motor and visual networks in AN patients compared to controls. In particular, AN patients showed reduced FC between primary somatosensory, and secondary visual and associative visual areas; and between primary motor, and secondary visual and associative visual areas. No differences were found in the DMN.	Reduced FC between sensorimotor and visual networks may suggest an altered visuospatial processing in AN patients, related to body image disturbances.
Graph analysis studies				
L	Kullmann et al. (2014)	degree centrality and effective connectivity	Reduced FC of the inferior frontal gyrus (IFG) bilaterally and altered effective connectivity (i.e. from the right IFG to the middle cingulate cortex, from the bilateral orbitofrontal gyrus to the right IFG and from the bilateral insula to the left IFG) in AN patients	Reduced connectivity within the cognitive control system of the brain and increased connectivity within regions important for salience processing.
M	Ehrlich et al. (2015)	Network-based statistic approach and regional homogeneity	A subnetwork of connections had decreased connectivity in AN patients, areas included the amygdala, thalamus, fusiform gyrus, putamen and the posterior insula.	The findings might reflect changes in the propagation of altering sensations to urgent homeostatic imbalances and pain-processes, which are known to be severely disturbed in AN and might explain the striking discrepancy between patients' actual and perceived internal body state.
N	Geisler et al. (2015)	7 global and 7 nodal graph metrics (see the paper for details)	Decreased connectivity strength and increased path length in the posterior insula and thalamus in AN patients.	Reduced local network efficiency in the thalamus and posterior insula may reflect a mechanism explaining the impaired integration of visuospatial and homeostatic signals in AN patients.
O	Lord et al. (2016)	Global and local network properties, including network-based statistics,	FC alterations were found in AN patients compared to controls including the insula and thalamus. These differences were consistent across the parcellation approaches.	Reduced FC in a subnetwork including the insula and thalamus (as found in this study independent of type of atlas) could be related to an altered processing of signals such as body size, hunger and pain.

	comparing two common parcellation approaches (i.e. anatomical and literature based analyses) (see the paper for details)

Note: Dorsal anterior cingulate cortex = dACC. Anorexia nervosa = AN. Eating disorder = ED. Functional connectivity = FC. Default mode network = DMN. Anterior cingulate cortex = ACC. Bulimia nervosa = BN. Inferior frontal gyrus = IFG.

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3-7
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	7
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	N/A
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	7-8
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	7-8
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	7-8
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	8
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	8
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	N/A
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	N/A
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	N/A

PRISMA 2009 Checklist

Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	N/A

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	N/A
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	N/A
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Fig S1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Table 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	N/A
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	N/A
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	N/A
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	N/A
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	N/A
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	17-18
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	18-19
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	23-24
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	N/A

PRISMA 2009 Checklist

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: **www.prisma-statement.org**.