



ELSEVIER

Contents lists available at ScienceDirect

Psychiatry Research: Neuroimaging

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

Amygdala alterations during an emotional conflict task in women recovered from anorexia nervosa

Lasse Bang ^{a,*}, Øyvind Rø ^{a,b}, Tor Endestad ^c^a Regional Department for Eating Disorders, Division of Mental Health and Addiction, Oslo University Hospital, Oslo, Norway^b Division of Mental Health and Addiction, Institute of Clinical Medicine, University of Oslo, Oslo, Norway^c Institute of Psychology, University of Oslo, Oslo, Norway

ARTICLE INFO

Article history:

Received 7 August 2015

Received in revised form

22 December 2015

Accepted 28 December 2015

Available online 29 December 2015

Keywords:

Functional magnetic resonance imaging

Cognitive control

Emotion

Limbic

Anorexia nervosa

ABSTRACT

The pathophysiology of anorexia nervosa (AN) is not completely understood, but research suggests that alterations in brain circuits related to cognitive control and emotion are central. The aim of this study was to explore neural responses to an emotional conflict task in women recovered from AN. Functional magnetic resonance imaging was used to measure neural responses to an emotional conflict task in 22 women recovered from AN and 21 age-matched healthy controls. The task involved categorizing affective faces while ignoring affective words. Face and word stimuli were either congruent (non-conflict) or incongruent (conflict). Brain responses to emotional conflict did not differ between groups. However, in response to emotional non-conflict, women recovered from AN relative to healthy controls showed significantly less activation in the bilateral amygdala. Specifically, while emotional non-conflict evoked significant activations of the amygdala in healthy controls, recovered AN women did not show such activations. Similar significant group differences were also observed in the hippocampus and basal ganglia. These results suggest that women recovered from AN are characterized by alterations within emotion-related brain circuits. Recovered women's absence of amygdala and hippocampus activation during non-conflict trials possibly reflects an impaired ability to process emotional significant stimuli.

© 2016 The Authors. Published by Elsevier Ireland Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Anorexia nervosa (AN) is a potentially fatal mental disorder that predominantly affects adolescent females (American Psychiatric Association, 2013). It is characterized by a relentless pursuit of thinness, severe food restriction, and extremely low body weight. Patients with AN have an intense fear of weight gain and a distorted view of their own body; viewing themselves as fat despite being emaciated. Furthermore, patients are characterized by personality traits such as perfectionism, neuroticism and obsessive-compulsiveness (Cassin and von Ranson, 2005), and they display high anxiety (Holtkamp et al., 2005; Pollice et al., 1997).

The pathophysiology of AN is not completely understood, but available evidence suggests that alterations in brain circuits related to cognitive control and emotion are central (Kaye et al., 2013). Cognitive control refers to higher order cognitive functions such as working memory, monitoring, mental flexibility, planning and inhibition, and enables regulation of behavior, cognition and

emotions in accordance with current goals (Chan et al., 2008; Miller and Cohen, 2001). The neurocircuitry underlying these functions mainly resides in prefrontal and anterior cingulate cortices, which monitor and exert top-down control over other brain circuits (Miller and Cohen, 2001). For instance, inadequate top-down control from the prefrontal cortex over subcortical (e.g., limbic) structures is associated with poor regulation of behavior and emotion (Heatherington and Wagner, 2011). In a similar vein, some have raised the possibility that an imbalance between cognitive control and emotion circuits underlies the pathophysiology of AN (Holliday et al., 2005; Kaye et al., 2013; Marsh et al., 2009), which could be associated with patients' extraordinary ability to inhibit incentive motivational drives (i.e. hunger), and the difficulties with emotional regulation that they display (Kaye et al., 2013).

Studies challenging cognitive control have reported that AN is associated with alterations in prefrontal and anterior cingulate cortices (Ehrlich et al., 2015; Lao-Kaim et al., 2015; Oberndorfer et al., 2011; Sato et al., 2013; Wierenga et al., 2014, 2015; Zastrow et al., 2009). For example, women recovered from AN show increased activation in the lateral prefrontal cortex during monetary decision tasks (Ehrlich et al., 2015; Wierenga et al., 2015), possibly

* Correspondence to: Regional Department for Eating Disorders, Oslo University Hospital, P.O. Box 4956 Nydalen, N-0424 Oslo, Norway.
E-mail address: Bang.Lasse@ous-hf.no (L. Bang).

reflecting elevated cognitive control processes. Studies investigating cognitive-behavioral flexibility in ill AN patients have also reported alterations in the prefrontal cortices, although results are mixed, with some showing decreased activation in patients (Sato et al., 2013; Zastrow et al., 2009), and others showing both decreased and increased activations (Lao-Kaim et al., 2015). It has also been demonstrated that while patients with AN have similar neural activation to healthy controls during low-demanding inhibitory trials, they exhibit decreased activation in the prefrontal and anterior cingulate cortex during high-demanding inhibitory trials (Wierenga et al., 2014). A similar demand-specific alteration of prefrontal cortices has been shown in recovered AN patients (Oberndorfer et al., 2011). These studies suggest AN individuals require less inhibitory resources to maintain performance as inhibitory demand increases. Interestingly, studies have also reported altered functional connectivity within cognitive control circuits during rest in AN individuals, but results are mixed: some report increased functional connectivity (Boehm et al., 2014; Cowdrey et al., 2012), while others report decreased connectivity (Gaudio et al., 2015; Kullmann et al., 2014b). These inconsistencies may be due to small sample sizes, or differences in sample characteristics. In sum, there are clear indications that both ill and recovered AN individuals are characterized by aberrations in cognitive control circuits.

Ample evidence suggests that AN is also associated with functional alterations within emotion circuits related to the perception and processing of emotionally salient stimuli. The majority of this research has been performed using symptom-provocation paradigms, where stimuli are AN-specific (i.e., images of food and bodies). When exposed to such stimuli, patients with AN relative to healthy controls exhibit greater activation in widespread cortical and subcortical brain circuits (Zhu et al., 2012), including anterior cingulate (Ellison et al., 1998; Uher et al., 2004), prefrontal (Ellison et al., 1998; Miyake et al., 2010; Uher et al., 2004), and amygdala cortices (Ellison et al., 1998; Joos et al., 2011; Miyake et al., 2010; Seeger et al., 2002; Vocks et al., 2010, 2011). These hyperactivations have been interpreted as representing heightened negative emotional arousal. The alterations in prefrontal and anterior cingulate cortices may indicate that compensatory control mechanisms are mobilized, for example to regulate amygdala activation. Consistent with this notion, Pruis et al., (2012) showed that negative emotional distractors (images of bodies) during a working memory task were associated with greater amygdala activation and reduced medial prefrontal cortex activation in recovered AN patients compared with healthy controls. This might point to a failure of prefrontal circuits to adequately inhibit amygdala activation. As most studies of emotion processing in AN have employed disorder-specific stimuli, it remains unclear to what extent the reported alterations are restricted to the processing of such stimuli, or are indicative of a general deficit in emotion processing.

Collectively, these studies indicate that the pathophysiology of AN is associated with alterations within both emotion and cognitive control circuits. However, few studies have attempted to characterize these alterations during tasks that require cognitive control in the presence of emotional stimuli. The aim of the present study was to explore this in women recovered from AN. To achieve this, an emotional conflict task was presented during functional magnetic resonance imaging (MRI). Performance on this task relies on cognitive control processes such as conflict detection and inhibition (Etkin et al., 2006). To our knowledge, this is the first study of AN to challenge cognitive control in the context of emotional stimuli unrelated to AN symptomatology.

2. Methods

2.1. Participants

We recruited 22 adult women recovered from AN and 21 age-matched healthy control women, all right-handed. Current and lifetime DSM-IV diagnoses (American Psychiatric Association, 2000) were determined with the Structured Clinical Interview for DSM-IV Axis I Disorders version I/P (First et al., 2002), which was administered to all participants no more than 1 week before the MRI session. During this interview, the status of AN recovery was evaluated (see below), and other clinical characteristics were obtained.

Women in the recovered AN group were included if they had a lifetime history of AN to DSM-IV criteria (American Psychiatric Association, 2000), which included (a) a weight below 85% of that expected based on height and age, (b) intense fear of weight gain or becoming fat, and (c) body image disturbances, or undue influence of body shape or weight on self-evaluation, or denial of the seriousness of their low body weight. Similar to other studies (Pruis et al., 2012), we excluded the amenorrhea criterion which was also removed in the DSM-5 (American Psychiatric Association, 2013). We also subtyped participants into restricting versus binge-eating/purging type, based on the presence of bingeing or purging behavior during the AN period (American Psychiatric Association, 2013). Only women recovered from AN were included in this study. Recovery was operationally defined as having maintained a body mass index above 18.0 for the past 12 months, and abstinence from bingeing and purging behavior, excessive or compulsive exercising behavior, and no severely restricted food intake for the past 12 months. Exclusion criteria for these women included the following: lifetime history of a psychotic disorder, substance abuse or dependence, or the presence of any Axis I disorder the past 12 months.

Exclusion criteria for women in the control group included the following: lifetime history of any Axis I disorder, current use of psychoactive medications, and a first-degree relative with a history of an eating disorder. Furthermore, we excluded control women who reported bingeing and purging behavior, excessive and compulsive exercising, severely restricted food intake, or had a body mass index below 18.0 for the past 12 months. Women in both groups were excluded if they reported any major medical illnesses, history of severe head trauma, or any contraindications to magnetic resonance imaging (MRI).

Three of the recovered AN women were using psychoactive medications (one for insomnia, and two for depressive symptoms), but results did not change when these women were excluded, so they were included in the final analyses. This study was approved by the Regional Ethics Committee in Norway. After complete description of the study, written informed consent was obtained from all participants.

2.2. Behavioral measures

Immediately before the MRI session, all participants completed the following self-report questionnaires: Spielberger State-Trait Anxiety Inventory (STAI; Spielberger et al., 1970), Difficulties in Emotion Regulation Scale (DERS; Gratz and Roemer, 2004), Beck Depression Inventory (BDI; Beck et al., 1961), and Eating Disorder Examination-Questionnaire (EDE-Q; Fairburn and Beglin, 2008). Following the MRI session, participants were weighed in order to calculate their body mass index.

2.3. Emotional conflict task

The emotional conflict task was similar to that used in previous

studies (Etkin et al., 2006). Monochromatic photographs of seven male and seven female actors with happy or fearful expressions from the NIMSTIM database (Tottenham et al., 2009, <http://www.macbrain.org/resources.htm>), overlaid with the word “FEAR” or “HAPPY,” were presented. A total of 160 trials were presented, for which half (80 trials) was congruent (i.e. non-conflict, expression of actor matched the word), and the other half (80 trials) was incongruent (i.e. conflict, expression of actor did not match the word). Subjects were instructed to ignore the word, and to indicate the emotional expression of the actor, as fast and accurately as possible. As previous studies have illustrated, incongruent trials generate emotional conflict, which is associated with slower responses and increased activity in the prefrontal cortex (Etkin et al., 2006, 2010). E-prime (Sharpsburg, PA) was used to present the experimental task during scanning, and record responses. Trials were presented in a pseudorandom order, counterbalanced across conditions for gender, actor, facial expression, and overlaid word. There were no direct repetitions of the same actor. Stimuli were presented for 1000 milliseconds (ms) with a random interstimulus interval between 3000–5000 ms. Responses were made using the right index and middle finger.

2.4. MRI data acquisition

Images were recorded with a 3 T Achieva MRI scanner (Philips, Eindhoven) equipped with an eight-channel Philips SENSE head coil. Functional images were acquired using a T2*-weighted single-shot echo-planar sequence (repetition time/echo time=2000/30 ms, flip angle=80°; field of view=240 × 240 mm², matrix=80 × 80). For each participant, 34 axial slices covering the whole brain were acquired in an interleaved order, aligned with the anterior commissure/posterior commissure line (voxel size=3 × 3 × 3 mm³, slice thickness=3 mm, slice gap=0.5 mm). Approximately 400 volumes were acquired for each participant. The five first volumes of each run were discarded to avoid T1 saturation effects. High-resolution structural images were also acquired using a T1-weighted multi-shot turbo-field-echo sequence (repetition time/echo time=6.7/3.1 ms, flip angle=8°, field of view=256 × 256 mm², matrix=256 × 213), recording 170 sagittal slices (voxel size=1.0 × 1.2 × 1.2).

2.5. MRI data analysis

MR images were preprocessed using the Statistical Parametric Mapping 8 (SPM8) toolbox (<http://www.fil.ion.ucl.ac.uk/spm>) implemented in MATLAB (MATLAB and Statistics Toolbox Release 2012b, The Mathworks, Inc., Natick, MA, USA). Functional images were slice-time corrected and realigned to the mean scan. They were then spatially normalized and bias-field corrected using high-dimensional diffeomorphic anatomical registration through exponentiated Lie algebra (DARTEL, Ashburner, 2007). The resulting modulated images were then smoothed with a 10-mm full-width at half-maximum kernel. There were no differences between recovered AN women and healthy controls in total gray or white matter volumes, and total brain size was similar across groups (unpublished observations).

A fixed effects model was created for each participant. Regressors for the onsets of emotional conflict and non-conflict trials were created, and convolved with the canonical hemodynamic response function. Incorrect trials, and trials with a response time below 200 or above 1200 ms (5.2% of all trials) were separately modeled as a regressor of no interest. Additional regressors corresponding to the six movement parameters were also included (there was no displacement above 3 mm). A 128-s temporal high-pass filter was applied to the data, and serial correlations were accounted for by using an autoregressive model. All models were globally scaled, and masked using the respective participant's

segmented gray matter images (to restrict the statistical parametric maps to gray matter). *T*-contrasts for emotional conflict and non-conflict trials over baseline were separately specified, and the resulting contrast images were submitted to a 2 × 2 (group × condition) factorial model. As this model did not include the appropriate error terms to test the main effect of group, a separate two-sample *t*-test was used to investigate this main effect. Four a priori regions of interest (ROIs) were specified based on previous research: inferior frontal gyrus, middle frontal gyrus, anterior cingulate cortex and amygdala (see Supplemental material: Methods and Fig. S1). A maximum probability atlas (www.brain-development.org) consisting of 83 regions hand-drawn on 30 MR images was used to specify the ROIs (Gousias et al., 2008; Hammers et al., 2003).

For the main effect of condition, we first performed a whole-brain analysis, followed by post hoc paired samples *t*-tests to show the neural activation associated with the two conditions. For the main effect of group and the group × condition interaction effect, ROI analyses followed by exploratory whole-brain analyses were conducted. For the ROI analyses, we used small volume corrections, thresholded at voxel-level $p < 0.05$ family-wise error-corrected. All whole-brain analyses were thresholded at voxel-level $p < 0.001$ uncorrected for multiple comparisons, with a minimum cluster size of 20 voxels. To facilitate interpretation of interaction effects, we extracted the raw β -weights from voxels showing a significant group × condition interaction for all participants using the Marsbar toolbox (Brett et al., 2002), and analyzed these with *t*-tests. For all statistically significant effects, peak-voxel activations are reported.

3. Results

3.1. Demographic and clinical characteristics

Recovered AN and healthy control women were of similar age, but body mass index was significantly higher for the controls (see Table 1). The two groups were matched for education, employment and civil status (χ^2 tests; $p > 0.05$). The recovered AN women scored higher (i.e., more pathological scores) on all self-report questionnaires (see Table 1). All participants scored below the empirically established clinical cut-off value of 2.5 on the EDE-Q (Rø et al., 2015), indicating non-pathology. Within the recovered AN group, age of AN onset ranged from 11 to 32 years ($M = 17.36$, $SD = 4.17$), lifetime lowest observed weight in relation to that expected for age and height ranged from 47% to 85% ($M = 71.84$, $SD = 9.19$), duration of recovery ranged from 12 to 192 months ($M = 51.62$, $SD = 42.70$), and illness duration (operationalized as duration of last AN episode) ranged from 6 to 120 months ($M = 32.86$, $SD = 27.47$). Half of the AN cases had a history of AN binge-eating/purging subtype, while the remaining half had a history of AN restricting subtype. The majority of the recovered AN women ($n = 19$ and 86%) reported that they received treatment for their eating disorder.

3.2. Behavioral results

Response times (RTs) were calculated for all correct trials with an RT between 200 and 1200 ms. Frequencies of correct and incorrect responses were converted to represent percentage correct responses. These data were analyzed with two-way analysis of variance (ANOVA) models. The task evoked emotional conflict (main effect of condition), indicated by slower RTs for incongruent ($M = 693.60$ ms, $SD = 69.27$ ms) compared with congruent trials ($M = 650.04$ ms, $SD = 61.24$ ms; $F(1,41) = 103.71$, $p < 0.001$, $\eta_p^2 = 0.72$).

Table 1
Participant characteristics.

Characteristic	Recovered AN (n=22)		HC (n=21)		Two-sample t-test		
	Mean (range)	SD	Mean (range)	SD	t (df)	p	d
Age	27.32 (20–38)	5.14	26.00 (19–35)	4.71	0.96 (41)	.386	0.27
BMI ^a	20.39 (18.06–23.79)	1.66	21.85 (18.32–25.62)	1.76	–2.70 (38)	.010	–0.85
STAI trait	38.77 (20–68)	11.48	28.67 (20–42)	6.42	3.54 (41)	.001	1.09
STAI state	32.14 (20–47)	8.16	26.10 (20–38)	5.22	2.88 (41)	.006	0.88
DERS total	77.36 (40–141)	24.47	62.24 (42–96)	14.61	2.45 (41)	.019	0.75
BDI	6.36 (0–36)	7.94	1.86 (0–8)	2.73	2.47 (41)	.018	0.76
EDE-Q global	0.84 (0.00–2.49)	0.74	0.20 (0.00–0.65)	0.17	3.90 (41)	<.0001	1.19

Abbreviations-AN, anorexia nervosa; HC, healthy controls; STAI, Spielberger State-Trait Anxiety Inventory; DERS, Difficulties with Emotional Regulation; BDI, Beck Depression Inventory; EDE-Q, Eating Disorder Examination-Questionnaire; d, Cohen's d effect size.

^a Data not available for three recovered anorexia nervosa women.

For accuracy, there was a similar effect of emotional conflict, where incongruent trials ($M=93.58\%$, $SD=0.06\%$) were associated with lower accuracy compared with congruent trials ($M=96.95\%$, $SD=0.03\%$; $F(1,41)=22.00$, $p < 0.001$, $\eta_p^2=.35$). These data are in line with previous studies using the same task (Etkin et al., 2006; Jarcho et al., 2013). For both RTs and accuracy, we failed to detect a main effect of group or a group \times condition interaction effect ($p > 0.05$).

3.3. MRI results

There was a main effect of condition in widespread frontal, parietal, and temporal areas. Post-hoc paired samples *t*-tests revealed that emotional conflict (incongruent > congruent) was associated with increased activation in the inferior frontal gyrus, middle frontal gyrus, supplementary motor area (dorsomedial prefrontal cortex), middle temporal gyrus, insula and in a large cluster extending from the precentral to postcentral gyrus (see Supplemental material, Table S1). This is in line with previous studies, which also showed increased activation in the insula, dorsomedial prefrontal cortex, and parietal cortex in response to emotional conflict (Etkin et al., 2010; Jarcho et al., 2013). Further, emotional non-conflict (congruent > incongruent) was associated with increased activation in the precuneus/cuneus, posterior cingulate cortex, and fusiform gyrus.

ROI and whole-brain analyses showed no main effect of group. This suggests that the stimuli in the task (i.e., words and faces) were associated with similar neural responses in both groups. No interaction effect was observed within the ROIs in the anterior cingulate cortex, inferior frontal gyrus, or middle frontal gyrus.

However, there was a significant group \times condition interaction effect in the bilateral amygdala ROIs ($p < 0.05$, family-wise error-corrected, see Table 2 and Fig. 1). Post-hoc two-sample *t*-tests of the β -weights (extracted from voxels showing a significant interaction effect within left and right amygdala ROIs) revealed that this effect was due to lower activation in the left ($t[41]=-2.35$, $p=0.023$, $d=-0.72$) and right ($t[41]=-2.66$, $p=0.011$, $d=-0.81$) amygdala during congruent trials in recovered AN women compared with healthy controls (see Fig. 1). Specifically, one-sample *t*-tests showed that while healthy controls exhibited significant activation in both left ($t[20]=2.14$, $p=0.045$) and right ($t[20]=2.58$, $p=0.018$) amygdala during congruent trials, this activation was absent in the recovered AN group ($p > 0.05$). In contrast, both groups showed an absence of amygdala activation during incongruent trials ($p > 0.05$).

Exploratory whole-brain analyses showed a similar group \times condition interaction effect in a large cluster centered in the right amygdala ($p < 0.001$ uncorrected for multiple comparisons, see Table 2 and Fig. 2). However, this cluster extended outside the amygdala, into the hippocampus and basal ganglia, including the

Table 2
Brain regions showing a group \times condition interaction.

Brain region	Peak MNI coordinates			Cluster size		
	x	y	z	(# voxels)	F (df=41)	z
Region of interest analyses						
Left amygdala	–27	–3	–21	19	19.94	3.84
Right amygdala	24	–3	–24	21	19.07	3.76
Whole-brain analyses						
Right amygdala ^a	24	–6	–9	85	24.27	4.19

Abbreviations-MNI, Montreal Neurological Institute. Region of interest analyses are based on small volume corrections, thresholded at voxel-level $p < 0.05$, family-wise error corrected. Whole-brain analyses are thresholded at voxel-level $p < 0.001$, uncorrected for multiple comparisons, with a minimum cluster size of 20 voxels.

^a Cluster extending into the hippocampus, globus pallidus, and putamen.

globus pallidus and putamen. No other clusters showed a significant group \times condition effect in the whole-brain analysis.

We investigated if the amygdalar hypoactivations during congruent trials were similar for women with a history of restricting AN versus binge-eating/purging AN subtype, by performing additional *t*-tests on the extracted β -weights from left and right amygdala. When compared separately with healthy controls, both AN subtypes showed similar and-in all but one comparison-significant hypoactivation in left (binge-eating/purging AN: $t[30]=-2.55$, $p=0.016$; restricting AN: $t[30]=-1.27$, $p=0.215$) and right (binge-eating/purging AN: $t[30]=-2.16$, $p=0.039$; restricting AN: $t[30]=-2.17$, $p=0.038$) amygdala during congruent trials. Moreover, there were no differences between AN subtypes in terms of amygdalar activations during congruent trials in left ($t[20]=1.35$, $p=0.193$) and right ($t[20]=0.20$, $p=0.843$) amygdala, indicating that the amygdalar alterations are present in recovered AN women regardless of AN subtype history.

To investigate potential associations between the observed AN-related amygdalar alterations and participant characteristics, β -weights from the left and the right amygdala during congruent trials were separately correlated (using Spearman r_s) with the following variables: age, body mass index, STAI, BDI, DERS, and EDE-Q. This was performed both on the whole sample and separately for the two groups. For the whole sample, the DERS (total score) showed an inverse association with activation in the left amygdala during congruent trials ($r_s=-0.341$, $p=0.025$, see Fig. S2). Within each group separately, this association showed the same direction, but was not statistically significant (recovered AN: $r_s=-0.233$, $p=0.296$; healthy controls: $r_s=-0.321$, $p=0.155$). We then redid the ROI and whole-brain analyses including this variable as a covariate, which did not significantly alter our results. None of the other participant characteristics showed a significant

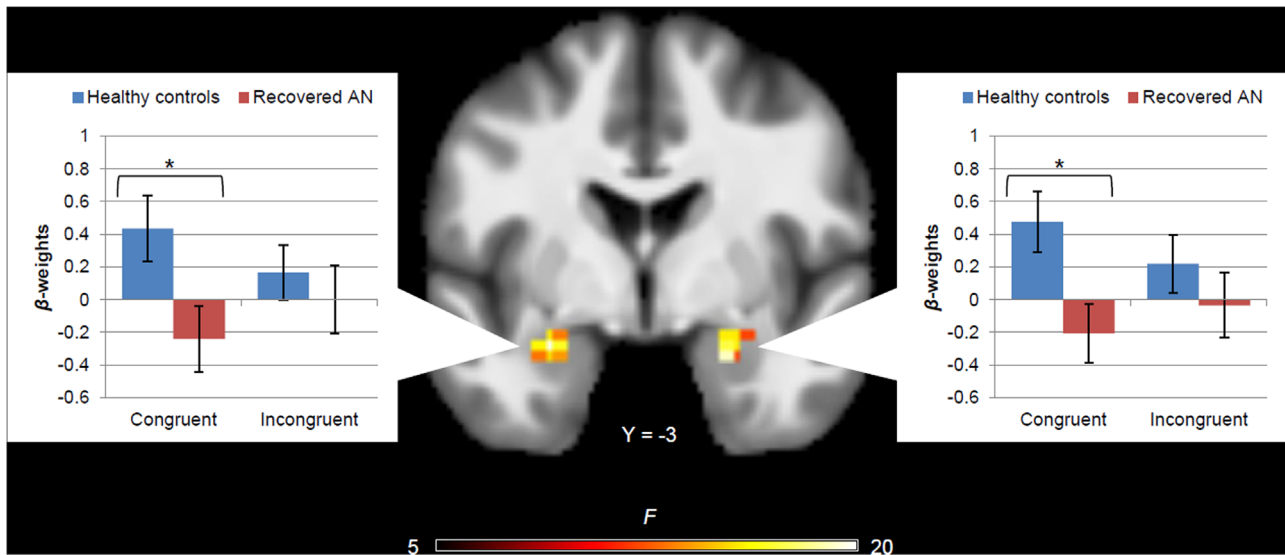


Fig. 1. Amygdala activations associated with emotional conflict (incongruent trials) and non-conflict (congruent trials). Bars show mean β -weights with standard error of mean ($*=p < 0.05$). Clusters contain voxels showing a group \times condition interaction effect thresholded at $p < 0.05$ family-wise error corrected, and are overlaid on a group average anatomical image displayed in neurologic convention (left side of image corresponds to left brain hemisphere). Abbreviations-AN, anorexia nervosa.

association with the amygdalar activations (all $p > 0.05$). Within the recovered AN group, β -weights from the left and the right amygdala during congruent trials were also correlated with illness duration, recovery duration, age of AN onset, and lowest weight ever, but none of these associations reached statistical significance (all $p > 0.05$).

4. Discussion

Using an emotional conflict task, we demonstrated bilateral amygdala alterations in women recovered from AN. Specifically, while emotional conflict evoked similar brain activation across both groups, emotional non-conflict evoked greater amygdalae activations in healthy controls compared to recovered AN women. This was due to activation of the amygdalae during non-conflict in healthy controls, and lack of this activation in recovered AN women. Similar alterations were also observed in the hippocampus and basal ganglia. Despite the differential neural response,

behavioral performance was similar between groups, showing that recovered women's performance was unaffected. Contrary to our expectations, there were no group differences within the dorso-lateral prefrontal or anterior cingulate cortices.

The amygdala is a complex structure important in detecting relevant or biological significant stimuli (Sander et al., 2003). As such, it is highly responsive towards all emotional stimuli, particularly emotional faces (Sergerie et al., 2008). Functional alterations of the amygdalae appear to characterize anxiety-prone individuals, and patients with anxiety disorders (Etkin and Tor, 2007; Stein et al., 2007). In our study, healthy controls exhibited enhanced amygdalae activation during non-conflicting relative to conflicting emotional stimuli. Behaviorally, both healthy controls and recovered AN women responded faster in the absence of emotional conflict, indicating that conflict is associated with higher cognitive load.

These results are in line with earlier research showing that as cognitive load increases, emotional information is suppressed (Okon-Singer et al., 2013). For instance, Kron et al. (2010) reported



Fig. 2. Cluster from the whole brain analysis ($p < 0.001$ uncorrected for multiple comparisons) showing a group \times condition interaction effect. Cluster shows regions less active in recovered anorexia nervosa women compared with healthy controls during non-conflict (congruent trials). The cluster is centered in the right amygdala, but extends into the hippocampus, globus pallidus, and putamen. Voxels are overlaid on a group average anatomical image displayed in neurologic convention (left side of image corresponds to left brain hemisphere).

that participants performing a high cognitive load task reported less intense negative and positive feelings following exposure to emotional images, compared with participants performing a low cognitive load task. Even subliminal emotional images interfere with performance during conditions of low, but not high cognitive load (Uher et al., 2014). Furthermore, Van Dillen and associates (Van Dillen et al., 2009) reported that performing a demanding arithmetic task following a negative mood-induction led to attenuated amygdalae activation, and reduced subjectively experienced negative emotions. Others have reported similar attenuation of the amygdalae during tasks of high cognitive load, and enhanced amygdala response during low cognitive load (Hariri et al., 2000; Liberzon et al., 2000; Mitchell et al., 2007). These studies suggest that emotion and cognition draw from a common pool of resources (Kron et al., 2010), and consumption of these reciprocally affects their manifestation. In these veins, the enhanced amygdala activation during non-conflict in healthy controls probably reflects the higher cognitive load during emotional conflict as opposed to non-conflict.

In contrast to the healthy controls, the recovered AN women failed to exhibit enhanced activation of the amygdala during emotional non-conflict. This effect was observed both in individuals with a history of restricting and binge-eating/purging AN subtype. This suggests that recovered AN individuals do not process the emotional information to the same extent as healthy controls, even when available cognitive resources permit them to. It is possible that women recovered from AN have an impaired ability to identify or process the emotional significance of stimuli, which do have some empirical support (Oldershaw et al., 2011). It is worth noting that the recovered AN women in our study did not exhibit differential neural responses to the words or faces per se, as there was no main effect of group. Furthermore, when groups were considered together, activation in the left amygdala during emotional non-conflict showed a significant inverse correlation with self-reported difficulties in emotion regulation. However, it is possible that this association is driven by the significant between-group differences in emotion regulation skills. Indeed, within each group separately, the association between left amygdala and emotion regulation was non-significant.

Our finding of decreased amygdala activation is in contrast to previous studies of AN, which have reported greater amygdala activation in AN individuals compared with healthy controls (Elison et al., 1998; Joos et al., 2011; Miyake et al., 2010; Puijs et al., 2012; Seeger et al., 2002; Vocks et al., 2010, 2011). However, these studies explored the processing of disorder-specific stimuli such as food and bodies, and the enhanced amygdala activation could be due to the greater emotional significance of such stimuli to AN individuals. Regardless, our results are in line with the notion that alterations of limbic circuits related to emotion are central in the pathophysiology of AN (Hatch et al., 2010; Lipsman et al., 2014; Southgate et al., 2005).

In addition to the amygdala alterations, we also found that emotional non-conflict evoked less activation in the hippocampus, globus pallidus, and putamen among recovered AN women compared with healthy controls. While the role of the hippocampus in this context probably reflects emotional processes similar to the amygdala, the involvement of the globus pallidus and putamen is not clear. Previous studies have reported basal ganglia alterations among individuals recovered from AN in response to taste (Wagner et al., 2008) and inhibition (Kullmann et al., 2014a) tasks, and in ill patients when viewing images of bodies (Fladung et al., 2010). It has been suggested that alterations within basal ganglia circuits are related to the pathophysiology of AN, by influencing how individuals with AN encode and process the reward value or affective valence of stimuli (Kaye et al., 2013). However, the exact nature of the basal ganglia alterations identified in the present

study is unclear. It is important to note that the alterations within the hippocampus and basal ganglia structures were only evident at a liberal threshold, and were part of a continuous cluster centered in the right amygdala.

Contrary to our hypotheses, we did not find any group differences in the cognitive control circuitry, including lateral prefrontal and anterior cingulate cortices. Several studies have reported that ill and recovered AN patients are characterized by both decreased (Oberndorfer et al., 2011; Sato et al., 2013; Zastrow et al., 2009) and increased (Ehrlich et al., 2015; Lao-Kaim et al., 2015) activation within these circuits during tasks challenging cognitive control. It is unclear why the present study failed to detect similar alterations. Two studies showed that alterations in cognitive control circuits were only evident as cognitive demand increased (Oberndorfer et al., 2011; Wierenga et al., 2014). It could be that our task was not sufficiently demanding for such alterations to be evident. Indeed, the task we employed was relatively slow-paced and participants made few errors. Alternatively, it is possible that the face-word stimuli used in the present study were not sufficiently salient to stress the cognitive control circuitry in the recovered AN group. Using an emotional Stroop task, Redgrave et al. (2008) were able to demonstrate widespread alterations within cognitive control circuits in AN patients. However, they used AN-related stimuli which are highly salient to AN individuals, which might put more strain on the cognitive control system. Furthermore, the emotional conflict task employed in the present study has not previously been used with AN samples, and it is possible that other tasks are more suited to explore the alterations within cognitive control circuits in AN. Our results stress the need for more studies investigating cognitive control circuits in ill and recovered AN patients, and to determine their role in the pathophysiology.

The present study has several limitations. The most important limitation pertains to the cross-sectional design, which does not enable us to differentiate brain scarring effects following prolonged emaciation from trait dispositions. Another limitation is our lack of a non-emotional control condition. Thus, it is not possible to say if the amygdala hypoactivation associated with the recovered AN group is specific to emotional stimuli. However, considering the role of the amygdala in signifying biological or emotional significance (Sander et al., 2003), it is likely that this effect is specific to emotional stimuli. Lastly, the present study has a number of possible confounders, including between-group differences in body mass index and self-reported anxiety, depressive symptomatology, eating disorder psychopathology, and emotion regulation difficulties. However, we did investigate the associations between these potential confounders and the amygdala activations during congruent trials, and only the association between self-reported emotion regulation difficulties and left amygdala was statistically significant, and including this self-report measure as a covariate did not significantly alter our results.

In conclusion, our study is the first to show functional amygdala alterations in women recovered from AN, in response to a task that is unrelated to AN symptomatology. This provides further evidence of alterations within limbic circuits in AN, which persist following recovery. Contrary to our expectations, we did not find evidence of alterations within cognitive control circuits. Further research is needed to determine the nature of the alterations within emotion and cognitive control circuits, the interplay between them, and to what extent they reflect trait dispositions.

Contributors

All authors were involved in planning the study. Data were collected by L.B, who also performed the analyses with

contributions from T.E. L.B wrote the manuscript with contributions from both co-authors.

Conflict of interest

The authors have no conflicts of interest.

Acknowledgments

We thank Deborah L. Reas, Ph.D., for proofreading the manuscript.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at <http://doi:10.1016/j.psychres.2015.12.008>.

References

- American Psychiatric Association, 2000. *Diagnostic and Statistical Manual of Mental Disorders*, fourth ed. Author, Washington, DC.
- American Psychiatric Association, 2013. *Diagnostic and Statistical Manual of Mental Disorders*, fifth ed. Author, Arlington, VA.
- Ashburner, J., 2007. A fast diffeomorphic image registration algorithm. *Neuroimage* 38, 95–113.
- Beck, A.T., Ward, C.H., Mendelson, M., Mock, J., Erbaugh, J., 1961. An inventory for measuring depression. *Arch. Gen. Psychiatry* 4, 561–571.
- Boehm, I., Geisler, D., King, J.A., Ritschel, F., Seidel, M., Araujo, Y.D., 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.* 9.
- Brett, M., Anton, J.-L., Valabregue, R., Poline, J.-B., 2002. Region of interest analysis using an SPM toolbox [abstract]. In: *Proceedings of 8th International Conference on Functional Mapping of the Human Brain*, Sendai, Japan.
- Cassin, S.E., von Ranson, K.M., 2005. Personality and eating disorders: a decade in review. *Clin. Psychol. Rev.* 25, 895–916.
- Chan, R.C.K., Shum, D., Touloupoulou, T., Chen, E.Y.H., 2008. Assessment of executive functions: Review of instruments and identification of critical issues. *Arch. Clin. Neuropsychol.* 23, 201–216.
- Cowdrey, F.A., Filippini, N., Park, R.J., Smith, S.M., McCabe, C., 2012. Increased resting state functional connectivity in the default mode network in recovered anorexia nervosa. *Hum. Brain Mapp.* 2014, 483–491.
- Ehrlich, S., Geisler, D., Ritschel, F., King, J.A., Seidel, M., Boehm, I., Breier, M., Clas, S., Weiss, J., Marxen, M., 2015. Elevated cognitive control over reward processing in recovered female patients with anorexia nervosa. *J. Psychiatry Neurosci.* 40, 307–315.
- Ellison, Z., Foong, J., Howard, R., Bullmore, E., Williams, S., Treasure, J., 1998. Functional anatomy of calorie fear in anorexia nervosa. *Lancet* 352, 1192–1192.
- Etkin, A., Egner, T., Peraza, D.M., Kandel, E.R., Hirsch, J., 2006. Resolving emotional conflict: a role for the rostral anterior cingulate cortex in modulating activity in the amygdala. *Neuron* 51, 871–882.
- Etkin, A., Prater, K.E., Hoelt, F., Menon, V., Schatzberg, A.F., 2010. Failure of anterior cingulate activation and connectivity with the amygdala during implicit regulation of emotional processing in generalized anxiety disorder. *Am. J. Psychiatry* 167, 545–554.
- Etkin, A.W., Tor, D., 2007. Functional neuroimaging of anxiety: a meta-analysis of emotional processing in PTSD, social anxiety disorder, and specific phobia. *Am. J. Psychiatry* 164, 1476–1488.
- Fairburn, C.G., Beglin, S.J., 2008. Eating disorder examination questionnaire (EDE-Q 6.0). In: Fairburn, C.G. (Ed.), *Cognitive Behavior Therapy and Eating Disorders*. Guilford Press, New York, pp. 309–313.
- First, M.B., Spitzer, R.L., Gibbon, M., Williams, J.B.W., Miriam, Gibbon, Janet B.W., Williams, 2002. *Structured clinical interview for DSM-IV-TR Axis I disorders, research version, patient edition. (SCID-I/P)*. Biometrics Research, New York State Psychiatric Institute, New York, NY.
- Fladung, A.-K., Grön, G., Grammer, K., Herrnberger, B., Schilly, E., Grasteit, S., Wolf, R.C., Walter, H., Wietersheim, Jv., 2010. A neural signature of anorexia nervosa in the ventral striatal reward system. *Am. J. Psychiatry* 167, 206–212.
- Gaudio, S., Piervincenzi, C., Zobel, B., Beomonte, Montecchi, F. Romana, Riva, G., Carducci, F., Quattrocchi, C. Cosimo, 2015. Altered resting state functional connectivity of anterior cingulate cortex in drug naïve adolescents at the earliest stages of anorexia nervosa. *Sci. Rep.* 5, 1–11.
- Gousias, I.S., Rueckert, D., Heckemann, R.A., Dyet, L.E., Boardman, J.P., Edwards, A.D., Hammers, A., 2008. Automatic segmentation of brain MRIs of 2-year-olds into 83 regions of interest. *Neuroimage* 40, 672–684.
- Gratz, K., Roemer, L., 2004. Multidimensional assessment of emotion regulation and dysregulation: development, factor structure, and initial validation of the difficulties in emotion regulation scale. *J. Psychopathol. Behav. Assess.* 26, 41–54.
- Hammers, A., Allom, R., Koeppe, M.J., Free, S.L., Myers, R., Lemieux, L., Mitchell, T.N., Brooks, D.J., Duncan, J.S., 2003. Three-dimensional maximum probability atlas of the human brain, with particular reference to the temporal lobe. *Hum. Brain Mapp.* 19, 224–247.
- Hariri, A.R., Bookheimer, S.Y., Mazziotta, J.C., 2000. Modulating emotional responses: effects of a neocortical network on the limbic system. *Neuroreport* 11, 43–48.
- Hatch, A., Madden, S., Kohn, M., Clarke, S., Touyz, S., Williams, L.M., 2010. Anorexia nervosa: towards an integrative neuroscience model. *Eur. Eat. Disord. Rev.* 18, 165–179.
- Heatherington, T.F., Wagner, D.D., 2011. Cognitive neuroscience of self-regulation failure. *Trends Cogn. Sci.* 15, 132–139.
- Holliday, J., Tchanturia, K., Landau, S., Collier, D., Treasure, J., 2005. Is impaired set-shifting an endophenotype of anorexia nervosa? *Am. J. Psychiatry* 162, 2269–2275.
- Holtkamp, K., Müller, B., Heussen, N., Renschmidt, H., Herpertz-Dahlmann, B., 2005. Depression, anxiety, and obsessionality in long-term recovered patients with adolescent-onset anorexia nervosa. *Eur. Child. Adolesc. Psychiatry* 14, 106–110.
- Jarcho, J.M., Fox, N.A., Pine, D.S., Etkin, A., Leibenluft, E., Shechner, T., Ernst, M., 2013. The neural correlates of emotion-based cognitive control in adults with early childhood behavioral inhibition. *Biol. Psychol.* 92, 306–314.
- Joos, A.A.B., Saum, B., van Elst, L.T., Perlov, E., Glauche, V., Hartmann, A., Freyer, T., Tüscher, O., Zeeck, A., 2011. Amygdala hyperreactivity in restrictive anorexia nervosa. *Psychiatry Res. Neuroimaging* 191, 189–195.
- 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.
- Kron, A., Schul, Y., Cohen, A., Hassin, R.R., 2010. Feelings don't come easy: studies on the effortful nature of feelings. *J. Exp. Psychol. Gen.* 139, 520–534.
- Kullmann, S., Giel, K.E., Hu, X., Bischoff, S.C., Teufel, M., Thiel, A., Zipfel, S., Preissl, H., 2014a. Impaired inhibitory control in anorexia nervosa elicited by physical activity stimuli. *Soc. Cogn. Affect. Neurosci.* 9 (7), 917–923.
- Kullmann, S., Giel, K.E., Teufel, M., Thiel, A., Zipfel, S., Preissl, H., 2014b. Aberrant network integrity of the inferior frontal cortex in women with anorexia nervosa. *NeuroImage: Clin.* 4, 615–622.
- Lao-Kaim, N.P., Fonville, L., Giampietro, V.P., Williams, S.C.R., Simmons, A., Tchanturia, K., 2015. Aberrant function of learning and cognitive control networks underlie inefficient cognitive flexibility in anorexia nervosa: a cross-sectional fMRI study. *Plos. ONE* 10, e0124027.
- Liberzon, I., Taylor, S.F., Fig, L.M., Decker, L.R., Koeppe, R.A., Minoshima, S., 2000. Limbic activation and psychophysiological responses to aversive visual stimuli: interaction with cognitive task. *Neuropsychopharmacology* 23, 508–516.
- Lipsman, N., Woodside, D.B., Lozano, A.M., 2014. Neurocircuitry of limbic dysfunction in anorexia nervosa. *Cortex* 62, 109–118.
- Marsh, R., Maia, T.V., Peterson, B.S., 2009. Functional disturbances within frontostriatal circuits across multiple childhood psychopathologies. *Am. J. Psychiatry* 166, 664–674.
- Miller, E.K., Cohen, J.D., 2001. An integrative theory of prefrontal cortex function. *Annu. Rev. Neurosci.* 24, 167–202.
- Mitchell, D.G.V., Nakkic, M., Fridberg, D., Kamel, N., Pine, D.S., Blair, R.J.R., 2007. The impact of processing load on emotion. *Neuroimage* 34, 1299–1309.
- Miyake, Y., Okamoto, Y., Onoda, K., Shirao, N., Okamoto, Y., Otagaki, Y., Yamawaki, S., 2010. Neural processing of negative word stimuli concerning body image in patients with eating disorders: an fMRI study. *Neuroimage* 50, 1333–1339.
- Oberndorfer, T.A., Kaye, W.H., Simmons, A.N., Strigo, I.A., Matthews, S.C., 2011. Demand-specific alteration of medial prefrontal cortex response during an inhibition task in recovered anorexic women. *Int. J. Eat. Disord.* 44, 1–8.
- Okon-Singer, H., Lichtenstein-Vidne, L., Cohen, N., 2013. Dynamic modulation of emotional processing. *Biol. Psychol.* 92, 480–491.
- Oldershaw, A., Hambrook, D., Stahl, D., Tchanturia, K., Treasure, J., Schmidt, U., 2011. The socio-emotional processing stream in anorexia nervosa. *Neurosci. Biobehav. Rev.* 35, 970–988.
- Pollice, C., Kaye, W.H., Greeno, C.G., Weltzin, T.E., 1997. Relationship of depression, anxiety, and obsessionality to state of illness in anorexia nervosa. *Int. J. Eat. Disord.* 21, 367–376.
- Pruis, T.A., Keel, P.K., Janowsky, J.S., 2012. Recovery from anorexia nervosa includes neural compensation for negative body image. *Int. J. Eat. Disord.* 45, 919–931.
- Redgrave, G.W., Bakker, A., Bello, N.T., Caffo, B.S., Coughlin, J.W., Guarda, A.S., McEntee, J.E., Pekar, J.J., Reinblatt, S.P., Verduzco, G., Moran, T.H., 2008. Differential brain activation in anorexia nervosa to fat and thin words during a stroop task. *Neuroreport* 19 (12), 1181–1185.
- Rø, Ø., Reas, D.L., Stedal, K., 2015. Eating disorder examination questionnaire (EDE-Q) in Norwegian adults: discrimination between female controls and eating disorder patients. *Eur. Eat. Disord. Rev.* 23, 408–412.
- Sander, D., Grafman, J., Zalla, T., 2003. The human amygdala: an evolved system for relevance detection. *Rev. Neurosci.* 14, 303–316.
- Sato, Y., Saito, N., Utsumi, A., Aizawa, E., Shoji, T., Izumiyama, M., Mushiaki, H., Hongo, M., Fukudo, S., 2013. Neural basis of impaired cognitive flexibility in patients with anorexia nervosa. *Plos. One* 8 (5), e61108.
- Seeger, G., Braus, D.F., Ruf, M., Goldberger, U., Schmidt, M.H., 2002. Body image distortion reveals amygdala activation in patients with anorexia nervosa-a

- functional magnetic resonance imaging study. *Neurosci. Lett.* 326, 25–28.
- Sergerie, K., Chochol, C., Armony, J.L., 2008. The role of the amygdala in emotional processing: a quantitative meta-analysis of functional neuroimaging studies. *Neurosci. Biobehav. Rev.* 32, 811–830.
- Southgate, L., Tchanturia, K., Treasure, J., 2005. Building a model of the aetiology of eating disorders by translating experimental neuroscience into clinical practice. *J. Ment. Health* 14, 553–566.
- Spielberger, C.D., Gorsuch, R.L., Lushene, R.D., 1970. *STAI Manual*. Consulting Psychologists Press, Palo Alto, CA.
- Stein, B.M., Simmon, N.A., Feinstein, S.J., Paulus, P.M., 2007. Increased amygdala and insula activation during emotion processing in anxiety-prone subjects. *Am. J. Psychiatry* 164, 318–327.
- Tottenham, N., Tanaka, J.W., Leon, A.C., McCarry, T., Nurse, M., Hare, T.A., Marcus, D. J., Westerlund, A., Casey, B.J., Nelson, C., 2009. The NimStim set of facial expressions: judgments from untrained research participants. *Psychiatry Res.* 168, 242–249.
- Uher, R., Brooks, S.J., Bartholdy, S., Tchanturia, K., Campbell, I.C., 2014. Increasing cognitive load reduces interference from masked appetitive and aversive but not neutral stimuli. *Plos. ONE* 9, e94417.
- Uher, R., Murphy, T., Brammer, M.J., Dalgleish, T., Phillips, M.L., Ng, V.W., Andrew, C. M., Williams, S.C.R., Campbell, I.C., Treasure, J., 2004. Medial prefrontal cortex activity associated with symptom provocation in eating disorders. *Am. J. Psychiatry* 161, 1238–1246.
- Van Dillen, L.F., Heslenfeld, D.J., Koole, S.L., 2009. Tuning down the emotional brain: an fMRI study of the effects of cognitive load on the processing of affective images. *Neuroimage* 45, 1212–1219.
- Vocks, S., Busch, M., Grönemeyer, D., Schulte, D., Herpertz, S., Suchan, B., 2010. Neural correlates of viewing photographs of one's own body and another woman's body in anorexia and bulimia nervosa: an fMRI study. *J. Psychiatry Neurosci.* 35, 163–176.
- Vocks, S., Herpertz, S., Rosenberger, C., Senf, W., Gizewski, E.R., 2011. Effects of gustatory stimulation on brain activity during hunger and satiety in females with restricting-type anorexia nervosa: an fMRI study. *J. Psychiatr. Res.* 45, 395–403.
- Wagner, A., Aizenstein, H., Mazurkewicz, L., Fudge, J., Frank, G.K., Putnam, K., Bailer, U.F., Fischer, L., Kaye, W.H., 2008. Altered insula response to taste stimuli in individuals recovered from restricting-type anorexia nervosa. *Neuropsychopharmacology* 33, 513–523.
- Wierenga, C., Bischoff-Grethe, A., Melrose, A.J., Grenesko-Stevens, E., Irvine, Z., Wagner, A., Simmons, A., Matthews, S., Yau, W.-Y.W., Fennema-Notestine, C., Kaye, W.H., 2014. Altered BOLD response during inhibitory and error processing in adolescents with anorexia nervosa. *Plos. One* 9 (3), e92017.
- Wierenga, C.E., Bischoff-Grethe, A., Melrose, A.J., Irvine, Z., Torres, L., Bailer, U.F., Simmons, A., Fudge, J.L., McClure, S.M., Ely, A., Kaye, W.H., 2015. Hunger does not motivate reward in women remitted from anorexia nervosa. *Biol. Psychiatry* 77 (7), 642–852.
- Zastrow, A., Kaiser, Stefan, 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.
- Zhu, Y., Hu, X., Wang, J., Chen, J., Guo, Q., Li, C., Enck, P., 2012. Processing of food, body and emotional stimuli in anorexia nervosa: a systematic review and meta-analysis of functional magnetic resonance imaging studies. *Eur. Eat. Disord. Rev.* 20, 439–450.