STRUCTURAL AND FUNCTIONAL BRAIN ALTERATIONS IN WOMEN RECOVERED FROM ANOREXIA NERVOSA

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Dissertation submitted for the degree of Ph.D. at the
Department of Psychology
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ACKNOWLEDGEMENTS

I am grateful to a number of people who have contributed to this doctoral thesis. First, I would like to express my gratitude to Oslo University Hospital and the Regional Department for Eating Disorders (RASP), for giving me the opportunity to develop the research project that eventually led to this doctoral thesis. I am grateful to the late Professor Bryan Lask, who first hired me as a research assistant, and encouraged me to pursue my own research interests and a Ph.D.

Thank you Heidi Langbakk-Skille, for being a supportive and solution-oriented leader. And thank you for reminding me of holidays and other important dates. Without you, I fear that I would have missed Christmas and Easter the last four years.

To Øyvind Rø, my main supervisor and research director at RASP. You have supported me and my work from the very beginning. I appreciate the sincere, caring, and stable presence you have had during this project. Thank you for your invaluable contributions, for your support and trust, and for providing me with the flexibility and autonomy to thrive as a researcher.

To Tor Endestad, my co-supervisor and invaluable source of knowledge for all things related to MRI. Your knowledge and experience have been crucial for this project. Over the years, you always brought new ideas to the table, directed and focused my thinking, and supported my work. Thank you for your contributions, enthusiasm and positivity.

I am indebted to Tommy Sinnes, Lars Christian Vold, Laura Anne Wortinger, and Grethe Løvland for assisting me with the collection of MRI data at all hours of the day, all days of the week. A special thanks to the user-organizations “Spiseforstyrrelsесforeningen” and “Rådgivning om Spiseforstyrrelser”, who were supportive of this project and helped me recruit participants. Also, I would like to express my sincere gratitude to all women who participated in this project.

To all my wonderful colleagues at RASP, past and current, who provided a stimulating and social workplace during my work with this thesis. Thanks particularly
to Alina Coman, Kristin Stedal, and Line Wisting, whom I shared office, drinks, and the occasional workshop with, and who helped me in various ways with this thesis. I am also grateful to my colleague Deborah L. Reas, who proofread papers 1 and 2, and helped me in numerous ways over the years.

And finally to Camilla Bergland, who has supported me since day one. I am forever grateful for your patience, care, love, and “power backrubs” during the past four years. It’s been a long haul, but thankfully you were there with me, every step of the way.
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<tr>
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<td>ACC</td>
<td>Anterior cingulate cortex</td>
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<tr>
<td>AN</td>
<td>Anorexia nervosa</td>
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<td>BMI</td>
<td>Body mass Index</td>
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<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<td>CW</td>
<td>Comparison women</td>
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<tr>
<td>DSM-IV</td>
<td>Diagnostic and Statistical Manual Fourth Edition</td>
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<tr>
<td>DSM-5</td>
<td>Diagnostic and Statistical Manual Fifth Edition</td>
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<tr>
<td>EDE-Q</td>
<td>Eating Disorder Examination-Questionnaire</td>
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<td>fMRI</td>
<td>Functional magnetic resonance imaging</td>
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<tr>
<td>FWE</td>
<td>Family-wise error correction</td>
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<td>FWHM</td>
<td>Full width at half maximum</td>
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<td>GM</td>
<td>Gray matter</td>
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<td>Mm</td>
<td>Millimeters</td>
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<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<td>Ms</td>
<td>Milliseconds</td>
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<td>PFC</td>
<td>Prefrontal cortex</td>
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<td>RAN</td>
<td>Recovered anorexia nervosa</td>
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<td>ROI</td>
<td>Region of interest</td>
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<td>RT</td>
<td>Response time</td>
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<tr>
<td>SPM</td>
<td>Statistical Parametric Mapping</td>
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<tr>
<td>VBM</td>
<td>Voxel-Based Morphometry</td>
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<td>WM</td>
<td>White matter</td>
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LIST OF PAPERS


Bang, Lasse; Rø, Øyvind; Endestad, Tor (2015). Amygdala alterations during an emotional conflict task in women recovered from anorexia nervosa. Psychiatry Research: Neuroimaging. 248 (126-133).

Bang, Lasse; Rø, Øyvind; Endestad, Tor (2016). Threat-detection and attentional bias to threat in women recovered from anorexia nervosa: Neural alterations in extrastriate and medial prefrontal cortices. Submitted.
ABSTRACT

Anorexia nervosa (AN) is a mental disorder that predominantly affects adolescent females. It is characterized by a relentless pursuit of thinness, severe food restriction, body image disturbances, and an extremely low body weight. There has been considerable interest in the neurobiology associated with AN, and a wealth of neuroimaging studies have shed light on the neural correlates of this disorder.

Studies have consistently shown that patients with AN are characterized by brain mass reductions. It is clear that these reductions are largely secondary to malnutrition and emaciation. However, the extent to which similar reductions are present following weight-gain and recovery is unclear, with some studies showing full normalization and others reporting persistent reductions, particularly in gray matter. These persistent reductions could reflect irreversible damage due to emaciation, or alternatively trait characteristics related to AN.

AN is also associated with functional brain alterations in widespread circuits, including parietal, limbic, and prefrontal cortices. Available evidence suggests that AN is characterized by an imbalance within or between brain circuits related to cognitive control and emotion. Such alterations may be associated with the emotional dysregulation and high anxiety that characterizes AN. Still, the nature of these functional brain alterations is not understood.

The overall aim of this doctoral thesis was to advance our understanding of the structural and functional brain alterations associated with AN. Specifically, we wanted to a) determine the presence of brain tissue reductions in women recovered from AN, and b) investigate the neural responses in prefrontal and limbic brain circuits during emotional tasks in women recovered from AN.

To this end, we used magnetic resonance imaging to measure brain structure and function. We measured neural responses to emotion-tasks which required emotional stimuli to be suppressed or ignored, thus hoping to shed light on neural responses involved in both bottom-up emotional arousal and top-down control over emotion.
Women recovered from AN were compared to comparison women similar in age and education.

In *Paper 1*, we reported that brain tissue volumes in women recovered from AN were similar to comparison women. This contributes to the inconsistent research literature, and is in accordance with several previous studies. These findings suggest that women recovered from AN have normal brain volumes, and that the brain tissue reductions observed during the acute stage of AN are not trait characteristics, but transient reductions secondary to emaciation and malnutrition. In *Paper 2*, we showed that women recovered from AN were characterized by altered amygdala responses during an emotional conflict task. This indicates that AN is associated with functional alterations in the emotion-circuitry. In *Paper 3*, we showed that women recovered from AN were characterized by diminished responses to threat stimuli in the extrastriate cortex, which may reflect altered saliency processing. Furthermore, these women exhibited increased responses in the medial prefrontal cortex when an attentional reorientation away from threat stimuli was required. This hyperactivation could reflect heightened cognitive control in the presence of distracting emotional stimuli.

This thesis contributes to the existing research literature of AN by advancing our understanding of the neurobiological underpinnings of AN.
1. INTRODUCTION TO THESIS

With the advent of modern neuroimaging techniques, considerable effort has been made to investigate the neurobiological correlates of mental disorders. Understanding the neuropathophysiology (the neurobiological correlates of mental disorders) can contribute to the overall understanding of etiological mechanisms by which such disorders develop. This in turn, has potential implications for nosology and treatment. In the past decades, a wealth of studies has used neuroimaging to study the neurobiological correlates of anorexia nervosa (AN). These have shown that AN is associated with both structural and functional brain alterations. Still, the neuropathophysiology and etiology of this disorder is not understood. The extent to which structural and functional brain alterations are secondary to emaciation and malnutrition, or reflect trait characteristics of AN is unclear.

The overall aim of this doctoral thesis was to investigate the neuropathophysiology of AN, and in so doing advance our understanding of AN. The three papers included in this thesis all employ magnetic resonance imaging to measure brain structure and function in women recovered from anorexia nervosa.

The following sections will provide a general introduction to AN, and an overview of current knowledge about the neuropathophysiology. The methodology underlying the three papers included in this thesis will then be described, followed by a summary of the findings. For specific details regarding the methodology and results of the three studies included in this thesis, the reader is referred to the specific papers. Last, the discussion will deliberate on the findings from the papers, and on overarching methodological issues regarding this thesis.
2. BACKGROUND

2.1 Anorexia Nervosa

AN is a mental disorder that predominantly affects adolescent girls, although it can also occur in males and at any age. It is characterized by a relentless pursuit of thinness, severe food restriction, body image disturbances, and an extremely low body weight. Patients with AN have an intense fear of weight-gain and a distorted view of their own body; often viewing themselves as fat despite being emaciated. It has been demonstrated that starvation and emaciation have profound psychological effects on humans (Keys, Brožek, Henschel, Mickelsen, & Taylor, 1950), and these secondary effects play an important role in AN symptomatology. AN belongs to a category of mental disorders referred to as ‘eating disorders’, alongside other disorders including bulimia nervosa and binge-eating disorder. As this thesis focused on AN, all subsequent sections will deal exclusively with this particular eating disorder.

2.2 Diagnostic classification

AN is diagnosed based on two classification systems; Diagnostic and Statistical Manual of Mental Disorders - DSM (APA – American Psychological Association, 2013), and the International Classification of Diseases - ICD (WHO – World Health Organization, 1992). In this thesis, the DSM was used. At the outset of this thesis, the current version of the DSM was DSM-IV, and this version was used to evaluate criteria for AN. The DSM-IV lists four criteria for AN:

A) refusal to maintain a minimal normal body weight, or failure to make expected weight gain during period of growth,
B) Intense fear of gaining weight or becoming fat, even though underweight,
C) body image disturbances, or an undue influence of body weight or shape on self-evaluation, or denial of the seriousness of the current low body weight, and
D) In postmenarcheal females, absence of three consecutive menstrual cycles (amenorrhea).
For criterion A, a body weight less than 85% of that expected (given gender, age and height) is considered below normal. The DSM-IV enables further subtyping of AN into two subtypes: Binge-eating/purging, and Restricting subtype. Binge-eating/purging AN subtype is characterized by regular episodes of binge-eating (eating a large amount of food with an accompanying sense of loss of control) and/or purging behaviors (e.g. vomiting, laxative misuse). Restricting AN subtype is characterized by absence of such behaviors.

In 2013, the DSM-5 version was published, with several changes to the diagnostic criteria for AN. The major changes included removing a specific weight threshold for criterion A, and removing criterion D (amenorrhoea) altogether. In addition, minor adjustments were made in the wording of the criteria. The majority of literature presented in the following sections have used the DSM-IV definition of AN.

2.3 Prevalence & incidence

International studies have reported that the overall point-prevalence of AN among young females is 0.3%, with an incidence of eight per 100 000 persons per year (Hoek, 2006). The overall incidence rates in Europe have remained stable since the 1970’s, but incidence rates have increased in 15-19 year old girls (Smink, Van Hoeken, & Hoek, 2012). It is not known whether this increase reflects earlier detection or earlier age of onset. It could also be attributed to a general increase in diagnosed mental disorders (Steinhausen & Jensen, 2015). AN affects more females than males, with a ratio of 10:1 (APA, 2013). Few estimates of prevalence and incidence have been reported using the current DSM-5 criteria. Two recent studies have shown that compared to the DSM-IV, lifetime prevalence of AN among females increase by 50-60% when applying DSM-5 criteria (Mustelin et al., 2016; Smink, van Hoeken, Oldehinkel, & Hoek, 2014) .

There are no reliable or up-to-date prevalence estimates of AN in Norway. One study relying on self-report measures estimated that AN has a point-prevalence of 0.3% and a lifetime prevalence of 0.4% among Norwegian females (Götestam & Agras, 1995). A later study using diagnostic interviews found AN and bulimia nervosa combined had a lifetime prevalence of 3%, and a 12-month prevalence of 1.2%
among Norwegian females (Kringlen, Torgersen, & Cramer, 2001). Rates were considerably lower for Norwegian males, where AN and bulimia nervosa had a combined lifetime prevalence of 0.2%, and 12-month prevalence of 0.0%. Both these Norwegian studies used DSM-III criteria for establishing AN diagnosis, so rates of AN according to DSM-5 criteria are likely higher today.

2.4 Treatment, course and outcome

Treatment for AN involves a combination of renourishment and psychotherapy. In some cases, treatment must also addresses somatic complications secondary to emaciation and malnutrition to ensure medical stabilization. There is limited evidence to suggest one psychotherapeutic approach is more advantageous than others. Current evidence favors family-based treatment for adolescent patients, but for adults no specific approach have unequivocally shown to be more efficacious or effective (Watson & Bulik, 2013). One recent randomized controlled trial (Zipfel et al., 2014) compared focal psychodynamic therapy, cognitive behavior therapy, and optimized treatment as usual in adult outpatients with AN, and found no significant differences in outcome.

There is also limited evidence for pharmacological treatments. Both atypical antipsychotic and antidepressant medications have been evaluated, but findings are inconsistent and do not point to a specific treatment effect for AN (Frank & Shott, 2016; Watson & Bulik, 2013). One meta-analysis (Lebow, Sim, Erwin, & Murad, 2013) concluded that atypical antipsychotics had no statistically significant beneficial effect on weight-gain or AN psychopathology. However, these medications were associated with significant reductions in depressive symptoms, but also a significant increase in anxiety. In sum, evidence for pharmacological treatment of AN is inconclusive.

Treatment outcome is often unsatisfactory. According to one study (Löwe et al., 2001) showed that at follow-up 21 years after first hospitalization, 51% of patients were fully recovered, 21% were partially recovered, and 10% still met AN criteria. In a review of outcome studies, Steinhausen (2002) reported that on average less than 50% of patients have a good outcome, while 20% remain chronically ill. Prognostic
risk factors for poor outcome include purging behaviors, chronicity of illness, older age of onset, and obsessive-compulsive personality traits (Steinhausen, 2002).

Medical complications are common, and can adversely affect almost every organ system (Mehler & Brown, 2015). This includes dermatological, gastrointestinal, cardiovascular, endocrine and metabolic, hematologic, opthalamic, and pulmonary complications. Osteoporosis or osteopenia affect the majority of patients with AN, and cardiovascular complications such as bradycardia and hypotension are also common.

Partly due to these serious complications, mortality rates are high. Patients are 5.2 times more likely to die prematurely from any cause, and 18.1 times more likely to die by suicide, compared to 15-34 year old females in the general population (Keshaviah et al., 2014). Approximately 20% of patient deaths are attributable to suicide (Arcelus, Mitchell, Wales, & Nielsen, 2011).

2.5 Psychiatric comorbidity

Psychiatric comorbidity is common in patients with AN (Zipfel, Giel, Bulik, Hay, & Schmidt, 2015). According to one study, almost 50% of adolescent patients with first-onset AN have a lifetime history of another psychiatric disorder (Bühren et al., 2014). Depression is one of the most frequently comorbid psychiatric disorders. Fernandez-Aranda and colleagues (2007) reported that 73% of patients with AN had a lifetime history of major depressive disorder, and that 76% of comorbid cases developed both disorders during a 3 year window. Rates of anxiety disorders are also elevated in AN (see Swinbourne & Touyz, 2007 for a review). One study reported that 64% of AN individuals had a lifetime history of one or more anxiety disorders (Kaye, Bulik, Thornton, Barbarich, & Masters, 2004). The most common disorders were obsessive-compulsive disorder (41%) and social phobia (20%). Indeed, onset of an anxiety disorder, particularly obsessive-compulsive disorder, has been shown to increase risk for later development of AN (Meier et al., 2015).

Additionally, personality disorders show high comorbidity with AN. A meta-analysis (Farstad, McGeown, & von Ranson, 2016) showed that avoidant, obsessive-
compulsive, borderline and paranoid personality disorders are frequently diagnosed in AN patients. The distribution of specific personality disorders differed between the AN subtypes, and those with binge-eating/purging subtype showed particular high rates of personality disorders.

Other frequent comorbid disorders include autism-spectrum disorder (Koch et al., 2015; Zucker et al., 2007) and body dysmorphic disorder (Hartmann, Greenberg, & Wilhelm, 2013), which generally has received less attention than the other comorbid conditions mentioned.

2.6 Cognitive and emotional impairments

AN is associated with a range of neuropsychological and emotional impairments that may play an important role in the development and maintenance of the disorder.

Patients with AN show decreased performance on neuropsychological tests of cognitive flexibility and central coherence (Stedal, Frampton, Landrø, & Lask, 2012; Stedal, Rose, Frampton, Landrø, & Lask, 2012). Some of these weaknesses - particularly cognitive flexibility – appear to persist following recovery (Danner et al., 2012; Tchanturia, Morris, Surguladze, & Treasure, 2002; Tenconi et al., 2010). Interestingly, unaffected sisters of patients with AN show similar difficulties, raising the possibility that impaired cognitive flexibility and central coherence are endophenotypes of AN (Holliday, Tchanturia, Landau, Collier, & Treasure, 2005; Tenconi et al., 2010).

A range of emotional processing disturbances have also been described in AN. Anxiety (Kaye et al., 2004) and emotional dysregulation (Haynos, Roberto, Martinez, Attia, & Fruzzetti, 2014) is elevated, which persist following recovery. A wide range of socio-emotional processing impairments have also been reported (see Oldershaw et al., 2011 for a review), including impaired emotion recognition and emotional theory of mind capabilities. While deficits in emotion recognition appears to persist following recovery, emotional theory of mind seems to normalize (Oldershaw et al., 2011). Moreover, both ill and recovered AN patients show attentional bias to faces signifying threat (Cardi et al., 2015; Cardi, Matteo, Corfield, & Treasure, 2012; Harrison,
Tchanturia, & Treasure, 2010), indicating a cognitive system biased towards the processing of threat stimuli. However, not all studies have found such an attentional bias in AN (Schneier et al., 2016; Schober et al., 2014). The fact that some of these impairments (e.g. emotion recognition and attentional bias) are observed following recovery suggests they reflect trait characteristics of AN.

2.7 Etiology

Etiology is concerned with the causes and origins of disorders. It is related to the crucial, but challenging question: why (and how) do some people develop AN, while others do not? AN is a multifactorial disorder whose etiology is bound to be complex (Schmidt, 2003). While the etiology of AN is poorly understood, it is clear that biological, psychological and sociocultural factors are all important. Below, current knowledge of AN etiology is reviewed.

2.7.1 Environmental factors

AN is associated with cultural contexts where thinness is valued (APA, 2013). In Western cultures, thinness is the prevailing body ideal for women, and sociocultural models of AN have viewed this idealization of thinness as a specific risk factor for the development of eating disorders (Striegel-Moore & Bulik, 2007). This is exemplified in a naturalistic study where the introduction of Western television programs was found to increase levels of eating disorder psychopathology in Fijian schoolgirls (Becker, Burwell, Herzog, Hamburg, & Gilman, 2002). Idealization of thinness variables, including media exposure and perceived pressure to be thin have all been found to prospectively predict increased levels of eating disorder psychopathology (Culbert, Racine, & Klump, 2015). The extent to which such variables predict eating disorder diagnoses however, has not been established, warranting further research (Culbert et al., 2015).

The relatively low frequency of AN even in cultures where there is an idealization of thinness could be partly explained by varying degrees of internalization of the thinness ideal in individuals (Striegel-Moore & Bulik, 2007). Internalizing the thinness-ideal presumably leads to an evaluation of one’s own body in light of the ideal. Indeed, thin-ideal internalization is associated with increased negative affect, dieting
behavior, body dissatisfaction, and eating disorder psychopathology (Stice, 2002). However, these associations are small in magnitude, probably reflecting the fact that relationships between such variables are complex.

Stressful life events also increase risk for AN, and can in some cases trigger the onset of the disorder. These include normal but stressful events like moving from home, starting college etc. It also includes adverse life events such as childhood sexual abuse (Brewerton, 2007; Carter, Bewell, Blackmore, & Woodside, 2006), other traumatic events including abuse (Brewerton, 2007), and being bullied in childhood (Engström & Norring, 2002; Kaltiala-Heino, Rissanen, Rimpelä, & Rantanen, 2003).

2.7.2 Gender, age, and ethnicity

AN predominantly affects females (Jacobi, Hayward, de Zwaan, Kraemer, & Agras, 2004). This gender difference is often attributed to the idealization and internalization of Western female thin body ideals. AN typically debuts in adolescence, and onset later in life is uncommon (Striegel-Moore & Bulik, 2007). Adolescence is a period where profound changes take place, and bodily changes associated with puberty (e.g. weight-gain and changes to body composition) may induce risk for AN. Additionally, adolescence may increase risk for AN by increasing the susceptibility for sociocultural pressures to be thin. Ethnicity is also a commonly reported risk factor for AN (Jacobi et al., 2004). However, while AN and other eating disorders historically have been regarded as affecting predominantly White females, the picture is slightly more complicated, and varies depending on the cultural context and outcome measured (Jacobi et al., 2004).

2.7.3 Temperamental factors

Perplexingly, although almost all females living in Western societies are exposed to the thinness ideal, only a small percentage ever begin to engage in behaviors such as extreme dieting and over-exercise that can lead to AN. Therefore, the individuals who do develop AN must be characterized by certain vulnerability traits. Several personality features are associated with heightened risk for AN. These include high perfectionism and neuroticism, and low extraversion (Farstad et al., 2016). An
anxious dispositional style has been shown to prospectively predict development of AN (Bulik et al., 2006). Perfectionism is a particularly potent risk factor for AN (Jacobi et al., 2004), and may also serve to maintain the disorder; one study showed that decreased levels of perfectionism was associated with shorter illness duration (Nilsson, Sundbom, & Hägglöf, 2008). Patients are also characterized by high obsessionality (Holtkamp, Müller, Heussen, Remschmidt, & Herpertz-Dahlmann, 2005; Pollice, Kaye, Greeno, & Weltzin, 1997), which is interesting in light of the high comorbidity rates between AN and disorders characterized by obsessional symptoms, such as obsessive-compulsive disorder.

2.7.4 Genetic factors

Genetic factors play a substantial role in vulnerability for AN (Yilmaz, Andrew Hardaway, & Bulik, 2015). AN aggregate in families, indicating shared familial liability factors (Steinhausen, Jakobsen, Helenius, Munk-Jørgensen, & Strober, 2015; Strober, Freeman, Lampert, Diamond, & Kaye, 2000). Moreover, twin-studies have estimated the heritability for AN to be around 48-74% (Yilmaz et al., 2015). Despite considerable effort, the specific genetic variations underlying AN risk have not been identified. Three genome-wide association studies of AN have conducted (Boraska et al., 2014; Nakabayashi et al., 2009; Wang et al., 2011), but all have failed to detect reliable associations between AN and genetic variations. However, these studies were all underpowered, so amassing bigger samples is a priority within this field (Boraska et al., 2014).

It is possible that the genetic variations underlying risk for AN is related to vulnerability traits such as anxiousness. Also, they could be related to biological factors that determine how an individual physiologically responds to dieting and emaciation. For certain individuals, possibly those at greatest genetic risk, extreme dieting can lead to a cascade of biological and psychological changes that culminate in an eating disorder. Also, it is now recognized that genetic and environmental factors interact in a complex manner via epigenetic processes. Environmental factors can alter the genetic expression and increase AN risk (Campbell, Mill, Uher, & Schmidt, 2011). Few studies have investigated gene x environment interactions in
eating disorders, so it is currently not known what role epigenetic factors play in the development of AN (Campbell et al., 2011).

2.8 Neuropathophysiology of AN

Modern inquiries into the neuropathophysiology of AN started over 60 years ago, when post mortem studies of deceased patients with AN documented brain mass reductions, with prominent sulci and small gyri (Gagel, 1953; Martin, 1958; cited from Wagner et al., 2006). Reduced brain mass was confirmed in later neuroimaging studies, first in case studies (Heinz, Martinez, & Haenggeli, 1977) and next in larger case-control studies (Krieg, Pirke, Lauer, & Herbert, 1988). The extent to which these reductions reversed following weight-restoration was unclear at this point (Krieg et al., 1988). From the late 1980’s and onward, there has been considerable interest in functional brain aberrations in AN. Studies have shown that AN is associated with brain hypometabolism of glucose (Delvenne et al., 1995) and reduced regional cerebral blood flow (Gordon, Lask, Bryant-Waugh, Christie, & Timimi, 1997). In the 1990’s, the first Magnetic Resonance Imaging (MRI) studies of AN were conducted. These investigated both brain structure (Katzman et al., 1996) and function (Ellison et al., 1998), and were followed by a surge of MRI studies into AN neuropathophysiology. There has also been considerable interest in neurotransmitter function, with studies showing altered serotonin and dopamine functioning in AN (Kaye, Wierenga, Bailer, Simmons, & Bischoff-Grethe, 2013). Today, the majority of brain research in the eating disorders field employs MRI to study brain structure and function.

In the following sections, an overview of structural and functional brain changes associated with AN will be introduced, focusing on findings from MRI studies.

2.8.1 Structural brain alterations

Studies have consistently showed that AN is associated with brain mass reductions, which are related to loss of both gray (GM) and white matter (WM) volumes, alongside enlarged cerebrospinal fluid (CSF) cavities (Titova, Hjorth, Schioth, & Brooks, 2013; Van den Eynde et al., 2012). On average, GM is reduced by approximately 6% and WM by 4% compared to healthy controls (Seitz et al., 2013).
These reductions seem to be more pronounced in adolescents compared to adults (Seitz, Herpertz-Dahlmann, & Konrad, 2016). Several studies have used voxel-based morphometry (VBM) to characterize the local distribution of GM loss. These studies commonly report regional GM reductions, often in the anterior cingulate cortex (Friederich et al., 2012; Joos et al., 2010; McCormick et al., 2008), supplementary motor area (Amianto et al., 2013; Bär, de la Cruz, Berger, Schultz, & Wagner, 2015; D’Agata et al., 2015; Friederich et al., 2012), precuneus (Bär et al., 2015; Gaudio et al., 2011; Joos et al., 2010), and striatum (Boghi et al., 2011; Friederich et al., 2012). A meta-analysis of 9 VBM studies concluded that AN was associated with GM reductions in the hypothalamus, inferior parietal lobe, and striatum. Widespread cortical thinning is also observed in AN (Bär et al., 2015; King et al., 2015).

Although considerable research effort has been devoted to determining whether structural brain alterations persist following recovery from AN, extant findings are equivocal. Longitudinal studies of patients have shown normalization of WM but persistent loss of GM at follow-up (Castro-Fornieles et al., 2009; Katzman, Zipursky, Lambe, & Mikulis, 1997; Roberto et al., 2011). However, some of these did report an increase in global GM volume with weight-restoration (Castro-Fornieles et al., 2009; Roberto et al., 2011), raising the possibility that given enough time and weight-gain, full GM normalization will occur.

However, regional GM volume reductions have been reported in cross-sectional studies of individuals recovered from AN (Frank, Shott, Hagman, & Mittal, 2013a; Friederich et al., 2012; Joos et al., 2011a; Lambe, Katzman, Mikulis, Kennedy, & Zipursky, 1997; Mühlau et al., 2007). The specific location of these reductions vary between studies, but several report reduced volumes in mid-line areas including the anterior cingulate (ACC) cortex (Friederich et al., 2012; Mühlau et al., 2007) and supplementary motor area (Castro-Fornieles et al., 2009; Friederich et al., 2012). These reductions either reflect irreversible damage caused by emaciation, or trait characteristics of AN.

In contrast, many studies show no GM reductions in recovered AN patients (Chui et al., 2008; Lázaro et al., 2013; Mainz, Schulte-Rüther, Fink, Herpertz-Dahlmann, & Konrad, 2012; Wagner et al., 2006). This implies that GM reductions observed during
the acute phase of the illness are reversible, and thus secondary to effects of AN. Indeed, Bernardoni and colleagues (2016) showed that cortical thickness and subcortical volumes increase rapidly following weight-restoration, and were no different from comparison women or long-term recovered AN patients at discharge. The increase in GM thickness and volumes were predicted solely by weight-restoration.

The inconsistent findings regarding the presence of GM volume reduction in recovered patients may be due to heterogeneity in study design. This includes differences in the operationalization or duration of recovery, or variations in the length of follow-up interval or degree of weight-restoration in longitudinal studies. Also, several studies are limited by small sample sizes.

In sum, it seems that at some of the brain mass reductions observed during the ill state are reversible with weight-restoration, particularly WM, and to a large extent GM. Findings regarding the presence of reduced regional GM volumes following recovery are inconsistent. Establishing this is important, as it will advance our understanding of the structural brain changes associated with AN. If irreversible damage to brain tissue occurs during AN, the extent and clinical significance of this must be determined. If however, such regional GM reductions reflect traits, their role in AN symptomatology should be explored. As current findings are inconsistent, more studies are warranted to determine the presence of regional GM loss following recovery, (Van den Eynde et al., 2012).

2.8.2 Functional brain alterations

Studies of brain function in AN have predominantly used functional MRI (fMRI) to measure the neural responses to a variety of stimuli and tasks among patients with AN (see Kaye et al., 2013; Pietrini et al., 2011 for reviews). Available evidence suggests that alterations in brain circuits related to cognitive control, reward, and emotion are central (Kaye et al., 2013). Given that this thesis focused on brain circuits and cognitive processes related to emotion and cognitive control, the following sections will focus on literature relevant for these processes. However, it is worth noting that several studies have shown alterations in the reward-circuitry as
well (e.g. insula and striatum) in response to palatable taste stimuli (see Wierenga et al., 2014b for an overview of this field).

**Cognitive control:** Studies challenging cognitive control have reported that AN is associated with alterations in the lateral prefrontal cortex (PFC) and ACC. One study (Lao-Kaim et al., 2015) showed that cognitive set-shifting (a measure of cognitive flexibility) evoked greater activation in lateral PFC and parietal circuits in patients compared to controls. Moreover, activation in these circuits was lower in patients when no set-shifting was required. However, another study using the same task reported that patients had *lower* activation in the lateral PFC during set-shifting (Sato et al., 2013). Lower frontal activation in patients during set-shifting was also reported by Zastrow and colleagues (Zastrow et al., 2009), not in the lateral PFC but in the ACC and putamen. The reason for these discrepant findings is unclear.

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 lateral PFC and ACC during high-demanding inhibitory trials (Wierenga et al., 2014a). A similar demand-specific alteration of PFC has been shown in recovered AN patients, suggesting it reflects a trait characteristic (Oberndorfer, Kaye, Simmons, Strigo, & Matthews, 2011). These studies indicate that AN individuals require less inhibitory resources to maintain performance as inhibitory demand increases.

Of note, studies have also shown altered functional connectivity within cognitive control circuits during rest in AN individuals, but results are mixed: some report increased functional connectivity (Boehm, 2014; Cowdrey, Filippini, Park, Smith, & McCabe, 2012a), while others report decreased connectivity (Gaudio et al., 2015; Kullmann et al., 2014). Together, these studies point to aberrations within lateral PFC and ACC circuits related to cognitive control processes. However, it is unclear to what extent these alterations are associated with excessive or impaired cognitive control.

**Emotion:** Visual presentation of food stimuli evokes altered neural responses among AN women. In AN, such stimulation is associated with hyperactivations in the medial
PFC and posterior cingulate / precuneus, and hypoactivation in the lateral parietal lobe, orbitofrontal cortex, and lateral PFC (García-García et al., 2013). Hyperactivations in limbic circuits, including the amygdala (Ellison et al., 1998; Joos et al., 2011b), and insula (Ellison et al., 1998; Kim, Ku, Lee, Lee, & Jung, 2012) have also been observed. Based on what is known about the functions of these circuits (Bishop, 2007; Etkin, 2009), hyperactivations in medial PFC and limbic circuits may reflect heightened negative emotional arousal towards food stimuli, while hypoactivations in lateral PFC and parietal cortices may reflect decreased cognitive control, perhaps over emotion. As medial PFC circuits is also involved in regulatory functions (Etkin, Egner, & Kalisch, 2011), they may also reflect emotion-regulation processes.

Similar neural alterations are observed in response to visual presentations of body stimuli. A meta-analysis (Zhu et al., 2012) showed that body-stimuli evoke greater activation in the insula and uncus in patients with AN compared to healthy controls, and less activation in parietal cortices. Some studies also report amygdala hyperactivations among AN women in response to such stimuli (Miyake et al., 2010; Vocks et al., 2010). Again, these alterations may reflect heightened emotional arousal (Zhu et al., 2012).

Together, these studies suggests that AN is associated with alterations in circuits involved in emotion. Exaggerated activation of these circuits may be associated with clinical features of AN, including poor emotion regulation and high anxiety. In a similar vein, Kaye and colleagues (Kaye et al., 2013) suggests an imbalance between ventral limbic (involved in reward and emotion) and dorsal cognitive control circuits (involved in cognitive control) characterizes women with AN. Emotional dysregulation in AN may then be associated with a skewed balance between these circuits, where the dorsal control system is unable to adequately dampen or modulate negative emotion signals from the limbic system.

However, as most studies of the neural correlates of emotion processing in AN have employed disorder-specific stimuli (e.g. food and bodies), it remains unclear to what extent the reported alterations are restricted to the processing of such stimuli, or are indicative of a general impairment in emotion processing related to the primary
neuropathophysiology and etiology of AN. Obviously, images of food and bodies evoke different connotations and associations among AN women compared to controls. Hence, the observed neural alterations in emotion-circuits among AN women may not necessarily reflect a general dysregulated emotion circuitry. Investigating general properties of the emotion circuitry in AN, for instance how it handles and regulates general salient and emotional stimuli is important in order to shed light on the neuropathophysiology and etiology.

There is a distinct lack of studies investigating the neural responses to general (i.e. disorder non-specific) stimuli in AN. There is evidence suggesting that general emotional stimuli is associated with altered neural responses in AN (Fonville, Giampietro, Surguladze, Williams, & Tchanturia, 2014; Uher et al., 2004; Via et al., 2015). For example, one study (Via et al., 2015) found that patients relative to controls exhibited reduced activation in the dorsomedial PFC in response to facial stimuli signifying social acceptance, and increased activation in visual areas during facial stimuli signifying social rejection. Such findings suggest that the neural circuitry underlying the processing of general emotional stimuli is altered in AN. In contrast, other studies have failed to detect altered neural responses to general emotion stimuli among AN women (Cowdrey, Harmer, Park, & McCabe, 2012b; Uher et al., 2003), perhaps underscoring the importance of the type of experimental tasks used while recording neural activation. More studies are needed to investigate the emotion-circuitry in AN. Also, few studies have attempted to investigate the neural responses to tasks that require cognitive control in the presence of emotional stimuli, which would shed light on the interplay between prefrontal and limbic circuits.

2.9 Synopsis and introduction to aims

In sum, despite significant research into the neuropathophysiology associated with AN, the neural underpinnings of AN is not understood. It is clear that patients with AN show brain tissue reductions, some of which normalize following weight-restoration and recovery. A number of studies report regional GM reductions in women recovered from AN, which could reflect irreversible damage subsequent to emaciation, or alternatively trait characteristics of these women. However, there is
conflicting evidence regarding the presence of such reductions in women recovered from AN, and more studies are warranted.

AN is also associated with functional brain alterations in PFC, parietal, and limbic circuits related to cognitive control and emotion. Some of these appear to reflect trait characteristics, but their role in the psychopathology of AN remains unclear. An imbalance within / between these circuits may underlie vulnerability for AN, and be related to clinical features such as anxiousness and poor emotional regulation skills. However, as most studies of the neural correlates of emotion processing in AN have employed disorder-specific stimuli (e.g. food and bodies), it remains unclear to what extent the reported alterations are restricted to the processing of such stimuli, or are indicative of a general impairment in emotion processing related to the primary neuropathophysiology and etiology of AN. Also, few have attempted to investigate the neural responses to tasks that require cognitive control in the presence of emotional stimuli.

In sum, despite significant progress the neuropathophysiology of AN is not understood. The nature of the structural and functional brain alterations, how they relate to AN psychopathology, and whether they reflect state or trait characteristics, remains unclear. To advance our understanding of AN, more studies are needed to investigate these issues.
3. RESEARCH AIMS

The overall aim of this doctoral thesis was to investigate the neuropathophysiology of AN, and in so doing advance our understanding of AN. We wanted to characterize brain structure in women recovered from AN, and probe functional characteristics of emotion-related brain circuits in these women.

Specifically, we aimed to:

1: Determine the presence of brain tissue reductions in recovered AN women.

2: Investigate neural responses to an emotional conflict task in recovered AN women.

3: Investigate neural correlates of threat-detection and attentional bias to threat in recovered AN women.
4. MATERIALS AND METHODS

4.1 Sample

To circumvent the confounding effects of active AN and emaciation, we only included women recovered from AN. In total, we included 22 adult women recovered from AN and 22 comparison women (CW), all right-handed (see Table 1 for participant characteristics). Half of the recovered AN women \((n = 11)\) had a history of AN binge-eating/purging subtype, while the remaining half \((n = 11)\) had a history of AN restricting subtype. All papers in this thesis are based on the same sample, with the exception of one CW, who due to technical difficulties during recording of the fMRI images was not included in Papers 2 and 3.

Both groups were recruited through flyers (distributed at universities and Oslo University Hospital), an internet discussion forum, the Oslo University Hospital webpage, and universities’ Facebook pages. Additionally, two user-organizations aided in the recruitment of recovered AN women, by informing their members through member magazines, websites, and meetings.
Table 1. Participant characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>RAN (n = 22)</th>
<th>CW (n = 22)</th>
<th>Two-sample t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>27.32 ± 5.14</td>
<td>26.14 ± 4.64</td>
<td>0.80 (42)</td>
</tr>
<tr>
<td>BMI (kg/m²)¹</td>
<td>20.39 ± 1.66</td>
<td>21.85 ± 1.76</td>
<td>-2.70 (38)</td>
</tr>
<tr>
<td>BDI</td>
<td>6.36 ± 7.94</td>
<td>1.77 ± 2.69</td>
<td>2.57 (42)</td>
</tr>
<tr>
<td>EDE-Q global score</td>
<td>0.84 ± 0.74</td>
<td>0.19 ± 0.17</td>
<td>4.04 (42)</td>
</tr>
<tr>
<td>STAI state score</td>
<td>32.14 ± 8.16</td>
<td>25.86 ± 5.21</td>
<td>3.04 (42)</td>
</tr>
<tr>
<td>STAI trait score</td>
<td>38.77 ± 11.48</td>
<td>28.36 ± 6.43</td>
<td>3.71 (42)</td>
</tr>
<tr>
<td>Lowest lifetime weight²</td>
<td>71.84 ± 9.18 (range: 46 – 85)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age of AN onset</td>
<td>17.36 ± 4.17 (range: 11 – 32)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of illness (months)³</td>
<td>32.86 ± 27.47 (range: 6 – 120)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of recovery (months)³</td>
<td>51.62 ± 42.70 (range: 12 – 192)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ Data not available for three recovered anorexia nervosa women and one comparison woman.
² Percentage of ideal weight, taking into account height, age and gender.
³ Data not available for one recovered anorexia nervosa woman.

Abbreviations: BDI = Beck depression inventory; BMI = Body mass index; d = Cohen’s d effect size; CW = Comparison women; EDE-Q = Eating disorder examination-questionnaire; RAN = Recovered anorexia nervosa; SD = Standard deviation; STAI = State-trait anxiety inventory.

4.2 Clinical assessment

Current and lifetime DSM-IV diagnoses (APA, 2000) were assessed with the Structured Clinical Interview for DSM-IV Axis I Disorders version I/P (First, Spitzer, Gibbon, & Williams, 2002). Lifetime histories of the following disorders were assessed: major depressive disorder, dysthymia, bipolar disorder, schizophrenia, alcohol and substance abuse/dependence, anxiety disorders (including obsessive-compulsive disorder and post-traumatic stress disorder), somatoform disorders, and eating disorders (AN, bulimia nervosa, and binge-eating disorder). During this interview, status of AN recovery was evaluated, and clinical characteristics related to the AN period were obtained (e.g. AN subtype, recovery duration).
Recovery was operationally defined as having maintained a body mass index (BMI) above 18 for the past 12 months, and abstinence from binge-eating and purging behavior, excessive or compulsive exercising behavior, and no severely restricted food intake for the past 12 months. This operationalization of recovery is similar to ones used by previous investigators (e.g. Wagner et al., 2006). The behavioral diagnostic items from the Eating Disorder Examination interview (Cooper & Fairburn, 1987) were adapted and used to assess presence of eating disorder psychopathology the previous year, and to evaluate fulfillment of our criteria for AN recovery.

4.3 Inclusion criteria

Women in the recovered AN group were included if they had a lifetime history of anorexia nervosa according to DSM-IV criteria (APA, 2000), excluding the amenorrhea criterion. They were also required to fulfill our criteria for AN recovery. Exclusion criteria for these women included: lifetime history of a psychotic disorder, substance abuse or dependence, or the presence of an Axis-I disorder the past 12 months.

Exclusion criteria for CW included: lifetime history of an Axis I disorder, current use of psychoactive medications or substances, and a first-degree relative with a history of an eating disorder. The background for excluding women who had a first-degree relative with a history of an eating disorder stems from preliminary evidence that neuropsychological impairments of AN probands are shared with first-degree relatives (Holliday et al., 2005), and because AN susceptibility is genetically influenced (Bulik, Slof-Op't Landt, van Furth, & Sullivan, 2007). Furthermore, we excluded CW who reported binge-eating and purging behavior, excessive or compulsive exercising, severely restricted food-intake, or had a BMI below 18 for the past 12 months.
4.4 Data-collection procedure

All participants were first screened via e-mail, and then interviewed over phone to evaluate potential inclusion. A total of 60 participants were interviewed and considered for inclusion. Included participants were booked for an MRI scan no more than 1 week following the interview. See Figure 1 for a flow-chart detailing the exclusion of participants following the interviews.

At the day of the MRI-scan, participants first completed a set of self-report measures, which included self-reported height (which was used to calculate BMI along with their objective weight). Prior to the MRI-scan, participants were instructed on the two experimental tasks, and rehearsed them until they had thoroughly understood them. All structural and functional MR images were then collected. Following the MRI-scan, participants were weighed.
Figure 1. Flow-chart depicting exclusion of participants. As showed, the majority of exclusions were made following the interview. Following MRI session, one recovered AN woman was excluded due to low current weight. Additionally, due to technical difficulties, fMRI images were not acquired for one CW. The final sample size therefore differs for Paper 1 and Papers 2-3. RAN = Recovered AN; CW = Comparison women.

Ethical considerations: Studies in this thesis were approved by the Regional Ethics Committee in Norway. After complete description of the study, written informed consent was obtained from all participants. Participants who reported considerable psychological problems during the diagnostic interview were contacted by the main supervisor (a psychiatrist), and the need for further referral was evaluated. All participants were compensated with a gift card.

4.5 Self-report measures

All participants completed the following self-report questionnaires: Spielberger State-Trait Anxiety Inventory (Spielberger, Gorsuch, & Lushene, 1970), Difficulties in Emotion Regulation Scale (Gratz & Roemer, 2004), Beck Depression Inventory (Beck, Ward, Mendelson, Mock, & Erbaugh, 1961), and Eating Disorder Examination-
Questionnaire (EDE-Q; Fairburn & Beglin, 2008). These measures are well-established and commonly used in the eating disorder field. All of them (Beck, Steer, & Brown, 1996; Håseth, Hagtvet, & Spielberger, 1990; Rø, Reas, & Lask, 2010), with the exception of the DERS, have been properly translated to Norwegian and tested for reliability and validity. The Norwegian version of the DERS however, has not undergone formal translation and psychometric evaluation, but was nonetheless included due to its relevance. However, based on the sample in this thesis, the validity of the Norwegian version of DERS is supported by the expected positive correlations between DERS and total score on the BDI, STAI, and EDE-Q (all \( r_s = .55 - .77, p < .001 \)). Furthermore, the DERS differentiated between the recovered AN women and CW, in accordance with previous studies. The Cronbach’s \( \alpha \) for the DERS total score in our sample was .95, indicating excellent internal consistency.

4.6 fMRI tasks

For Papers 2-3, a task was presented to participants during acquisition of fMRI images to evoke neurocognitive responses. E-prime 2.0 software (Psychology Software Tools, Pittsburgh, PA) was used to create these tasks, and to present the tasks and record responses during MRI data acquisition. For both tasks, responses were made using the right index and middle finger on a two-button response-box. Detailed descriptions of these tasks are described in Papers 2 and 3. Below follows a brief description of the tasks.

4.6.1 Paper 2: Emotional conflict task

The purpose of this task was to elicit neural responses related to the inhibition of emotional material and the resolution of emotional conflict. This task relies on both emotion processing and cognitive control over emotion. We created the task to be largely identical to the one originally described by Etkin and colleagues (2006), with the exception that we used facial images from the NIMSTIM database (Tottenham et al., 2009). Trials consisted of a central happy or fearful face, overlaid with either the word “FEAR” or “HAPPY”. Participants were instructed to ignore the word, and to indicate the emotional expression of the actor, as fast and accurately as possible. Thus, the task requires one to inhibit the word. There were two conditions; congruent trials (emotional non-conflict) where expression of actor matched the word, and
incongruent trials (emotional conflict) where expression of actor did not match the word.

In this task, we were mainly interested in the neural responses to incongruent relative to congruent trials. As previous studies have illustrated, incongruent trials generate emotional conflict, which is associated with slower responses times (RTs) and increased activity in the PFC (Etkin et al., 2006; Etkin, Prater, Hoeft, Menon, & Schatzberg, 2010; Jarcho et al., 2013). The slowing of responses and increased PFC activation during emotional conflict probably reflects heightened cognitive control processes, necessary to suppress the word and resolve the word-face conflict. Emotional conflict have previously been associated with altered neural responses in the dorsomedial PFC among clinical samples (Etkin et al., 2010).

4.6.2 Paper 3: Dot-probe task

The well-established dot-probe task (Macleod 1986) was used to elicit neural responses associated with threat-detection and attentional bias to threat. The task involves attentional processes related to both the detection and inhibition of an emotional stimulus. Trials consisted of brief presentations (500 milliseconds [ms]) of a pair of faces (cues) on the left and right side of the screen. One of the images was then replaced by an asterisk (probe). Participants were instructed to determine the location of the probe (left or right) as quickly and accurately as possible. Each pair displayed either an angry-neutral combination, or a neutral-neutral combination of the same actor. There were three conditions: congruent trials in which the probe replaced the angry face in an angry-neutral face pair, incongruent trials in which the probe replaced the neutral face in an angry-neutral face pair, and control trials in which a neutral-neutral face pair was replaced by a probe in either the left or right side of the screen. Congruent and incongruent trials differed only in the location of the probe.

In this task, we were interested in the neural responses to the angry faces (regardless of probe location), and attentional bias to angry faces. Attentional bias is observed when emotional (angry face) relative to neutral (neutral face) stimuli interferes with behavioral performance, leading to increased RTs. Such bias reflects an information-processing style favoring threat (i.e. emotionally negative) stimuli. An
attentional bias to threat has consistently been associated with anxiety (Bar-Haim, Lamy, Pergamin, Bakermans-Kranenburg, & H. Marinus, 2007), and a similar bias is associated with AN (Cardi et al., 2015; Cardi et al., 2012; Harrison et al., 2010). This bias to threat may occur as a result of amplification of threat-signals in limbic circuits, and/or by attenuation of the regulatory functions of PFC (Bishop, 2007). Similar to the emotional conflict task in Paper 2, the dot-probe task involves suppressing irrelevant emotional stimuli.

4.7 MRI methods

4.7.1 Introduction to MRI

MRI can be used to study both structural and functional characteristics of the brain, by measuring how radio frequency pulses act upon hydrogen nuclei in the brain (Logothetis & Wandell, 2004). MRI machines contain a strong homogenous main magnetic field. When placed in the bore of an MRI-machine, hydrogen nuclei in the body tend to align either parallel or antiparallel to this main field, at which point they achieve a low-energy state. When introducing a radio frequency pulse that resonates with hydrogen nuclei, the nuclei absorbs the energy and spins away from their low-energy state and enters a high-energy state. When the radiofrequency pulse is turned off, the hydrogen nuclei will return to their low-energy state, and realign with the main magnetic field. The time taken to return to the original state is called relaxation time, and varies depending on which tissue the nuclei are located in. As nuclei relax and realign with the main magnetic field, they emit the energy originally absorbed, which is measured by coils. This basic measurement underlies MR imaging.

Gradient magnets are used to locally alter the main magnetic field, which enables one to select which specific slice of tissue (and hydrogen nuclei) to record. Measuring MRI signals in many slices and reconstructing the signals into a spatial dimension give rise to the 3D images of the brain commonly associated with MRI. By varying the timing and amplitude of the gradient magnets and the radiofrequency pulse according to specific parameters, structural and functional properties of the brain can be measured. For example, one may choose parameters that maximize differences.
between GM and WM in order to later analyze morphometric characteristics of these tissues.

In the case of fMRI, these basic principles are applied to indirectly measure neural activation. When neurons fire, local blood flow to those neurons is increased, supplying them with oxygen-rich blood (the hemodynamic response). This association between neural activity and local supply of oxygenated blood underlies the ability to measure neural activation through fMRI (Huettel, Song, & McCarthy, 2004, pp. 127-156). Oxygen-rich blood and oxygen-poor blood has different magnetic properties, and MRI can measure the signal associated with oxygenated blood, which is called the blood-oxygen-level-dependent (BOLD) signal. Although the BOLD signal is an indirect measure of neural activity and is not completely understood (Arthurs & Boniface, 2002), it is closely associated with neural activity (Logothetis, Pauls, Augath, Trinath, & Oeltermann, 2001).

In fMRI studies, participants typically perform some task while the BOLD signal in the brain is continually measured over time. Through statistical analyses of the BOLD images, one can associate certain tasks and cognitive processes with increased or decreased BOLD signal, and thereby make inferences regarding the underlying neural activation. However, it is important to reiterate that the BOLD signal is an indirect measure of neural activity, and inferences of observed BOLD signals are strictly speaking made regarding the hemodynamic correlates of neural activity. Also, the hemodynamic response measured with the BOLD signal reflects lags behind neural activity: it begins approximately 2 seconds later and peaks 6-9 seconds after the onset of the neural event (Logothetis & Wandell, 2004). Alongside the fact that the sampling rate of BOLD sequences is about 2 seconds, the temporal resolution of fMRI as a measure of neural activity is inferior compared to some other neuroimaging techniques (e.g. electroencephalography).

However, MRI produces images of high spatial resolution, allowing for highly accurate localization. In structural MRI, the resolution is typically around 1-2 millimeter (mm)³, while it is slightly lower in fMRI, typically around 3mm³. Due to the high spatial resolution of MRI images, the availability of MRI machines, and the
noninvasive nature of the procedure, MRI is currently the most popular and widespread method for measuring brain structure and function.

### 4.7.2 MRI data acquisition

All MR images were recorded with a 3 Tesla Achieva MRI scanner (Philips, Eindhoven), located at Rikshospitalet, Oslo University Hospital. All MR images were collected during a single session, lasting approximately 40 minutes. For the functional images (Papers 2-3) the five first volumes of each run were discarded to avoid T1 saturation effects.

**Paper 1:** High-resolution structural images were acquired using a T1-weighted multi-shot turbo-field-echo sequence (TR/TE = 6.7/3.1 ms, flip angle = 8º, FOV = 256 x 256 mm, matrix = 256 x 213), recording 170 sagittal slices covering the whole brain (voxel size = 1.0 x 1.2 x 1.2).

**Paper 2:** Functional images were acquired using a T2*-weighted single-shot echo-planar sequence (TR/TE = 2000/30 ms, flip angle = 80º; FOV = 240 x 240 mm, matrix = 80 x 80). For each participant, 34 axial slices covering the whole brain were acquired in an interleaved order (voxel size = 3 x 3 x 3 mm).

**Paper 3:** Functional images were acquired using a T2*-weighted single-shot echo-planar sequence (TR/TE = 2100/30 ms, flip angle = 80º; FOV = 240 x 240 mm, matrix = 80 x 80). For each participant, 36 axial slices covering the whole brain were acquired in an interleaved order (voxel size = 3 x 3 x 3 mm).

### 4.7.3 MRI preprocessing

Preprocessing of fMRI data (Papers 2-3) were conducted using the Statistical Parametric Mapping (SPM8, [http://www.fil.ion.ucl.ac.uk/spm](http://www.fil.ion.ucl.ac.uk/spm)) toolbox. Preprocessing of structural images (Paper 1) were conducted using the Voxel-Based Morphometry (VBM8, [http://dbm.neuro.uni-jena.de/vbm8](http://dbm.neuro.uni-jena.de/vbm8)) toolbox; an extension of SPM8. Both toolboxes are implemented in MATLAB (MATLAB and Statistics Toolbox Release 2012b, The Mathworks, Inc., Natick, MA, US).
**Paper 1:** Images were segmented into different tissue classes using the ‘new segment’ procedure in SPM8. GM images were then normalized to the MNI template, bias-field corrected, and modulated using the DARTEL (Ashburner, 2007) toolbox for SPM8. During this stage images were resampled to 1.5 isotropic voxels. DARTEL has been shown to provide good normalization compared to other approaches (Klein et al., 2009). To reduce variability between participants, and to render the data more normally distributed, the modulated GM images were smoothed with a 10 mm full width at half maximum (FWHM) kernel. Calculation of global raw WM, GM, and CSF volumes were performed with VBM8.

**Papers 2 and 3:** To account for timing differences in slice acquisition, slice-timing correction was applied to all volumes. This has been shown to significantly increase the robustness of the data analyses (Sladky et al., 2011). To correct for head motion, images were realigned to the mean scan using a two-pass procedure. At this point, movement was manually inspected, but no movement among participants exceeded 3 mm so all images were included. Images were then spatially normalized to the MNI template, and bias-field corrected using DARTEL. Images were resampled to 3 mm isotropic voxels. The resulting modulated images were then smoothed with a 10 mm FWHM kernel.

4.8 Statistical analyses

All analyses were performed using SPM8 (implemented in MATLAB) and IBM SPSS Statistics version 21 for Windows (IBM Corp. in Armonk, NY). For Papers 2-3, behavioral performance (accuracy and RT) was analyzed using ANOVA models in SPSS. For MRI data, all regions of interest (ROIs) were defined using the ‘Hammers atlas’ (Gousias et al., 2008; Hammers et al., 2003), available from [www.brain-development.org](http://www.brain-development.org). This is a maximum probability atlas consisting of a large number of regions which have been hand-drawn on 30 MR images. Probabilistic atlases are more likely to be representative of population anatomy compared to single-brain atlases. The amygdala, ACC, and PFC (inferior, middle, and superior frontal gyrus) ROIs were all defined using this atlas. Detailed descriptions of the analyses are presented in Papers 1-3. The following sections briefly summarize the main statistical analyses for each paper separately.
Paper 1: To investigate potential group differences in global WM, GM and CSF volumes, between-group t-tests were performed on the raw tissue volumes. VBM was used to investigate regional GM volumes. In VBM, local concentration of GM is compared on a voxel-wise level between groups (Ashburner & Friston, 2000). VBM is fully automated, and does not rely on a trained operator to perform manual tracing of images. It allows for voxel-wise comparisons of local GM volumes, which can pick up subtle differences in brain morphometry. The ACC and supplementary motor area were defined as a priori ROIs. These regions were chosen because several studies have reported local GM reductions in these areas, among both ill (e.g. Friederich et al., 2012; Joos et al., 2010) and recovered AN (e.g. Friederich et al., 2012; Mühlau et al., 2007) patients. Exploratory whole-brain VBM analyses were also performed. T-tests in SPM were used to compare regional GM volumes between groups.

The issue of multiple comparisons and inflation of type-I error rates were addressed by using a combination of small volume corrections and family-wise error (FWE) correction. Results were presented at both corrected and uncorrected thresholds.

Paper 2: Regressors corresponding to the onset of emotional conflict and non-conflict trials were created and convolved with the canonical hemodynamic response function. A 128-seconds temporal high-pass filter was applied to remove low-frequency drifts in the data. A random-effects factorial 2 x 2 (group x condition) model was defined, which allowed us to test the main effect of condition and the condition x group interaction effect. As this model (as implemented in SPM8) do not provide the valid error terms for testing main effects of between-subject factors, a separate two-sample t-test was used to investigate main effect of group. Using these models, we tested whether emotional conflict and/or non-conflict was associated with altered neural responses in recovered AN women. Four a priori ROIs were specified in the analyses; the inferior frontal gyrus, middle frontal gyrus, ACC and amygdala. These were chosen on the basis that emotional conflict has been shown to activate these regions (PFC), and because these regions have previously been implicated in AN neuropathophysiology (Kaye et al., 2013). Exploratory whole-brain analyses were also performed. As in Paper 1, small volume corrections and FWE were used to correct alpha levels, both for the ROI and whole-brain analyses.
Paper 3: Regressors for the onsets of congruent, incongruent, and control trials were created, and convolved with the canonical hemodynamic response function. A 128-seconds temporal high-pass filter was applied to remove low-frequency drifts in the data. We were interested in both the neural response to the angry faces (incongruent + congruent] > control trials), and attentional bias to angry faces (incongruent > congruent trials). T-tests in SPM were defined to test for between-group differences in neural responses to these events. Four a priori ROIs were specified; the inferior frontal gyrus, middle frontal gyrus, superior frontal gyrus, and amygdala. These regions were chosen because they have previously been implicated in the neuropathophysiology of AN (Kaye et al., 2013), and because they are known to be involved in threat-detection and attentional bias to threat (Bishop, 2007).

To correct our alpha level for multiple comparisons, alpha levels were determined with Monte Carlo simulations using the AlphaSim implementation in the REST toolbox (Song et al., 2011, www.restfmri.net). This provides an empirical way of directly estimating the whole-brain alpha level, given a specific combination of uncorrected voxel-level alpha levels and cluster extents. It is likely to provide more accurate alpha level correction compared to FWE and other corrections.

4.9 Results

Summary of Paper 1: Research regarding the presence of regional GM volumes in women recovered from AN is inconsistent. Our results showed that global and regional GM volumes were similar between recovered women and CW. These results are in line with previous findings showing that brain tissue volumes increase with weight-restoration, and are normal in women recovered from AN. This indicates that AN is not associated with irreversible brain damage subsequent to emaciation, nor trait characteristics involving regional GM reductions. However, our findings are in contrast to several studies where regional GM volumes have been reported following AN recovery.
However, we did find positive correlations between lowest lifetime weight and regional GM volumes in the insula and precuneus among recovered AN women. Reductions in these regions have previously been reported in AN. Also, similar correlations between regional GM volumes and lowest lifetime weight have been reported in previous studies. Whether such associations reflect irreversible brain reductions in patients with severe AN, or trait characteristics of those women who attain a severely low body weight is unclear.

Summary of Paper 2: There were no group differences in behavioral performance, but emotional conflict relative to non-conflict was associated with increased RTs for both groups. In terms of neural activation, there was no main effect of group, indicating that overall, the task stimuli evoked similar brain responses in both groups. However, there was a group x condition effect, where women recovered from AN displayed less activation in the amygdalae during non-conflict trials. Similar alterations were observed in the hippocampus and striatum. Brain responses to emotional conflict did not differ between groups. In contrast to our expectations, no group differences emerged in the PFC.

Amygdala alterations have previously been described in AN, but our study is the first to report such alterations in response to a task that is unrelated to AN symptomatology. Our findings indicate that AN is associated aberrations within limbic circuits involved in emotion, and may reflect an impaired ability to process emotional stimuli.

Summary of Paper 3: There were no group differences in behavioral performance, and no attentional bias to threat in the recovered AN group. Failure to detect an attentional bias to threat effect in AN is in line with some prior studies, although several have reported such an effect in both ill and recovered AN women. In response to angry faces, women recovered from AN showed less activation in the extrastriate cortex compared to CW. This may reflect impaired reward or saliency processing of faces. Indeed, previous research have shown that AN is associated with impaired emotion recognition capabilities.
In response to incongruent relative to congruent trials (attentional bias to threat), women recovered from AN exhibited increased activation in the medial PFC compared to CW. While CW showed decreased activation during incongruent compared to congruent trials, recovered AN women showed no differentiated response to the two conditions. This may reflect increased compensatory cognitive control processes that serve to counter the attentional bias to threat and maintain behavioral performance. If so, this could explain why some studies report attentional bias to threat in AN while others do not, as the ability of the compensatory mechanisms to engage cognitive control may vary between individuals and experimental contexts. The medial PFC contrast activation showed a significant negative correlation with lowest lifetime weight in the recovered AN group, indicating it has some relevance to illness severity. Contrary to our hypothesis, no differential amygdala activation between groups was observed.
5. DISCUSSION

5.1 Summary of findings

The overall aim of this doctoral thesis was to investigate the neuropathophysiology of AN, and in so doing advance our understanding of AN. We wanted to characterize brain structure in women recovered from AN, and probe functional characteristics of emotion-related brain circuits in these women.

Paper 1 demonstrated normal brain tissue volumes in recovered AN women, indicating that AN is not associated with irreversible brain tissue damage or trait-like reductions of localized gray matter volumes, as some previous studies have suggested (Friederich et al., 2012; Mühlau et al., 2007). In Papers 2-3, we reported that recovered AN women are characterized by functional alterations in the medial PFC and amygdala, both part of the emotion-circuitry of the brain. As the alterations were observed in women recovered from AN, they may reflect trait characteristics associated with AN. Although our study design prohibits conclusions regarding causality, we speculate that these traits are associated with vulnerability for AN. They could reflect dispositional styles involving how the brain processes and regulates emotional signals, which may relate to psychological constructs such as anxiousness, attentional bias to threat, and emotional dysregulation. However, the exact nature of these alterations is not clear. Detailed discussions of the results of Papers 1-3 are presented in the papers. The following sections will provide a broader discussion regarding the findings, and methodological considerations which results must be viewed in light of.

5.1.1 Presence of structural brain alterations following recovery from AN?

Paper 1 showed that recovered AN women and CW had similar brain tissue volumes. This included both global and regional GM volumes. This is in accordance with several studies (Bernardoni et al., 2016; Chui et al., 2008; Lázaro et al., 2013; Mainz et al., 2012; Wagner et al., 2006), and suggests that the brain tissue reductions frequently observed during the acute stage of AN does not persist following recovery. However, as this was a cross-sectional investigation, we cannot be certain that the recovered AN women were in fact characterized by brain tissue reductions when ill.
However, such reductions among ill AN patients have consistently been reported in a wealth of studies (Titova et al., 2013; Van den Eynde et al., 2012), so it is feasible to assume that they were present in our sample as well. Moreover, clinical characteristics of our recovered AN group showed that all had significantly low body weights when ill, and many were ill for years. However, absence of evidence is not evidence of absence, and we cannot definitively exclude the possibility that there are regional GM reductions in women recovered from AN associated with irreversible brain damage or trait characteristics.

Indeed, our findings are inconsistent with some previous studies where local GM reductions in recovered individuals were observed (Castro-Fornieles et al., 2009; Frank et al., 2013a; Friederich et al., 2012; Katzman et al., 1997; Mühlau et al., 2007; Roberto et al., 2011). The reasons for these discrepant findings are unclear, but could be related to differences in study design, including sample size, inclusion criteria, and operationalization and duration of recovery. In our study, we used recovery criteria similar to that of Wagner and colleagues (2006), who also reported normal GM volumes in recovered individuals. Moreover, the duration of recovery in our study was minimum one year, and on average four years, which may be sufficient for normalization of brain tissue. As there is no universal definition of recovery from AN, studies vary in how they operationalize recovery, and also in how long participants must have maintained recovery for inclusion in the study.

For example, Mühlau and colleagues (2007) defined recovery as a BMI above 17 and regular menses for at least 6 months, and they observed regional GM reductions in recovered women. Participants fulfilling these criteria may not be sufficiently recovered for the brain changes associated with the acute stage to have reversed. Another study (Friederich et al., 2012) defined recovery as a BMI above 17.5, normal eating patterns for one year, and regular menstruation, which is similar to our own definition. Still, these authors reported regional GM reductions.

Our null-finding could also be due to low statistical power. Although this is difficult to ascertain, it is worth mentioning that previous studies with sample sizes comparable to or smaller than our own have reported GM alterations in recovered AN individuals. For example, Friedrich et al. (2012) included 13 recovered AN women and 14 CW,
Lambe et al. (Lambe et al., 1997) included 12 recovered AN and 18 CW, Frank et al. (Frank et al., 2013a) included 24 recovered AN and 24 CW, and Mühlau et al. (Mühlau et al., 2007) included 22 recovered AN and 37 CW. All these studies reported regional GM reductions in the recovered AN group, indicating our study was sufficiently powered to detect similar effects. It should also be acknowledged that a publication bias may exist, in which more studies showing differences in brain volumes are published compared to those who do not find any differences.

There is also the possibility that persistent GM reductions are present in subgroups of AN individuals, for instance those with particularly severe AN or who were emaciated for a prolonged period. In our study lowest lifetime weight was positively correlated with GM volumes in the precuneus and insula. This finding is interesting, as regional GM reductions in both the insula (Brooks et al., 2011) and precuneus (Gaudio et al., 2011; Joos et al., 2011a) have been reported in previous AN studies. Moreover, functional alterations have also been described in these regions (García-García et al., 2013). However, it should be noted that these correlations did not survive correction for multiple comparisons, and so must be interpreted with caution, as they could reflect spurious findings.

Also, women recovered from AN may be characterized by other structural brain changes that were not considered in Paper 1. For example, increased cortical folding in the lateral PFC have been observed in patients (Schultz et al., 2015). Reduced cortical thickness have also been described in ill patients (Bär et al., 2015), although these appear to normalize following weight-gain according to one study (Bernardoni et al., 2016). Moreover, there is increasing evidence that both ill and recovered AN women are characterized by alterations in white matter integrity (Frank, Shott, Hagman, & Yang, 2013b; Kazlouski et al., 2011; Yau et al., 2013). Intriguingly, some of these alterations correlates with anxious traits in AN women (Kazlouski et al., 2011), and similar associations have been reported in healthy individuals (Westlye, Bjørnebekk, Grydeland, Fjell, & Walhovd, 2011). As these morphological characteristics were not considered in Paper 1, it is not known whether our recovered AN women are characterized by similar structural brain alterations. Future studies should continue to characterize structural brain alterations in both ill and recovered AN women.
5.1.2 Functional alterations in limbic and prefrontal circuits?

In Papers 2-3, we investigated recovered AN women’s neural responses to tasks containing general emotional stimuli. For both tasks, the emotional stimuli were distracting and served to bias the bottom-up system. According to our reasoning, these tasks require the recruitment of cognitive control processes while performing the tasks, as inhibitory processes must be engaged to suppress the irrelevant emotional stimuli. We hypothesized that recovered AN women would be characterized by hyperactivations in both bottom-up (i.e. amygdala) and top-down (i.e. lateral PFC) circuits. We partly confirmed our hypotheses for both studies.

In Paper 2, we showed that women recovered from AN were characterized by amygdala hypoactivations during emotional non-conflict. During emotional conflict however, there were no differences between groups in neural activation. We suggested these results reflected impaired emotional processing of emotional stimuli. These results may have some association with the impaired emotional recognition abilities in AN, which also persist following recovery (Oldershaw et al., 2011). Amygdala hypoactivation have previously been observed in AN patients, but in response to food stimuli (Holsen et al., 2012). A more common finding is that food and body-related stimuli evokes exaggerated amygdala responses in AN women (Joos et al., 2011b; Miyake et al., 2010; Vocks et al., 2010). Why we found decreased as opposed to increased amygdala activation is not clear, but suggests that amygdala alterations in AN differ between experimental task contexts and stimuli. To our knowledge, the study in Paper 2 is the first to report amygdala alterations to general emotional stimuli among AN women. But because of this, and because no other studies of AN have used tasks similar to the one in Paper 2, it is difficult to directly compare our results to previous studies.

We also found a similar hypoactivation in the hippocampus and striatum during non-conflict in recovered AN women. However, this cluster did not survive alpha level correction for multiple comparisons, and so should be interpreted with caution. Also, hypoactivation in these areas were observed in a continuous cluster which partly covered both the hippocampus and striatum. As we used a large smoothing kernel
(10 mm), the extent to which there were true hypoactivation in both regions is unclear.

In Paper 3, we reported that women recovered from AN showed hypoactivation in the extrastriate cortex in response to angry faces, and hyperactivation in the medial PFC in response to incongruent relative to congruent trials. The differential extrastriate cortex activation could reflect altered emotion or reward response to emotional faces. It is not associated with facial stimuli per se however, because the extrastriate cortex alteration was observed for angry faces relative to neutral faces. When considering all face stimuli (angry and neutral) together, we found no altered neural responses in AN women (results not included in Paper 3). Therefore, the extrastriate cortex in recovered AN women seemed to respond differentially according to the emotional valence of the face.

The alteration in the medial PFC was associated with the location of the probe in relation to the angry faces (i.e. attentional bias). In models of attentional bias, if the attentional system preferentially processes threat stimuli, it will automatically direct attention towards such stimuli - even though they are irrelevant to the task at hand. We suggested the PFC alteration observed in recovered AN women is associated with compensatory mechanisms, reflecting greater attentional control to counter the attentional bias to threat. However, recovered AN women did not show a behavioral attentional bias to threat. This is in line with some previous studies (Schneier et al., 2016; Schober et al., 2014), but inconsistent with others (Cardi et al., 2012; Harrison et al., 2010). In Paper 3 we provide an account for these inconsistent findings. Additionally, it is worth noting that the dot-probe task (or other tasks of attentional bias to threat) has not been used among AN women in an MRI context before. It could be something regarding our experimental setup that led to an absence of a behavioral attentional bias to threat effect (assuming it does exist in recovered AN women). An MRI environment could influence behavioral performance on the basis of a range of factors; noise levels, participants are lying down, the task is projected on a mirror etc. Also, the dot-probe task was always presented after the emotional conflict task (Paper 2). In hindsight, the order of the tasks should have been counterbalanced across participants to avoid systematic effects related to fatigue or emotional
habituation on one task. Thus it cannot be ruled out that the sequential order of tasks may have influenced results in Paper 3.

Together, Papers 2 and 3 showed that women recovered from AN are characterized by altered neural responses to emotion tasks that do not involve disorder-specific stimuli. This supports the notion that AN neuropathophysiology is related to a general aberration in the emotion circuitry. Our hypotheses were thus partially confirmed; we observed both limbic and PFC alterations; but in each paper separately.

Interestingly, even though both tasks used facial stimuli, different neural alterations were observed. This underscores the importance of the overall task context. We did not observe any AN alterations in the lateral PFC. In both Papers 2-3, we showed that the tasks were in fact associated with responses in the lateral PFC, but these were not different between groups. The reason for this might be that the tasks were not demanding enough for lateral PFC alterations in AN to be observed. Some previous studies do suggest that lateral PFC alterations in AN are only evident during high cognitive demand (Oberndorfer et al., 2011; Wierenga et al., 2014a). Alternatively, AN-alterations in these circuits may only be evident in tasks of “cool” cognitive processes, such as set-shifting or go/no-go tasks (e.g. Lao-Kaim et al., 2015; Wierenga et al., 2014a), that do not include emotion stimuli.

In sum, findings from Papers 2 and 3 provide some support to the hypothesis that AN is associated with alterations in both limbic and PFC circuits, related to emotion and cognitive control over emotion (Kaye et al., 2013). Future research should further study the alterations within and between these circuits. It may be prudent to use tasks that rely heavily on both, for example by using highly salient stimuli during high task demand.

5.2 Methodological considerations

5.2.1 Interpretation of MRI findings

The interpretation of MRI findings, particularly fMRI, is not straightforward. This is especially true when dealing with clinical populations, where patient-specific brain alterations must be interpreted in light of symptomatology, and there is a range of
possible confounders. It is also important to reiterate that fMRI measures hemodynamic responses, which provides an indirect measure of neural activity. Moreover, although MRI has an excellent spatial resolution, a single voxel still contains thousands of neurons, which are connected in complex circuits. This, along with the preprocessing steps that increase the smoothness of MRI data, means that inferences are made on the level of circuits (e.g. as opposed to single cell level). It is uncertain exactly what type of inferences can be made on this circuit level, or indeed if mental disorders can actually be characterized or differentiated sufficiently on this level.

Also, the functional specialization of different brain regions and circuits is limited; a given brain region supports many different cognitive processes. This complicates interpretation of findings. Commonly, results from fMRI studies are interpreted by means of reverse inferences, in which a cognitive process is inferred from activation in certain brain regions (Poldrack, 2006). Such inferences are deductively invalid, but are often used to make sense of a particular activation pattern. Although often necessary, this can lead to misinterpretation of findings. It is therefore crucial that neuroimagers are aware of these potential pitfalls in interpretation.

A related point is that both structural and functional brain alterations implicated in AN are also associated with other mental disorders. For instance, functional alterations in PFC and limbic circuits are also observed in anxiety disorders (Etkin, 2007). Few studies include a psychiatric comparison group, so the extent to which such alterations are specific for certain disorders, or shared, is largely unknown. Future studies should include such comparison groups to more precisely describe the functional brain alterations in mental disorders, and to what extent they reflect disorder-specific symptomatology or general emotional distress.

The translational value of MRI research is largely unrealized in psychiatry. This is obviously related to the complexity of the brain and the disorders themselves, but also reflects some of the challenges to the interpretation of findings from such studies, highlighted in this section. Lastly, interpretation of MRI findings must be viewed in light of methodological considerations, which are discussed in the following sections.
5.2.2 MRI methods

Over the years, neuroimaging (fMRI in particular) have been criticized, and the validity of results from such studies put into question (Poldrack et al., 2016). Common criticisms include potential for spurious findings, low statistical power, and low reproducibility of findings.

Multiple comparisons: A frequent criticism of MRI analysis relate to the huge number of statistical tests and subsequent inflation of type-I error rates (incorrectly rejecting the null hypothesis, i.e. detecting false positives). This can lead to the reporting of spurious findings, and low reproducibility of findings. As such, adjusting the alpha levels or restricting the amount of tests to minimize this problem is an important step in MRI analysis. While there is agreement that some form of alpha level correction should be applied, there is no gold-standard for how to perform this correction. There are numerous ways to carry this out, including FWE correction, false-discovery rate correction, and Monte Carlo simulations where a combination of uncorrected alpha levels and cluster sizes are considered together. Reducing the amount of statistical tests can also ameliorate the risk of type-I errors, and can be performed by defining a priori ROIs.

In this thesis, results at both corrected and uncorrected alpha levels were reported. Alpha level correction were performed using voxel-wise FWE levels (Papers 1-2), and by performing a Monte Carlo simulation that determined the combination of voxel-wise uncorrected alpha levels and minimum cluster sizes required to achieve an overall corrected alpha level of \( p < .05 \) (Paper 3). Also, ROIs were used for all studies to increase power and reduce amount of comparisons, although whole-brain searches were also performed to ensure we did not miss any significant effects outside our ROIs. For the fMRI studies (Papers 2-3), appropriate steps were taken to exclude all non-GM voxels from analyses. This included using a GM mask created for each participant, and by excluding voxels based on signal intensity (to further remove non-GM voxels).
However, it has been shown that popular fMRI software packages such as SPM do not adequately correct for multiple comparisons, either by being too conservative or too liberal (Eklund, Nichols, Andersson, & Knutsson, 2015; Eklund, Nichols, & Knutsson, 2015). As Papers 1-2 relied on the FWE correction implemented in SPM, it is possible that alpha level corrections were not accurate. However, it has been noted that fMRI research have mostly focused on type-I errors, with the unintended consequence of increasing type-II errors (Lieberman & Cunningham, 2009). Obviously, overlooking true effects by implementing too strict alpha levels is undesirable. Therefore, in this thesis we also reported results at alpha levels uncorrected for multiple comparisons.

**Statistical power:** Another common criticism of MRI studies concern statistical power. According to one empirical investigation (Desmond & Glover, 2002), 12 subjects are needed to detect true voxel-wise effects using liberal (i.e. uncorrected) alpha thresholds. With more realistic thresholds that adjust for multiple comparisons, this figure doubles. However, this estimation is likely inaccurate. It has been shown that fMRI studies are generally underpowered (Button et al., 2013). In fact, most studies today are only powered to detect large effects (Poldrack et al., 2016). A solution to this problem is obviously to increase power by including more participants, which can be guided by a priori power calculations. In psychiatry, the problem is often that due to low base rate of certain mental disorders it is challenging to include a sufficient number of participants.

For this thesis, no a priori power calculation was performed. An initial pilot study did investigate the feasibility of our experimental tasks, and the extent to which neural activation differences could be observed with the different conditions. This pilot study was performed on CW only. In the analyses, we used ROIs and presented results at uncorrected thresholds as well as corrected ones, all steps which can increase power. Considering what we now know about power in MRI studies, it is plausible that Papers 1-3 in this thesis were only sufficiently powered to detect effects sizes of medium to large magnitude. Therefore, the effects observed in Papers 2-3 may reflect the biggest between-group differences, while smaller effects may have gone undetected. This is a particular concern for Paper 1, where no significant between-group difference emerged in GM volumes. It is possible that the study was
underpowered, and that small differences would be evident with larger samples. However, as already mentioned, previous studies with sample sizes comparable to or smaller than our own have reported GM alterations in recovered AN individuals, indicating our study was sufficiently powered to detect similar effects.

**Analytical flexibility:** In MRI analysis, a considerable number of preprocessing and analysis steps of data are performed, and choices must be made at each step which leads to a significant number of possible configurations of these operations (Poldrack et al., 2016). These configurations can influence the final results, and lead to low reproducibility of findings across studies. For this thesis, we used conventional preprocessing and analysis steps similar to other studies. We also attempted to describe these steps in detail in Papers 1-3, so it would be clear to others how we handled and processed data. When in doubt regarding some of the choices, expert advice was acquired from software manuals, research articles, and online web forums.

5.2.3 Definition of AN recovery

The rationale for studying women recovered from AN is to exclude the potential confounding effects of malnutrition, emaciation, and other state effects related to active AN. Neuroimaging studies often recruit recovered women with the goal of elucidating trait characteristics that are unrelated to such confounders. Consequently, studies need to operationalize AN recovery. However, there is no universal definition of AN recovery (Couturier & Lock, 2006). This complicates cross-study comparisons, as investigators often use different operationalizations. If patients are not adequately recovered, state effects may be present and confound findings. Additionally, there is the consideration that brain structure and function may need time to recover from secondary effects of active AN, so duration of recovery is important to consider.

Weight, eating disorder psychopathology, and psychosocial functioning are all potential markers of recovery. Rates of recovery vary widely dependent on the definition used, from 57 – 94%, underscoring the importance of the operationalization of recovery (Couturier & Lock, 2006). The optimal definition of recovery may also
depend on the specific goals of a study; a recovery definition used in a treatment study may not be equally suitable for a neuroimaging study.

The Morgan-Russell criteria is one of the earliest attempts to define recovery from AN (Morgan & Hayward, 1988). According to these criteria, good outcome (recovery) is defined as a weight within 15% of ideal body weight and regular menstrual cycles. The DSM-5 (APA, 2013) defines full remission as a sustained period of time in which none of the criteria for AN have been met. Both these sets of criteria fail to consider the presence and extent of eating disorder psychopathology (cognitions and behaviors) beyond the specific diagnostic criteria for AN, and ignores psychosocial functioning altogether. Also, they do not specifically state the required duration for which the criteria must be met. This is important for neuroimaging studies, because the body and brain may need time to restitute from secondary effects of AN.

Other definitions of recovery generally include a combination of minimum body weight (typically above BMI 18.5 or 85% of ideal weight) and absence of eating disorder behaviors (e.g. binge-eating, purging, restrictive food intake). Some period of time (e.g. 3 months or 1 year) is usually specified, for which the recovery criteria must be met. Psychological recovery, inferred by a score below a certain threshold on eating disorder measures (e.g. EDE-Q) is not always included, despite some emphasizing its importance (Couturier & Lock, 2006).

In this thesis, recovery was defined based on a combination of weight and eating disorder behaviors (including restrictive food-intake, excessive exercise, binge-eating, and purging). Only participants who had met these criteria for the duration of one year prior to the study were included. We felt these criteria were sufficiently conservative to avoid potential confounders related to active AN. They were also similar to previous neuroimaging studies (Wagner et al., 2007; Wagner et al., 2006). In line with many other studies, we did not consider eating disorder cognitions (e.g. over-evaluation of body weight and shape) in the definition of recovery. No universal definition of “excessive” or “pathological” eating disorder cognitions exist. Many use cut-off values on various measures, but ultimately any threshold will be arbitrary. We would also argue that presence of eating disorder cognitions, such as over-evaluation of body weight and shape, desire to have a flat stomach, or fear of
becoming fat, are common among young females, and their presence in former patients does not necessarily indicate active illness or non-recovery.

Bardone-Cone et al. (2010) compared partially recovered to fully recovered women. Their definition of partial recovery included a BMI above 18.5, and absence of binge-eating, purging, and fasting for past three months. Full recovery was defined using the same criteria, in addition to EDE-Q scores (on all subscales) within 1 standard deviation (SD) of community norms. This definition considers the extent of eating disorder cognitions. Both groups had psychosocial functioning similar to controls. However, the partially recovered group scored similar to ill patients on body image concerns, which shows that despite absence of pathological behaviors and normal weight, eating disorder cognitions may be present.

When applying the recovery criteria specified by Bardone-Cone (Bardone-Cone et al., 2010) to the sample included in this thesis, 82% \((n = 18)\) of the recovered AN women would be considered fully recovered. Fourteen percent \((n = 3)\) would be considered partially recovered as they scored below 1 SD on one or more of the EDE-Q subscales, and 5% \((n = 1)\) would not be considered recovered due to a BMI below 18.5 (and scored below 1 SD on one or more of the EDE-Q subscales). Overall, this illustrates that the majority of our sample would be considered fully recovered if conservative criteria for eating disorder cognitions were included. Moreover, none scored above 1 SD of the EDE-Q global score, and all scored below the suggested Norwegian EDE-Q cut-off value (Rø, Reas, & Stedal, 2015), again suggestive of recovery. Also, we required participants to have maintained their recovery status for one full year, compared to only three months in the Bardone-Cone criteria.

Although a BMI of 18.5 is commonly used as a minimum BMI criterion in the definition of recovery, we used a threshold of 18. This threshold was selected purely on the basis that one CW and one recovered AN woman had current BMI’s between 18.0-18.5. We felt excluding these two for weighing less than 0.5 BMI step below the conventional cut-off was overly conservative, and so allowed them to participate by lowering our BMI threshold to 18. Also, a BMI difference of 0.5 is quite small, and corresponds to a difference in weight of approximately 1 kilogram.
5.2.4 DSM-IV to DSM-5

At the outset of this thesis, the DSM-IV was the current version of the DSM manual. Criteria from this version (excluding the amenorrhoea criterion) were used to establish lifetime axis-I disorders (including AN). During the course of this thesis, the DSM-5 version was published. However, this has minimal implications for the interpretation of the papers in this thesis. Apart from minor changes to the wording of the criteria, the changes from DSM-IV to 5 consisted of removal of a specific weight threshold criterion, and exclusion of the amenorrhoea criterion.

Inclusion criteria in this thesis required that recovered AN women had a weight below 85% of that expected during their AN period. Only one participant was excluded on this basis. Moreover, the amenorrhoea criterion was not considered at all, in accordance with DSM-5 criteria. Therefore, there would have been minimal differences in the acquired sample had DSM-5 criteria been employed.

Lifetime axis-I disorders other than AN were also evaluated for all participants. The impact of using DSM-5 as opposed to DSM-IV criteria for this evaluation on our sample is not clear. However, lifetime history of axis-I disorders other than AN were not considered in analyses. It is possible that the use of DSM-5 criteria would have influenced which participants were included and excluded based on presence or absence of lifetime axis-I disorders.

5.2.5 Other lifetime disorders

A potential confounding factor in this thesis and other studies of AN is the presence of psychiatric comorbidity. In AN, such comorbidity is common; it is therefore important to be aware of the possibility that this can influence results. In this thesis, lifetime presence of axis-I disorders were evaluated for all participants. All CW women with lifetime presence of these disorders were excluded. For recovered AN women, those who reported presence of such disorders during the past year were excluded. Recovered AN women who reported such disorders at an earlier point in their life were included, and the specific comorbidity was recorded. Of the 22
recovered AN women, 91% had a lifetime history of another mental disorder, most commonly major depressive disorder (unpublished observations, see Figure 2).

These exclusion criteria ensured that results were not confounded by the presence of a current (or recent) mental disorder. However, the extent to which previous mental disorders in the recovered AN women influenced our results cannot be ascertained. However, it is unfeasible to include women with a history of AN only, as psychiatric comorbidity rates in AN are high. Also, recruiting such a group would potentially reduce the generalizability of our findings.

However, we did not evaluate all axis-I disorders, nor axis-II disorders. Several disorders which were not evaluated, such as autism-spectrum disorder and certain personality disorders, are frequent comorbid conditions of AN. Therefore, it is possible that some individuals had a current or lifetime history of mental disorders which were not evaluated. However, it seems unlikely that this could have significantly influenced our results, as we had a decent sample size and our main findings were robust. Also, inspection of the parameter estimates (e.g. β-weights) did not reveal extreme outliers, suggesting that results were not driven entirely by a few extreme cases (e.g. those with a specific comorbidity). The choice to focus on a subset of axis-I disorders was made to evaluate some of the most common disorders, both in the general population and in AN populations (i.e. depression and anxiety disorders). Also, certain disorders were evaluated because they could heavily influence our MRI results (i.e. substance-abuse, psychotic disorders).
Figure 2. Frequency of lifetime history of axis-I disorders in women recovered from AN. MDD = Major depressive disorder; BED = Binge-eating disorder; OCD = Obsessive compulsive disorder; PTSD = Post-traumatic stress disorder; GAD = Generalized anxiety disorder.

5.2.6 Generalizability

This thesis sought to investigate the neurobiological alterations associated with AN. Therefore, our target population was individuals who develop AN. The generalizability of our findings depends on whether or not this population was indeed sampled, and the extent to which our sample is representative of this population.

First, neither CW nor recovered AN women were randomly sampled from the underlying populations. To reach a sufficient number of recovered AN women, a targeted recruitment approach is required. Participants were recruited through universities, hospitals, and an online web forum. Additionally, recovered AN women were recruited through two user-organizations. These recruitment channels were chosen because they were a convenient way to reach a large number of young adults. However, such recruitment will most likely introduce selection biases. In this thesis, predominantly young adults with (or in) higher education were reached with this recruitment approach.

Second, the inclusion criteria will also introduce a selection bias for both groups. For CW, we excluded those with a lifetime history of a mental disorder, which obviously does not reflect the general population. However, this was done to avoid confounders which could have influenced our group comparisons. For recovered AN women, only
those with a prior history of a DSM-IV diagnosis of AN were recruited. The generalizability of our findings are dependent upon the definition of AN, which is provided by diagnostic manuals. As already mentioned, the differences between the DSM-IV and DSM-5 are minimal, so it is doubtful that the version of the DSM manual had significant effects on generalizability.

In this thesis, recovery from AN was operationalized on the basis of weight and AN-related behaviors. The definition of recovery influences the extent to which findings are generalizable to all individuals who develop AN. Our definition of recovery is similar to previous studies, and conservative in that it requires an absence of pathological behaviors and a weight above a given threshold, over a period of one year. Moreover, we excluded recovered AN women who had an axis-I disorder the previous year. Thus, the findings in this thesis may only be generalizable to women who are long-term recovered from AN, and not those in partial recovery, or those who are not recovered according to our specific criteria. It is also not known if our findings are generalizable to patients with active AN.

When recruiting recovered women, there is a concern that one is selectively recruiting a subset of individuals who are systematically different from the underlying population of recovered women, for instance only those who have a very good AN outcome. Comparing our sample characteristics to samples in other studies can shed light on this issue. The characteristics of our sample are largely comparable to prior neuroimaging studies where young adult samples of recovered AN women were included (Cowdrey et al., 2012b; Pruis, Keel, & Janowsky, 2012; Wagner et al., 2007; Wagner et al., 2006). For example, mean BMI of recovered AN women in our sample was 20.4, compared to 20.7 (Wagner et al., 2007), 21.2 (Wagner et al., 2006), 21.3 (Cowdrey et al., 2012b), and 21.5 (Pruis et al., 2012) in previous studies. Our finding of slightly lower BMIs for recovered AN women compared to CW is not uncommon (Pruis et al., 2012; Wagner et al., 2007). Mean age of AN onset in our sample was 17 years, which is typical of AN and similar to the studies by Wagner et al. 2007 (mean 16 years) and Wagner et al. 2006 (mean 16 and 18, depending on AN subtype). Average recovery duration in our study was 52 months (45 when excluding one outlier case who had been recovered for 192 months), which is comparable to Wagner et al. 2007 (who reported 48 months), but slightly longer than Wagner et al.
2006 (who reported 29 and 39 months, depending on AN subtype). These cross-study similarities suggest that our sample represents a typical distribution of recovered individuals, given our definition of recovery. This supports the generalizability of our findings.

The recovered AN women in our sample consisted of both binge-eating/purging and restricting AN subtypes (n = 11 for both subtypes). Some studies recruit only one specific AN subtype. Thus, it is unclear if our findings are equally generalizable to both AN subtypes. However, in Papers 1-2 we did not find any evidence for structural or functional brain differences between AN subtypes. Still, our studies were likely underpowered to detect such differences, considering that there was only 11 participants for each AN subtype.

However, we did not find any evidence of differences between AN subtypes in Papers 1-3, indicating our findings reflect general AN alterations and is not restricted to subtypes.

Lastly, all participants in this thesis were female. This is true for most studies of AN, and is due to the low frequency of AN in males. Therefore, the extent to which findings in this thesis - indeed in most prior neuroimaging studies - are generalizable to males is unclear. This might depend on the extent to which eating disorder psychopathology and etiology differs between males and females, which is largely unknown.

5.2.7 State vs. trait

A considerable challenge is to delineate state and trait aspects of AN. We know that starvation and subsequent emaciation have profound somatic and psychological sequelae (Keys et al., 1950). When identifying some brain alteration in AN individuals, it is difficult to ascertain whether this is related to trait characteristics of AN (which may be related to etiology), or state effects of being ill and underweight. For any observed alteration in brain structure or function, there is a range of possibilities regarding the origin of this alteration:
1. The alteration was present premorbidly, and is thus considered a trait characteristic of AN. The trait is present following recovery.

2. The alteration was not present premorbidly, but is a correlate of the acute stage of AN. The alteration reverses following recovery.

3. The alteration was not present premorbidly, but was brought on by the disorder (e.g. due to prolonged emaciation). The alteration does not reverse following recovery and reflects a scar effect.

4. Complex interactions may also occur, for instance where an alteration was present premorbidly but is exacerbated by the onset of the disorder, and remains following recovery in its premorbid form.

To circumvent the confounding effects of malnutrition and emaciation, many studies include recovered women. However, although this circumvents any transient changes secondary to AN, it does not enable one to determine if the observed group differences reflect scars of the disorder or trait characteristics (i.e. to distinguish between points 1 and 3 above). Since prospective neuroimaging studies of AN are infeasible, delineating state and trait effects in AN remains a considerable challenge. As Papers 1-3 in this thesis were cross-sectional comparisons it is not possible to draw conclusions regarding causality or the origins of observed brain alterations.

The issue of state and trait effects is also related to the discussion regarding definition of recovery. When a significant group difference is revealed between recovered AN women and CW, lack of full recovery is often raised as a possible explanation for the difference. In other words, there is the possibility that state effects associated with AN are present even in recovered patients. For example, not enough time may have passed for the body and brain to recover from AN, there may be residual symptoms etc. However, the sample in this thesis had been recovered for many years on average, had normal BMIs, and had not engaged in pathological eating disorder behaviors during the past year. Moreover, they scored within non-clinical range on our measure of eating disorder psychopathology (EDE-Q). We therefore view it as unlikely that AN-related state effects influenced our results, and instead suggest that the alterations we observed reflect trait characteristics of AN.
5.3 Strengths and limitations

The studies included in this thesis have several strengths. All participants were thoroughly evaluated with a diagnostic interview, providing information on lifetime history of axis-I disorders. The inclusion criteria were also strict, and ensured that no CW had a lifetime history of any axis-I disorder, and furthermore that recovered AN women did not fulfill criteria for an axis-I disorder the previous year. The CW were comparable to recovered AN women in age and education, and all participants were female and right-handed. For the experimental tasks in Papers 2-3, we had a considerable number of separate trials, increasing power of our analyses. Furthermore, the recruitment of recovered AN women as opposed to ill patients can be viewed as a strength, as it circumvents the confounding effects of emaciation and malnutrition. Lastly, we used experimental tasks that were unrelated to food, body, or otherwise eating disorder specific stimuli. This represents a strength as the observed findings are unlikely to reflect neural correlates of merely having fulfilled criteria for AN.

Many of the limitations of the studies in this thesis have already been covered in the discussion of this thesis. First, the cross-sectional design does not enable us to differentiate brain scar effects from trait characteristics. Therefore, we cannot ascertain whether the observed functional brain alterations (Paper 2-3) were present before the onset of AN, or reflect scars of the disorder. Also, we cannot be certain that the recovered AN women were in fact characterized by brain tissue reductions during the acute stage of the disorder, which would have implications for the interpretation of findings in Paper 1. Secondly, our study had a modest sample size, and it is possible that our sample was underpowered to detect between-group differences in brain structure and function. This is particularly relevant for Paper 1, where we failed to detect any between-group differences in brain tissue volumes. This null-finding could be due to insufficient power, and it is possible that small effects would have emerged with larger samples.

Thirdly, this thesis has a number of possible confounders; including BMI, self-reported psychopathology, and emotion regulation difficulties. Moreover, three of the recovered AN women were taking psychoactive medications at the time of study.
inclusion. Such confounders may have influenced our results. This is more relevant for Papers 2-3, where we reported between-group differences, which may have some relation to unwanted confounding variables. However, we investigated correlations between these possible confounders and the neural responses that differed between groups, but few associations reached statistical significance. Those that did are discussed in the papers. Moreover, we examined whether the recovered AN women who were on psychoactive medications influenced our results by excluding them from the analyses. This did not significantly change our results. Fourthly, it is worth noting that evaluating recovery from AN was dependent on retrospective recall and self-report, and we cannot rule out the possibility of misreporting. As already discussed however, we our recovered AN women scored within non-clinical ranges on psychopathology measures, had normal current BMIs, and were comparable to other studies who recruited recovered patients.

5.4 Clinical and research implications

This thesis showed that women recovered from AN is not associated with reduced brain tissue volumes. These findings give cause for optimism by both patients and clinicians, and underscores the importance of weight-gain during treatment to restore brain tissue mass. Knowledge regarding the detrimental effects of emaciation, and their reversibility following recovery is important and should be integrated into patient psychoeducation. The functional brain alterations reported suggest that AN is related to certain neural characteristics associated with emotion, which could play a role in AN vulnerability. Although the translational value of such findings is currently poor, they contribute to a complex understanding of AN which is beneficial for both clinicians and patients. They show that AN is associated with an altered response to emotional stimuli, and could be used in psychoeducation to discuss the importance of emotion and emotion-regulation in AN. Increased knowledge of the neurobiological correlates of AN has the potential to inform etiological models of AN, and ultimately benefit treatment and diagnosis.

Our results suggest that future research into structural brain alterations in AN should focus on investigating the extent to which extreme severe AN leads to irreversible brain damage, or alternatively the extent to which such cases have trait-like
reductions in local GM which contributes to the severity. Our findings of altered neural responses to general emotion stimuli shows that neuropathophysiological aberrations in AN are not restricted to the processing of disorder-specific stimuli, but reflect a general alteration of the general emotion brain circuitry. Future research should attempt to further elucidate these neural alterations, and to determine how prefrontal and limbic circuits interact, and their role in AN vulnerability.
6. CONCLUSIONS

In this thesis, brain structure and function of women recovered from AN were compared to CW. We showed that women recovered from AN have similar global and regional brain volumes to CW, suggesting the observed brain reductions among ill patients are secondary to emaciation and malnutrition. We also showed that these women were characterized by altered neural responses to tasks involving emotional stimuli. These alterations were situated in limbic, prefrontal, and extrastriate cortices. Our findings provide some support to the hypothesis that AN is associated with alterations within / between limbic and prefrontal circuits involved in emotion and cognitive control. The fact that these changes were observed in a group of individuals who had been recovered for many years, suggest they are related to trait characteristics of AN. These traits may be related to vulnerability for AN. Further research is needed to shed light on the neuropathophysiology of AN, and to determine the nature and clinical significance of alterations in emotion and cognitive control circuits.
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