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Cerebral structural and functional changes in adolescent females with anorexia nervosa

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Abbreviations

AN	Anorexia nervosa
ANTROS	Anorexia nervosa Tromsø–Oslo study
BDI-II	Becks depression inventory II
BMI	Body mass index
BOLD	Blood-oxygen level dependant signal
CANTAB	Cambridge Neuropsychological Test Automated Battery
CSA	Cortical surface area
CTh	Cortical thickness
DMN	Default mode network
DSM-V	Diagnostic and Statistical Manual of Mental Disorders, fifth edition
DTI	Diffusion tensor imaging
EDE-Q	Eating disorders examination questionnaire
FDR	False discovery rate
eTIV	Estimated total intracranial volume
HC	Healthy controls
HPA	Hypothalamic-pituitary-adrenal
HS	Hippocampal subfields
fMRI	Functional magnetic resonance imaging
MINI	Mini-international neuropsychiatric interview
MRI	Magnetic resonance imaging
NPC	Non-parametric combination
OCD	Obsessive-compulsive disorder
OUS	Oslo University Hospital
RASP	Regional Section for Eating Disorders at the Oslo University Hospital
RS-fMRI	Resting-state fMRI
RSN	Resting-state network
RSS	Regional Center for Eating Disorders at the University Hospital of North Norway

STAI	State Trait Anxiety inventory
T_3	Thriiodothyronine
UNN	University Hospital of North Norway
WAIS	Wechsler adult intelligence scale
WISC	Wechsler intelligence scale for children
WM	White matter

List of papers

I. Myrvang A. D., Vangberg, T. R., Stedal, K., Rø, Ø., Endestad, T., Rosenvinge, J. H., Aslaksen, P. M. Hippocampal subfields in adolescent anorexia nervosa. Psychiatry Research Neuroimaging, 2018;282:24–30.

II. Myrvang A. D., Vangberg, T. R., Stedal, K., Rø, Ø., Endestad, T., Rosenvinge, J. H., Aslaksen, P. M. Cerebral cortical thickness and surface area in adolescent anorexia nervosa: Separate and joint analyses with a permutation-based nonparametric method. Int J Eat Disord, 2021;54:561–568.

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Summary

Anorexia nervosa (AN) is a serious eating disorder with great physiological consequences also affecting the brain. Cerebral structural changes are associated with acute AN. Brain volume reduction is found in several regions, but it is unclear if this is a global phenomenon and how other morphological measures are affected. Cortical thickness (CTh) and surface area (CSA) are morphometrics that are less studied and develop with different trajectories in adolescence, when AN normally debuts. Alterations in the brains functional networks have also been found in several studies, but findings are discrepant, and few studies have investigated the same networks and in relation to core symptoms of the disorder. A structure-function link is suggested but not established. The studies described in this thesis aimed to investigate different morphometrics and multiple brain networks to improve our understanding of how AN affects connectivity in the developing brain and associations to symptoms, age and structural brain alterations.

Thirty young females in an acute state of AN and healthy age-matched control participants were included in the study and underwent clinical screening and MR imaging. MR data was preprocessed and analyzed with state-of-the-art analysis methods.

Results showed that the hippocampus was reduced in AN, adjusted for whole brain volume and that most subfields were reduced. Investigations of cortical thickness and surface area showed overlap between the two morphometrics, but some areas differed. When combining these measures, most of the brain appear to be affected by structural alterations in AN. Among several functional networks studied, three exhibit reduced functional connectivity. Reduced connectivity in two networks was differently associated with age for AN and HC, and one of the networks was associated with cortical thickness.

The results indicate that the hippocampus and its subfields are reduced and appear to be more affected than the brain as a whole. Results strengthen previous findings regarding cortical thickness reduction and indicate that this reduction appears in most of the cortex. The study provides novel insight into cortical surface area reduction in adolescent AN and show with a novel combination measure that may supersede traditional volumetric methods, that most of the cortex appear to be affected. Functional results indicate that development of functional connectivity is disrupted in AN and that a regional structure-function link may exist.

Norsk sammendrag (Norwegian summary)

Anorexia nervosa er en alvorlig spiseforstyrrelse som i hovedsak rammer unge jenter. Strukturelle og funksjonelle hjerneforandringer er funnet i en rekke studier. Samlet sett tyder funnene på at pasienter med anoreksi har redusert hjernevolum sammenliknet med friske kontrolldeltakere, og at hjerneaktivitet i områder som kan knyttes til kjernesymptomer ved lidelsen er endret. De nevrobiologiske mekanismene bak disse forandringene er uklare. Strukturelle hjerneforandringer synes å finnes i store deler av hjernen, men det er uklart i hvilken grad ulike hjernestrukturer er affisert. Få tidligere studier har undersøkt andre mål på hjernestruktur enn volum. Blant studier som undersøker hjerneaktivitet hos pasienter med anoreksi, er det få som har sett på funksjonelle hjernenettverk. Videre er det svært få studier på anoreksipasienter som undersøker hvordan endringer i hjernens struktur og funksjon henger sammen.

Flere studier har funnet at hippocampus-volum er redusert hos voksne med AN, men få har undersøkt om den er spesielt utsatt sammenliknet med resten av hjernen. Formålet med den første studien presentert i denne avhandlingen var å undersøke hvordan hippocampus var affisert hos unge jenter med anoreksi. Hele hippocampus ble undersøkt samt dens bestanddeler og i relasjon til hele hjernens volum. Den andre studien tok sikte på å undersøke kortikal tykkelse og hjerneoverflate, samt et kombinert mål som kan gi et mer presist resultat enn volum. I den tredje studien ønsket man å undersøke konnektivitet i hjenenettverk og sammenhengen mellom nettverkene og sykdomsrelaterte faktorer, samt hjernestruktur.

Jenter i alderen 12–18 år med diagnosen anorexia nervosa ble inkludert i studien. Deltakerne gjennomgikk kliniske intervjuer, selvrapportering om psykisk helse, nevropsykologiske tester og MR-skanning. Friske kontrolldeltakere i samme alder gjennomgikk samme prosedyre. Strukturelle og funksjonelle MR-data ble samlet inn og analysert med nylig utviklede metoder som få studier hadde benyttet seg av tidligere.

Resultater viste at unge jenter med anoreksi hadde redusert hippocampus-volum også når det ble justert for reduksjon i hele hjernens volum. Videre viste resultater at både kortikal tykkelse og hjernens overflate var redusert, men i noe ulike områder. En ny metode som kombinerer mål på overflate og tykkelse viste at strukturelle forandringer forekom over nærmest hele korteks. Undersøkelser av hjernenettverk viste at jenter med anoreksi hadde redusert konnektivitet i tre hjernenettverk med anatomisk plassering i henholdsvis precuneus, hippocampus og nucleus accumbens. De to første nettverkene hadde ulik sammenheng med alder for pasientene og de friske kontrolldeltakerne. Precuneus-nettverket hadde sammenheng med kortikal tykkelse, som var redusert hos jentene med anoreksi.

Funnene fra denne studien viser med nye og mer presise metoder at mesteparten av hjernen er affisert i form av reduksjon av masse, men at strukturen hippocampus kan være mer sårbar enn resten av hjernen. Videre kan funnene fra fMRI-studien tyde på at aldersnormal hjerneutvikling er forstyrret ho ungdom med anoreksi og at strukturelle og funksjonelle hjerneforandringer kan henge sammen. Fremtidige studier bør undersøke denne sammenhengen nærmere i hele hjernen hos AN-pasienter i akutt fase av sykdommen og remisjon. Funn fra disse studiene gir ny innsikt i hvordan hjernens struktur og funksjonelle nettverk er påvirket hos ungdom med anoreksi.

1 Introduction

Anorexia nervosa (AN) is an eating disorder that normally debuts in adolescence and mainly affects females. A young girl battling AN will experience emotional and physiological strain and may be hospitalized for longer periods. Neuroscientific research from the past decades show that she is likely to have abnormal brain structure and functional organization. The structural alterations may be to such an extent that it is visible on an MR image but will regain its normal appearance if an age-normative body weight is restored, suggesting that the volumetric alterations are temporary and reversible. Knowledge about these structural and functional alterations is still limited, and the question of what constitutes the reported abnormalities remain unanswered. Investigating the structural and functional variables in the developing brain of young patients suffering from AN can give valuable insight into possible mechanisms underlying the course of this illness.

1.1 Anorexia nervosa

Anorexia nervosa is the most fatal of the eating disorders, where self-starvation may result in multiple organ deterioration in the worst consequence leading to death. With a lifetime prevalence between 0.9–2.2% (Keski-Rahkonen et al., 2007) and a yearly incidence of 8 per 100 000 persons (Hoek, 2006) it is a relatively rare disease. It often debuts in adolescence with a yearly incidence of about 17.5 per 100 000 persons in the age group 10–19 years. Females are 10 times more likely to be diagnosed than males (American Psychiatric Association, 2013). Core symptoms as defined in the American Psychological Associations diagnostic manual – DSM-5 are restriction of energy intake that leads to abnormally low body weight, intense fear of weight gain and a disturbed body image (American Psychiatric Association, 2013). The behavior that leads to weight loss or a failure to gain weight may also involve excessive exercise or compensatory behavior after food intake such as laxative use or purging. Remission rates for AN inpatients after 2–3 years may be as low as 29% (Clausen, 2008). A review conclude that remission rate increase with duration of follow-up – after 16 years 84% will have achieved remission (Keel & Brown, 2010). Psychotherapy is more effective compared to mere nutritional counselling, but there is no evidence to support one specific psychotherapeutic approach above another (Zeeck et al., 2018). In Norway and other countries, some form of psychotherapy is recommended (i.e., cognitive behavioral therapy, interpersonal therapy) and for children, family-based interventions are the most common treatments offered.

Comorbid mood disorders (anxiety and depression) and obsessive compulsive disorder (OCD) is prevalent in patients with AN, occurring in more than half of adult patients and perhaps as much as in 73% of adolescents (Salbach-Andrae et al., 2008). A personality disorder is found in about half of AN patients according to a meta-analysis, with “Cluster C” types (anxious, avoidant, dependent and obsessive compulsive disorders) most frequently observed (Martinussen et al., 2017).

The etiology of AN is unknown and likely to be multifactorial. A genetic predisposition is probable as twin studies suggest 48–76% heritability for a more or less broad phenotype of AN (Bulik, Slof-Op't Landt, van Furth, & Sullivan, 2007). As it often debuts in adolescence, a link with pubertal onset is suggested – both in terms of psychosocial and biological alterations occurring in this phase of development. Particularly for females, puberty may be a vulnerable phase for the systems regulating weight and appetite. The rise of leptin and estrogen levels contribute to drastic changes in metabolism, body composition, appetite regulation, mood and stress responsivity, and may be part of the explanation of why AN is so much more common in females than males (Connan, Campbell, Katzman, Lightman, & Treasure, 2003).

Separating causal and perpetuating factors – serving to maintain symptoms and a prolonged course of illness is difficult. For instance, studies of neuropsychological functioning in AN suggest that patients have reduced performance on tasks measuring cognitive flexibility, commonly measured by set-shifting tasks and visuospatial ability (Weider, Indredavik, Lydersen, & Hestad, 2015). Such neuropsychological deficits may be a phenotypical trait but may also be a consequence of neurobiological alterations resulting from the illness itself. A meta-analysis suggest that cognitive deficits are not as pronounced in children and adolescents patients giving support to the hypothesis that this is a result of the illness, perhaps after a prolonged course (Lang, Stahl, Espie, Treasure, & Tchanturia, 2014). These cognitive difficulties may be a contributing factor in the low remission rates observed during the first years of illness.

When the body is subject to extreme malnutrition, a series of physiological alterations occur. All bodily systems will experience an effect from nutrient restriction and severely low body weight and somatic symptoms range from dry hair and skin eczema to cardiovascular dysfunction, the latter likely to be the most common cause of death in the disorder (Sachs, Harnke, Mehler, & Krantz, 2016). A series of endocrine abnormalities are found in AN patients in a malnourished state. Three major endocrine pathways, the hypothalamic-pituitary-adrenal (HPA), the hypothalamic-pituitary-gonadal (HPG) and the hypothalamic-pituitary-thyroid (HPT) axis are commonly dysregulated in AN patients with low weight, and is associated with for instance amenorrhea, elevated cortisol levels and reduced thyroid function. Low levels of triiodothyronine (T_3) are often found in AN patients with severely low weight and indicate HPA-axis malfunction. Dysregulation in these systems and the resulting negative feedback loop may sustain for instance low appetite and high levels of stress and anxiety (Schorr & Miller, 2016).

AN is a disorder that is difficult to combat and full recovery takes time to achieve. Knowledge about the link between the physiological alterations in the acute state of AN and the psychological symptoms is scarce and may partly explain the reduced effectiveness of current treatments.

1.2 Neuroimaging in AN

1.2.1 Structural imaging

Numerus studies have reported cerebral structural alterations in patients with AN, implicating several anatomical regions, mostly revealing volume reduction. Results were synthesized in a meta-analysis and the conclusions drawn was that adult AN patients showed a gray matter (GM) reduction of 3.1% and adolescents a significantly larger reduction of 8.4%. White matter (WM) was reduced by 4% in adolescents (Seitz, Herpertz-Dahlmann, & Konrad, 2016). The same meta-analysis showed that after more than one year of remission the GM reduction was no longer significant. Most studies conducted in the past five years indicate that the "pseudoatrophy" is reversible, and only a few imply a remaining structural alterations in weight recovered patients (Joos et al., 2011).

Regarding affected anatomical regions, studies find varying dispersion and the overlap between studies is limited. Areas that have been found to be reduced in more than one study are for instance the cingulate gyri (Bär, de la Cruz, Berger, Schultz, & Wagner, 2015; Brooks et al., 2011; Gaudio et al., 2011), the precuneus (King et al., 2015; Seitz et al., 2015) and the hippocampi (Brooks et al., 2011; Burkert, Koschutnig, Ebner, & Freidl, 2015; Miles, Voineskos, French, & Kaplan, 2018). Two larger studies in adolescents conclude that the structural alterations are wide spread across the cortical mantle (King et al., 2015; Seitz et al., 2015). Still, questions of whether some brain regions are more vulnerable and what constitutes the structural alterations remain unanswered.

1.2.1.1 Cortical thickness and surface area

While most MRI-studies in AN patients investigate cerebral volume and report volume reduction, a few studies have investigated cortical thickness (CTh) alterations. A large study of CTh in adolescent AN showed cortical thinning in most anatomical areas that was no longer present after body weight normalization (King et al., 2015). Volumetric methods and measures of CTh differ in how they quantify cerebral mass. While volumetric methods register the number of voxels of different tissue type, CTh is based on a mapping of the WM and pial surface and the distance between the two. The latter may be advantageous as it better accounts for cortical folding, a process that continues throughout adolescence (White, Su, Schmidt, Kao, & Sapiro, 2010).

From fetal development through childhood, the cortex of the brain steadily increases in size, both in terms of CTh and cortical surface area (CSA). During the first few weeks of gestation neural cells migrate radially (towards the surface) and vertically (parallel to the surface) and these two processes are believed to result in the increase in cortical thickness and surface area respectively. CTh peaks around age 9–10, but CSA continues to increase for another couple of years, in some regions. After their peak, both CTh and CSA steadily decrease throughout adolescence, due to synaptic pruning – the loss of unnecessary connections between neurons (Wierenga, Langen, Oranje, & Durston, 2014). As

the different measurement methods may reflect different developmental processes, the investigation of these measures separately may be of great importance in developing adolescent AN samples. Very few studies have investigated CSA in AN patients and none in adolescents.

1.2.1.2 Subcortical structures

Volume reduction is consistently found in subcortical structures as well. To what degree structural alterations in the cortex and subcortical structures are result of the same phenomenon or differ has hardly been investigated. Two studies reported volume reduction in subcortical structures in acutely ill patients, but not in weight recovered patients, suggesting that the reduction in subcortical structures is state dependent (Friederich et al., 2012; Miles et al., 2018). Total hippocampus volume is often found to be reduced in AN patients. The hippocampi consist of several cell layers and its architecture is distinctly different from that of the cerebral cortex (Fanselow & Dong, 2010). Volume reduction in the hippocampus is known to be related to elevated cortisol levels in patients with Cushing's disease, and AN patients are found to present elevated cortisol levels (Mainz, Schulte-Ruther, Fink, Herpertz-Dahlmann, & Konrad, 2012). In adult AN patients, Burkert and colleagues investigated hippocampus volume and self-reported stress levels. They found a selective reduction of two subfields, and only a trend level relationship with stress (Burkert et al., 2015). They did not investigate if the hippocampal reduction could be explained by the global brain volume reduction found in their sample. As we do not know exactly what constitutes mass reduction in AN, it may be informative to closely examine subcortical structures such as the hippocampus, that may react differently to neurochemical imbalances compared to the cortex.

1.2.1.3 Clinical correlates to structural changes

Structural changes have been linked to certain BMI-factors, although many studies do not detect a link to BMI. A couple of studies have found that regional volume reduction is related to lowest lifetime BMI (Mühlau et al., 2007; Seitz et al., 2015) and other studies have found cerebral volume reduction to be related to weight loss and BMI at the time of admission (Bomba et al., 2013). Together with results from follow-up studies showing normal brain volumes in weight recovered patients, these findings strengthen the belief in that this is a phenomenon caused by the weight loss associated with AN. Furthermore, normal volumes have been found in atypical (non-underweight) AN patients (Olivo et al., 2018). However, disorder traits have also been linked to structural alterations, such as "drive for thinness" found by King et al., (2015) to be associated with cortical thickness in an occipito-temporal region.

1.2.2 Functional imaging

A substantial number of studies, mainly in adult patients, have conducted task-based fMRI paradigms with disease relevant stimuli such as food and body cues or cognitive tasks, linking several disease traits to altered activity in different brain regions (Olivo, Gaudio, & Schiöth, 2019a). Such findings

have increased our understanding about specific brain activity abnormalities but may be limited to the study condition and have reduced relevance for clinical presentations of the disorder.

When subjects are not actively exposed to external stimuli, brain activity can be measured at rest, so-called resting state fMRI (RS-fMRI). This method returns relatively consistent brain networks or resting state networks (RSNs) found across thousands of healthy subjects in several studies. The networks are believed to represent basal coactivation between brain regions that "work together" frequently. The method is further described in the method section. RS-fMRI studies in AN have focused on selected resting state networks that are linked to disorder relevant functions such as reward, body perception and executive control (Boehm et al., 2014; Favaro et al., 2012). These are somatosensory networks, fronto-parietal networks and the widely studied default mode network (DMN). Studies find both decreased and increased functional connectivity within and between RSNs, but findings are difficult to synthesize mainly due to heterogeneous RSN selection. A network that has been implicated more than once is DMN, one study reporting decreased connectivity (Gaudio et al., 2015) a second reporting increased connectivity (Boehm et al., 2014) in adolescent patients, and a third reporting increased coactivation in recovered adults (Cowdrey, Filippini, Park, Smith, & McCabe, 2014). There are relatively few resting-state studies in the field and thus difficult to interpret the discrepant findings, but they may be due to differing analysis methods and AN samples in different stages of the disease (King, Frank, Thompson, & Ehrlich, 2018).

1.2.2.1 Clinical correlates to functional changes

Investigations into clinical correlates to functional connectivity alterations have yielded few answers. One of the few studies that could establish a link between investigated covariates and RSNs found a negative correlation between connectivity in an executive control network and harm avoidance, drive for thinness, perfectionism and depression across the whole sample including adolescent AN patients and healthy controls (Gaudio et al., 2015).

A recent review of fMRI studies using block-design stimulus paradigms in adolescent AN patients proposes a disease model where delayed development and resulting cognitive impairment sustains AN symptoms (Olivo, Gaudio, & Schiöth, 2019b). The authors propose that cognitive inflexibility is a premorbid trait that in combination with early life stressors and psychological traits such as heightened harm avoidance and body shape concerns lead to self-starvation and low BMI. The low BMI will in turn disrupt hormonal regulation and normal brain maturation during puberty causing the functional changes observed.

1.2.3 Structure-function link in AN

From neurodegenerative disorders such as Alzheimers disease a relationship between structural mass reduction and functional alterations in terms of reduced connectivity in brain networks is established

(Jessica S Damoiseaux & Greicius, 2009). As the mass reduction in AN is unlikely due to apoptosis, this relationship cannot be inferred. Very few studies have investigated the possible relationship between structural and functional alterations in AN and results are conflicting. Scaife et al., (2017) reported that GM volume explained functional connectivity alterations in adult AN patients and two other studies did not detect a link between morphometrics and brain activity (Favaro et al., 2012; Seidel et al., 2019).

1.3 Summary and aims

Brain changes in AN have been widely studied, but as the brain is a complex organ, AN is a multifaceted disorder occurring in a period of brain development, there is need for a broad investigation of different brain measures, using novel techniques, reflecting different developmental processes. MR imaging can return different measures of cerebral structure that may be more or less related to the effects of AN at different stages in development and measures of functional activity in selected areas or the entire active or resting brain. Remaining questions in the field of brain imaging in AN include the question of how different structural properties such as cortical thickness and surface area and subcortical structures such as the hippocampus are affected and how they relate to age and other clinical measures. Findings regarding the relationship between structural alterations and clinical variables are discrepant and warrants further investigation. Furthermore, the field lacks a broad investigation into multiple brain networks and how they are altered in AN. Very little is known about how functional network alterations relate to development and structural alterations in AN, a disorder that normally debuts in adolescence.

The specific aims of the research papers were as follow:

Paper I – Hippocampal subfields in adolescent anorexia nervosa.

The aim of the study was to investigate the volume of the hippocampus and its subfields in adolescent patients with acute anorexia nervosa compared to healthy age-matched controls. We aimed to investigate whether the hippocampus was reduced beyond the general GM reduction that we expected to find in the AN group. Further we wished to investigate the relationship between the hippocampus and its subfields and clinical measures.

Paper II – Cerebral cortical thickness and surface area in adolescent anorexia nervosa: Separate and joint analyses with a permutation-based nonparametric method.

The aim of the study was to investigate the two developmentally distinct morphometrics CTh and CSA, separately and combined with a novel method in adolescent AN and their relationship with clinical variables.

Paper III – Altered functional connectivity in adolescent anorexia nervosa is related to age and cortical thickness.

The aim of this explorative study was to investigate multiple resting-state networks in adolescent AN patients compared to healthy age-matched controls (HC) and the relationship with age, eating disorder symptoms and regional structural alterations.

2 Methods

2.1 Study design and participants

This longitudinal study, named the Anorexia Tromsø-Oslo (ANTROS) study included adolescent females ages 11–19. At study 1 (S1) a total of 77 participants were included, 58 in Tromsø and the remaining in Oslo. Some of the included participants were not willing or unable to undergo MR imaging and were not a part of the study population used in the three papers described in this thesis. In total, 65 adolescent females underwent clinical screening, neurocognitive testing, and MRI scanning (Figure 1). At follow up (S2) 1–2 years after S1, 12 participants underwent the same procedure. The same sample was used in all three papers but differs slightly. For various reasons, most frequently related to corrupted MR images (typically due to motion), some participants were excluded before analyses for the three papers. Data from S2 was not used for the papers described in this thesis due to the insufficient sample size.

Patients in this study were included upon admission to inpatient care in one of two specialized clinics for eating disorders, the Regional Center for Eating Disorders (RSS) at the University Hospital of North Norway (UNN) or the Regional Section for Eating Disorders (RASP) at the Oslo University Hospital (OUS). To be included in the study, patients had to have been diagnosed with restrictive type anorexia nervosa by a specialist in clinical psychology or psychiatry. All patients met the DSM-5 criteria for restrictive type anorexia nervosa, (i.e., they did not report any binge-eating episodes during the past months). Exclusion criteria for patients were binge/purge episodes during the past month.

Healthy control participants (HC) were recruited from two local high schools in Tromsø, and controls below the age of 15 and Oslo controls were recruited by snowball sampling. In Tromsø and Oslo an e-mail was sent out to employees at UiT or RASP calling for study participants. Exclusion criteria for HC's were a history of eating disorders or a BMI <17.5 or >30.

Exclusion criteria for both groups were history of traumatic brain injury, psychosis, substance abuse, neurological disorder, bulimia nervosa, and use of antipsychotic medication.

2.2 Procedure

At both clinics (RSS and RASP), patients were included 2–6 weeks after admission after starting on meal plans, regularly weighing and psychotherapy (family-based therapy). Patients were included when their T_3 levels were above 3.0 ensuring that participants were not in an extremely malnourished state and presenting severe thyroid dysfunction. Patients were asked to participate by a member of their treatment team and received written information about the project. If they expressed interest in participating the researcher met them and gave an oral presentation of the project and consent form. Within the next 1–2 weeks, patients underwent clinical screening and neuropsychological testing at

their respective clinic, and MR imaging at the radiological department at UNN or OUS in Oslo. All participants were scanned in the evening between 3 and 8 pm. For patients, scanning did not interfere with scheduled mealtimes, ensuring that participation in the project did not disrupt weight rehabilitation.

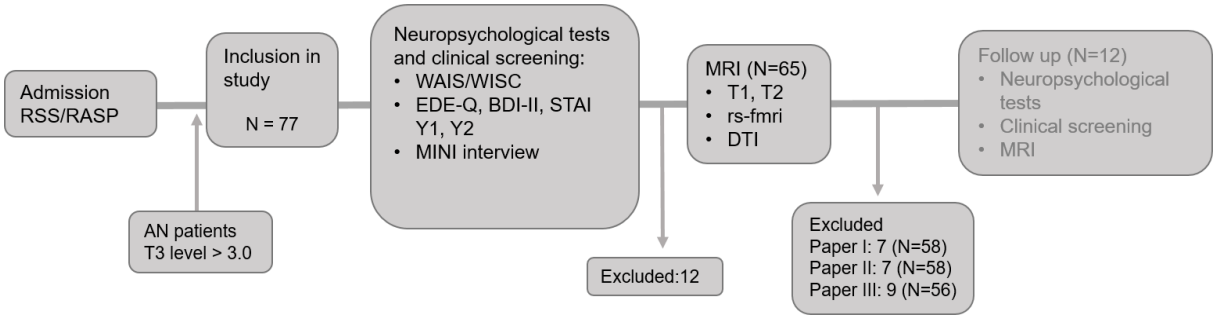

Figure 1: Procedure

2.3 Clinical screening

All participants were interviewed by a trained clinician using the MINI neuropsychological interview 6.0 to screen for other serious mental health disorders. MINI is a structured interview based on the DSM-IV axis II disorders that consists of 18 modules each regarding a psychiatric diagnose (i.e. major depression, social phobia, general anxiety disorder, eating disorders etc.) (Sheehan et al., 1998).

All participants filled out three different self-report forms measuring symptoms of depression, anxiety and eating disorders respectively. The Becks depression inventory (BDI II) is a widely used self-report measure of depression. It consists of 21 questions with four alternative answers indicating that symptoms are not present, present all the time, abstinent or severe. A total score is calculated and can vary between 0 and 64. Scores above 21 indicate moderate to severe depression and is characteristic for clinical populations (Beck, Ward, Mendelson, Mock, & Erbaugh, 1961).

The Eating disorders examination questionnaire (EDE-Q) is a well-established measure of eating disorder symptoms consisting of four subscales (restriction and concerns about eating, figure and weight) that together make up a global scale (Fairburn & Beglin, 2008). Although a clinical cut-off score of 4 for each subscale and the global scale is suggested for research studies, it has been shown that in clinical populations many patients diagnosed with eating disorders score below this cut-off (Rø, Reas, Stedal, & Reas, 2015).

To measure symptoms of anxiety, participants filled out the form State Trait Anxiety inventory (STAI). This 20 item instrument measures trait anxiety (form Y1) and state anxiety (form Y2), with total scores ranging from 20 to 80, and clinical populations usually score above 40 (Spielberger, Gorusuch, & Lushene, 1970).

2.4 Neuropsychological tests

All participants in the ANTROS study were tested with the age-appropriate IQ battery from Wechsler; WISC (Wechsler, 2003) was used for participants under 16 and WAIS (Wechsler, 2008) for participants aged 16 and up to ensure that participants had age-normal general cognitive abilities. A substantial body of research reveal cognitive dysfunction in adult AN patients (Stedal, Broomfield, Hay, Touyz, & Scherer, 2021). Findings regarding adolescent patients are less consistent, but quite a few studies indicate problems with visuospatial processing and executive function (Lang et al., 2014). Selected subtests from the Cambridge neuropsychological test automated battery (CANTAB) (De Luca et al., 2003) measuring executive functioning and visuospatial abilities. These data were not used for the papers described in this thesis.

2.5 Ethical considerations

The Norwegian committee for medical health research ethics (REC) approved the study (protocol no. 302969). Patients over the age of 16 can in Norway by law consent to medical interventions on their own behalf and could therefore consent to participating in the study. Patients under the age of 16 required additional parental consent to participate in the study. All participants gave written consent by signing a consent form.

2.6 Magnetic resonance imaging (MRI)

Magnetic resonance imaging is reliant on the magnetic properties of hydrogen nuclei in biological tissue. In response to the stationary magnetic field in the scanner and an applied radio frequency pulse that alters this magnetic field, protons will enter a high energy state. The time it takes to return to equilibrium will vary in different tissue type, giving rise to the contrasted images formed. Image formation is a complex process, and a detailed description is beyond the scope of this thesis. In short, gradient coils measure the released energy from protons in slices and the signal is reconstructed to an image. Depending on the timing and amplitude of RF pulse and gradient magnets, one can maximize the difference between for instance WM and GM, as in T1-weighted images used for structural data in this thesis. The challenges with the inhomogeneous magnetic field in the MR scanner are that it is susceptible to artefacts. Distortion (dropout and geometric) near the borders between air and tissue is the most common artefact caused by inhomogeneity in the border areas. Preprocessing of data is necessary to correct for artefacts and prepare the data for statistical analysis. Pipelines for preprocessing normally includes realignment, spatial normalization and smoothing. With realignment all images are realigned to one single reference image to correct for movement artefacts. To be able to compare images across subjects it is necessary to realign all subject images by spatially transforming all data into a common space. This is called spatial normalization. The most widely used templates for spatial normalization is the Montreal neurological institute (MNI) space. Spatial smoothing is done by

applying a filter to the images that removes high-frequency information and thus increases the signal-to-noise ratio for fMRI signals.

Functional magnetic resonance imaging (fMRI) generates functional images of the brain, by measuring the blood-oxygen level dependant (BOLD) signal. Upon neuronal activity there will be an increase of oxygen-rich blood and the deoxygenation of this blood can be measured in a similar manner to that described above for structural imaging. Traditionally in fMRI the BOLD signal is measured as a response to a stimulus. When subjects are not performing a task, fMRI can be used to measure BOLD-signal in the resting brain. Resting-state fMRI (RS-fMRI) is commonly conducted to reveal resting-state networks (RSN's) – spatially separated areas of the brain of which BOLD-signal correlates (Faro & Mohamed, 2010). Several RSN's that are consistent across trials and studies have been identified (J S Damoiseaux et al., 2006). The networks are linked to known sensory and cognitive domains such as vision, somatosensation and executive control. The most consistent network across studies is the default mode network (DMN). The DMN was first viewed as a "task negative" network, as it appeared to be supressed when subjects where performing tasks in the scanner, but during the past decade it has been suggested that it rather governs different levels of attention (Utevsky, Smith, & Huettel, 2014). In patient groups such as Alzheimer disease, schizophrenia and autism, researchers have found that RS-fMRI can distinguish patients from healthy controls (Lee, Smyser, & Shimony, 2013).

2.6.1 MRI data acquisition

The two scanners used in this study were a Siemens Skyra scanner and a Phillips Achieva, both 3T scanners. The scanning protocol included two structural sequences (high resolution 3D T1 and T2-weightet images) and a resting state fMRI scan (see scan parameters below). Total scan-time was 24.5 minutes. Participants were instructed to lie still and keep their eyes fixated on a cross during the fMRI scan.

2.6.1.1 MRI parameters (papers I–II)

Papers I and II used only the T1-weighted scans for the morphometric measures. In Tromsø a T1-weighted magnetization-prepared rapid gradient-echo (MPRAGE) sequence was performed, with the following parameters: Orientation = Sagittal; Slices = 176; Slice thickness = 1mm; Voxel size = 1x1x1, Repetition time (TR) = 2300ms; Echo time (TE) = 2.98ms; Field of view (FOV) = 256x256; Flip angle = 9º; and inversion time (TI) = 900ms, parallel excitation factor 2 (GRAPPA). In Oslo, a T1-weighted 3D-TFE sequence was used for acquisition with the following parameters: Orientation = Sagittal; Slices = 184; Voxel size = 1x1x1; Slice thickness = 1mm; TR/TE/T1 = 3000/2.3/853ms.; FOV = 256x256; Flip angle = 8º; and parallel excitation factor 2 (SENSE).

2.6.1.2 fMRI parameters (paper III)

The following parameters were used for functional imaging at both sites: A 2D, T2*-weighted gradient-echo sequence with echo-planar-imaging (EPI) readout. Voxel size: 3x3x3, matrix size: 80x80, TR: 2500ms., TE: 30ms., acquisition order: interleaved (43 slices), no. volumes: 288. Scan-time for fMRI sequence was 12.08 minutes

2.6.2 Preprocessing of structural data (papers I–II)

Surface reconstruction and volumetric segmentation was performed with FreeSurfer v6.0 software (http://surfer.nmr.mgh.harvard.edu) version 6.0; with the "recon-all" processing pipeline. The pipeline includes motion correction, normalization to Talairach space, intensity bias correction, skull-stripping, surface registration and segmentation. Visualization of the data and inspection for artefacts described above was performed before and between the different steps of preprocessing. Skull stripping can at times result in erroneous inclusion of skull of dura mater in the pial surface used to calculate CTh, CSA and volume. All images were checked after skull stripping, errors were manually edited for all individual subjects and erroneous cases were rerun before statistical analyses.

2.6.3 Preprocessing of functional data (paper III)

Functional and structural images were preprocessed with FSL FEAT (FSL ver. 5.0.11, fsl.fmrib.ox.ac.uk). The functional images were corrected for scan-to-scan motion, coregistered to the high-resolution anatomical image, warped to the MNI152 template. Spatial smoothing was performed with an 8mm FWHM Gaussian filter. Motion-related independent components were removed with ICA-AROMA (Pruim et al., 2015)

2.7 Statistical analyses

2.7.1 Descriptive data (papers I–III)

For all three papers, descriptive data was inspected on whether they fulfilled the assumptions of normality. Scatter plots, box plots, and QQ-plots were visually inspected, and Shapiro-Wilks tests were conducted to test normality. Group difference in sample characteristics were investigated with either one-way analysis of variance (ANOVA) or Mann-Whitney U-test using IBM SPSS 26.

2.7.2 Statistical analyses paper I

Using IBM SPSS 26, linear regression analyses were used to investigate the relationship between HS volumes and group affiliation (AN vs. HC) with age, depression score (BDI), estimated total intracranial volume (eTIV) – a common measure used to adjust for interindividual differences in scull and brain size, drug use and scanner site as additional covariates. Analyses were performed for all HS separately and results were corrected for errors of multiple comparisons with the false discovery rate (FDR). All analyses were reperformed with total brain volume (ventricles excluded) replacing eTIVas

a covariate to investigate if volume reduction in the hippocampus was explained by whole brain volume reduction. Group stratified linear regression analyses for whole hippocampus and HS that were significantly smaller in the AN group were conducted separately for the following clinical variables: BMI, BMI-SDS, BMI increase, weeks since admission, years since first GP consultation, subscales and global EDE-Q score, BDI-II score, STAI Y1 score.

2.7.3 Statistical analyses paper II

The software package Permutation analysis of Linear Models (PALM) was used to analyze CTh and CSA separately and combined, the latter with a nonparametric combination method described in (Winkler et al., 2016), using Fisher's method for combining p-values (Fisher, 1934). As statistical tests are usually performed on thousands of image units (i.e., voxels or vertices), MR analyses are prone to problems of multiple comparisons and false positive results. Permutation testing is a nonparametric method that provides better control of false positives (Winkler, Ridgway, Webster, Smith, & Nichols, 2014). The nonparametric combination method is a statistical method of combining p-values rather than morphometrics (CTh and CSA) that may supersede traditional volume methods (usually the product of surface area multiplied by thickness at each vertex) because it does not assume independence. This is advantageous when studying CTh and CSA that are highly related morphometrics, sharing the same environment (Winkler et al., 2018).

Analyses were performed in two stages; firstly, a model testing contrast between groups (HC>AN) with age as a nuisance variable was performed. Subsequently BMI was included in the model to investigate if group differences in CTh, CSA and the combination was explained by BMI. The same analyses were conducted in AN group only including BDI-II, STAI Y1 and Y2, and EDE-Q scores added as covariates, one by one to avoid multicollinearity. The Desikan-Kiliany atlas (Desikan et al., 2006) was used for anatomical labels.

2.7.4 Statistical analyses paper III

To detect functional components in the dataset, the software Group Independent Component analyses (GIFT) was used (http://icatb.sourceforge.net/). GIFT applies independent component analysis (ICA) to extract statistically independent sources of the BOLD signal. The algorithm can be run without defining an a priori number of components to extract, however, in a dataset with high variability this can result in an extremely high number of components each covering very small anatomical areas. As this would be difficult to interpret and uninformative, a decision was made to set component number to 25. A large body of RS-fMRI studies have reviled between 10 and 30 consistent brain networks (Beckmann, DeLuca, Devlin, & Smith, 2005; J S Damoiseaux et al., 2006; Smith et al., 2009) and the number 25 was chosen to capture as many of these networks as possible, but at the same time avoiding "splitting up" known networks. Of the 25 components one component was discarded as it clearly represented noise signal. One component, mainly located in the cerebellum appeared to involve signal

from the sinus (between cerebellum and cerebrum). A decision was made to discard two auditory networks, because at the time we were not familiar with any literature indicating abnormal brain activity in auditory brain regions. The remaining 21 networks resembled well known RSN's, a few however, appeared to have been "split up", such as the DMN and four components were identified as part of the DMN. A multivariate analysis was performed with all components and group affiliation, age and age*group interaction as covariates with MANCOVAN toolbox in GIFT. A second multivariate model was run with BMI as a covariate. In MANCOVAN, statistically significant components are selected automatically for further univariate analyses and univariate analyses are ran subsequently for each component and covariates.

2.7.5 Correction for between-site effect

For all three papers main analyses were rerun with HC participants and site as grouping variable, to investigate the potential confounding effect of scanner site. Mean BMI differed, though not statistically significantly, between AN patients in Tromsø and Oslo, and thus to avoid a potential confound of BMI only HC were used in these analyses. For paper I site was adjusted for in all analyses and main analyses were reperformed with participants from only the Tromsø scanner with highly similar results.

3 Results summary

3.1 Descriptive results

The AN group had significantly higher scores on self-report measures of symptoms of depression (BDI-II) and anxiety (STAI, Y1 & Y2) and eating disorder symptoms (EDE-Q). Patients had significantly lower BMI and BMI-SDS compared to HC. Results from neuropsychological tests did not show a significant group difference in IQ or executive functioning and working memory and were thus not included in any further analyses for the three papers included in this thesis.

3.2 Summary results paper I

Bilateral hippocampus volume and all but one hippocampal subfield was reduced in the AN group compared to healthy controls, adjusted for age, depression score and drug use. Results for nine subfields remained significant after adjustment for whole brain volume (reduced in AN group). Group stratified analyses of HS and clinical variables revealed a positive significant relationship between some of the subfields and scores on measures of anxiety (STAI) and depression (BDI-II).

3.3 Summary results paper II

Results showed significant reduction in both CTh and CSA in several cortical regions in AN compared to HC and the reduction was related to BMI. Different results for the two morphological measures were found in a few cortical regions, such as the superiorfrontal region. The joint NPC analyses showed significant group differences across most cortical regions. For the joint NPC analyses, some clusters including parietal and frontal regions remained significant when adjusting for BMI.

3.4 Summary results paper III

Results revealed significant group differences in five RSN's, and a relationship with clinical covariates in three of them. Univariate results revealed reduced intra-connectivity in a default mode network (DMN) located in the precuneus and two subcortical networks. Analyses of group*age interaction showed a significant interaction effect in two networks involving respectively the hippocampus and amygdala indicating a negative effect of age on intra-connectivity in the first and a positive effect in the latter for AN patients. Precuneus thickness was significantly associated with connectivity in the DMN (precuneus) network.

4 Discussion

The main aim of this thesis was to investigate different structural and functional properties in the brains of young females with AN compared to healthy age-matched participants. Results from paper I show that volume of the hippocampus is significantly reduced when adjusting for whole brain volume, and hippocampus reduction is thus not explained by general volume decrease. Hippocampus reduction appear to be unselective, as most subfields are significantly smaller in adolescent AN patients compared to healthy controls. In paper II a novel method was utilized to measure the combined effect of CTh and CSA, not previously investigated in AN. Results show that most anatomical regions of the cortex is affected in our AN sample, strengthening the notion that mass reduction in AN is a global phenomenon. CSA reduction, not previously investigated in adolescents, was found in large areas of the brain to an extent that has not been shown previously in any study. CTh was reduced in large areas of the brain, but some regions differed for CTh and CSA, for instance the superior frontal region. Results from paper III showed that three functional brain networks exhibited reduced internal connectivity in the AN group, and that hippocampus and amygdala connectivity was associated with age and precuneus connectivity with precuneus structure.

In all three papers, group differences between AN and HC has been shown for functional and structural brain metrics, strengthening previous findings in the field and bringing forth novel findings regarding the relationship between brain structure and function, and BMI and age, and lastly pointing to a possible structure-function link in adolescent AN patients.

4.1 Implicated brain regions

In the three papers included in this thesis, findings have implicated three brain regions in particular, namely the precuneus, the hippocampus and the amygdala. These regions are implicated in abundance in previous AN literature and our findings are discussed according to these results. Hippocampus reduction in AN is found repeatedly, particularly in the last few years. A recent review (Keeler et al., 2020), also citing paper I, conclude that there is cause to believe that the hippocampus is particularly vulnerable in AN patients. In this review authors highlight the importance of investigating the specific effects of AN on hippocampus. The hippocampus is known to play a key role in several cognitive processes such as learning and memory consolidation and is also highly related to emotions and mood (Fanselow & Dong, 2010). In patients with major depression, PTSD and adverse childhood experiences, hippocampal abnormalities are found (Keeler et al., 2020). As neuropsychological deficits and comorbid mood disorders is frequent in AN, the hippocampus is an area of interest. It is evident from the studies described in this thesis and several other studies in the field that the hippocampus is an important structure to consider in AN both in terms of structural and functional alterations.

In paper III we found that an amygdala network was differentially related to age in AN compared to HC, where increased activity was observed with increasing age. Amygdala volume reduction has been shown in multiple studies and was also present in our sample, although these results were not included in any of the papers. The amygdala is well known for its role in fear and anxiety and in relation to AN altered amygdala activity has been linked body image distortion (Gaudio & Quattrocchi, 2012). Connectivity is expected to increase with increasing age in networks involving both the hippocampus and amygdala and results from paper III indicate that the opposite is the case for the hippocampus network and that there may be an accelerated development in amygdala connectivity in AN patients. A hyperactivation of the amygdala is observed in healthy adolescents compared to adults and it is proposed that this is an essential factor in the debut of AN (Kappou et al., 2021). Findings from paper III may reflect this phenomenon.

Highly significant cortical thinning of the precuneus was present in the AN group in paper II, and reduced internal connectivity was found in paper III that was related to cortical thickness. The precuneus is a core region of the DMN, a network often found to have altered connectivity patterns in AN and other major psychiatric disorders (Olivo et al., 2019b). Precuneus thickness is expected to decrease during adolescence (Vijayakumar et al., 2016) and our findings indicate an accelerated thinning of the cortex in this area in AN. An association was found between the area of peak activity in the precuneus network and cortical thickness in this region, defined by the Desikan-Kiliani atlas implemented in FreeSurfer. As this was investigated across the whole sample, this may point to a general structure-function link in this region but may also reflect an association between the observed thickness reduction in AN and functional alterations. Few studies have investigated structure-function links in AN, and there may be regional variances to such a relationship. Results from the ANTROS study warrants further investigation into regional structure-function relationships. For instance, the insular cortex is often implicated in both structural and functional studies in AN patients and may represent an area of interest for future studies.

The precuneus is a region that is often associated with the integration of visual and somatosensory information and structural and functional alterations may be related to a core feature of AN, the disturbance in body image. A large study in adolescents presented a relationship with precuneus thickness and “drive for thinness” (King et al., 2015). Neither CTh in this area or functional connectivity was found related to eating disorder symptoms in the studies presented in this thesis.

4.2 Possible mechanisms

Attempts have been made to unveil the constitution of structural changes in AN patients, and several hypotheses have been proposed. Apoptosis is unlikely, as a normalization of brain structure is demonstrated in several studies when patients regain normal body weight (Seitz et al., 2016). Post mortem rodent examinations have shown reduced spine density and fewer dendritic branches in

malnourished rats (King et al., 2018). Also, the number of astrocytes, supporting cells that supply energy to neurons, were found to be 50% reduced in a study utilizing the activity-based anorexia animal model (Spadini, Ferro, Lamanna, & Malgaroli, 2021).

A selective hippocampus reduction, as found previously in adults (Burkert et al., 2015), affecting only some HS could indicate that certain cells are more vulnerable as the different HS are made up of different neuron types. The results from paper I showed an unselective hippocampus reduction. A more recent study investigating adults at different stages of the disorder also found that most subfields were reduced and was present in both patients with short and long duration of illness (Collantoni et al., 2020). These findings, along with the findings from paper I indicate that although the hippocampus appear to be particularly vulnerable, it does not seem that this is because of its diverse constitution.

Previously described dysregulations of endocrine systems, along with altered levels of neurotrophins such as brain derived neurotrophic factor (BDNF) and neurotransmitters such as serotonin may contribute to shrinking or stunted growth of neurons and supporting cells (King et al., 2018). The hippocampus is a structure in which neurogenesis occurs throughout adult life, and chronic stress has been found to impair this process (Keeler et al., 2020). It is sensitive to alterations in cortisol level and is highly innervated with serotonin receptors. A recent review of studies investigating hippocampal alterations in AN point to a relationship with cortisol levels (Keeler et al., 2020). Review authors report that all studies measuring cortisol level (N=5) found an increase in AN patients compared to HC, and two of them investigating longitudinally found a decrease from start to end of treatment. A few of the studies found a relationship with brain structure and one reported an inverse correlation between cortisol and hippocampus volume. Hippocampus abnormalities may be due to elevated cortisol levels and future studies should investigate this relationship more closely.

4.3 Comorbid depression and anxiety

Hypercortisolemia in AN may be due to secondary effects of starvation but may also be due to elevated stress and comorbid conditions of anxiety and depression. As hippocampus reduction is found in patients with major depressive disorder, we adjusted for BDI scores in paper I, and could conclude that depression symptoms were not driving the hippocampus reduction in AN. As depression and anxiety are associated with lower hippocampus volumes, the correlations between HS volumes and BDI and STAI were expected to be negative. Treatment and initial weight gain can in AN patients induce increased anxiety, shown in studies measuring refeeding and pre-meal anxiety over the course of treatment (Akgül, Bonny, Manos, Jackson, & Holland-Hall, 2021; Kezelman, Touyz, Hunt, & Rhodes, 2015). Patients reporting higher levels of depressive and anxious symptoms may be further along in weight rehabilitation and thus at a healthier weight. These unexpected findings may reflect the complexity of comorbidity in AN. Authors of the review of studies of hippocampal alterations

highlights a lack of stress-based neurobiological models of AN as is proposed for other psychiatric disorders (Keeler et al., 2020).

4.4 Development

The ANTROS study results point to a relationship between structural and functional brain changes and age in a sample that is known to experience its final growth spurt in brain development. In general, a drastic decrease in GM due to synaptic pruning and an increase in WM due to increased myelination is observed during the years between 11 and 22, but there are regional differences and as described in the introduction, different morphometrics develop somewhat differently (Vijayakumar, Op de Macks, Shirtcliff, & Pfeifer, 2018; Wierenga et al., 2014).

A widespread CSA reduction was found in our adolescent sample, contrary to previous findings in adults where no reduction or only small areas of reduction is detected (Leppanen, Sedgewick, Cardi, Treasure, & Tchanturia, 2019; Miles et al., 2018). This may indicate that the adolescent brain responds differently to AN than the adult, perhaps delaying normal brain development. The discrepant findings for CTh and CSA may point to this: Although the majority of regions were similarly affected in the AN group for both CTh and CSA, there were some differences. For instance, in the superior frontal region, we found that most of this region had a smaller CSA in patients, but CTh was not significantly lower, particularly in the left hemisphere. CSA and CTh is found to have opposite trajectories during adolescence in the superiorfrontal region where CSA increases and CTh decreases with increasing age (ages 11–20 years). It appears as though the expected increase in thickness and decrease in CSA is stunted in AN patients based on our results. In the study described in this thesis, the effect of age was adjusted for, but not investigated, and future studies are needed to confirm if AN indeed disrupts or delays cortical maturation.

Results from paper III show a significantly different relationship between connectivity and age in AN compared to HC in a bilateral hippocampus and a unilateral amygdala network suggesting a disruption of age-related development of connectivity. Previous functional studies have found hyperactivity in both the hippocampus and the amygdala in response to food cues, suggesting a heightened sensitivity in this region in patents (Kappou et al., 2021; Keeler et al., 2020). Whether hyperactivity in limbic systems presents a premorbid sensitivity that contribute to the development of AN or is a result of the cascade of endocrine and neurochemical consequences of either starvation or the stress and anxiety associated with AN and refeeding is not known.

Puberty is found to have a unique contribution to brain development, independent of chronological age. In a recent review, Vijayakumar and colleagues report evidence for associations between brain structure and functional connectivity and pubertal stage and/or sex hormone level that persist when adjusting for age (Vijayakumar et al., 2018). As AN can delay pubertal onset, it is probable that more

of the AN participants were prepubertal compared to the control group. Particularly regarding paper II and III, where age and brain development are central themes, pubertal stage would be an important measure. However, as pubertal status was not recorded for this study, a delayed onset of puberty in the AN group, and not chronological age may be driving this difference. Thus, pubertal status or pubertal onset may be a confounder of results in the ANTROS study.

4.5 BMI

Only paper II presents a significant effect of BMI that appear to explain most of the group difference observed. In paper I, BMI was investigated in groups separately and in the AN group it did not explain HS reduction Previous studies of hippocampus volume did not detect a relationship with BMI either, but a mor recent study did (Collantoni et al., 2020). It may be the case that the group difference in BMI drives a correlation, and the variability in the AN group may be too low to produce a significant correlation.

Patients in the ANTROS study were included 2–4 weeks after admission to inpatient care, a choice made to avoid the effects of acute starvation. At this point, patients had been on fixed meal plans and receiving treatment interventions for several weeks and had increased their weight substantially, albeit still severely underweight. In general, there is a lack of consensus in the field of AN research on if and how to define stages of the disorder, remission and relapse, and this is particularly problematic in MR research, as the brain is sensitive to changes in nutritional and hydrational status (King et al., 2018). Inclusion on the day of admission was an option, but the choice to wait until they had started treatment was not only to avoid starvation, but also due to ethical concerns. Most of the young patients were admitted for the first time and required time to adjust to the new environment with minimal disruption and activity. For papers I–II analyses were reran including the BMI difference score (subtracting BMI at admission day from BMI at the day of scanning) as a covariate to investigate if the weight increase during admission had affected results. This is recommended by renowned researchers in the field in a review directing future research in structural neuroimaging in AN (King et al., 2018). Results from all analyses including this variable were similar to the main findings, indicating that the BMI increase did not affect results. Furthermore, it indicates that the initial weight gain (mean increase reported in paper I was 0.9) is not related to either hippocampus volume, CTh, CSA or the combination of CTh and CSA. This may mean that regeneration of brain mass does not happen during the first 2–6 weeks of treatment and weight rehabilitation, but multiple imaging timepoints during admission are needed to confirm this. One longitudinal study showed that normalization was achieved after three months, and it is assumed that normal weight is a prerequisite (Bernardoni et al., 2016).

4.6 Limitations and strengths

As AN is mainly diagnosed in females, only females were recruited for this study, with the obvious caveat that the results cannot be generalized to male AN patients.

Regarding the scanning procedure it is recommended that participants are scanned at a fixed time of the day, preferably fasting to avoid effects of nutritional differences, and that hydrational status is measured (King et al., 2018). Participants in this study were scanned in the evening after 3pm and measures of hydration were not collected.

Historical measures of the illness such as lowest lifetime BMI, duration of illness and total weight loss prior to admission are in previous studies found to be related to morphometrics and connectivity (Bomba et al., 2015, 2013; Mühlau et al., 2007; Seitz et al., 2015), but these data were not recorded in the present study. The variable "years since first GP visit" was recorded as a year and calculated to whole years and most of the young patients in this sample had been to such a consultation in the previous year, leaving this variable greatly skewed towards one year.

The multicenter design of the study, particularly regarding the use of two different scanners, can cause a site effect. Due to a difference in mean BMI, though not statistically significant, in AN patients between the two sites, a possible scanner effect was only investigated in HC. As there was no such effect detected in the HC group, it is likely that it was not present in the AN group either.

In paper III, an a priori definition of number of components to extract was set to 25 and this seemed to result in a "splitting up" of the DMN into multiple networks. With a smaller number of components, results regarding the precuneus and DMN could hypothetically have been more informative given that the DMN would have been more "intact".

Probably due to the rarity of the disorder, only a handful of studies include more than 30 adolescent AN participants and thus the sample size in this study could be considered a strength. Furthermore, as most studies are conducted in adults, the narrow age group ranging from 12–18 years is also a strength to this study. However, no a priori power analyses were conducted.

Robust and state-of-the-art analysis methods are utilized in all three papers. For paper I-II recently developed software not previously reported in the field was used, and for paper III we conducted a largely data driven analysis of which there are very few in the field.

5 Conclusion

Taken together, results from the ANTROS study show widespread, global GM reduction with a new and robust method, and point to a particular hippocampus affliction in young AN patients, both in

terms of structure and function. A disruption of brain maturation is indicated by findings from paper II–III. Different anatomical location of CTh and CSA reduction may reflect a disruption of the different development trajectories for the two morphometrics. Furthermore, a developmental decrease in connectivity in a hippocampus network and accelerated connectivity in a network involving the amygdala in AN patients compared to HC indicate an abnormal age-related development in these networks. Lastly, a rarely investigated structure-function link warrants future investigations into regional relationships between structural and connectivity changes.

The papers described in this thesis provide novel findings of associations between brain alterations and covariates and provide a basis for further investigation into mechanisms involved. Particularly the relationship between functional alterations and age in limbic structures emphasizes the importance of disentangling the effects of starvation and stress as adolescence is a critical period for maturation and development of connectivity in these regions. As discussed in this thesis, with the knowledge about the effects of puberty in brain development, it is of key importance that future studies include measures of pubertal status when considering the effect of AN on brain development. Also, the importance of including measures of cortisol is highlighted by this discussion, as AN may be a disorder with a stress-based neurobiological underpinning. Future studies should also include detailed measures of illness history, as results in the field so far indicate that the stage of disorder is of essence regarding brain changes and a key to understanding these changes is to map the course of illness and cerebral morphometrics and connectivity.

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Paper I

Hippocampal subfields in adolescent anorexia nervosa

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Hippocampal subfields in adolescent anorexia nervosa

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ABSTRACT

Patients with anorexia nervosa (AN) exhibit volume reduction in cerebral gray matter (GM), and several studies report reduced hippocampus volume. The hippocampal subfields (HS) are functionally and structurally distinct, and appear to respond differently to neuropathology. The aim of this study was to investigate HS volumes in adolescent females with restrictive AN compared to a healthy age-matched control group (HC). The FreeSurfer v6.0 package was used to extract brain volumes, and segment HS in 58 female adolescents (AN = 30, HC = 28). We investigated group differences in GM, white matter (WM), whole hippocampus and 12 HS volumes. AN patients had significantly lower total GM and total hippocampal volume. No group difference was found in WM. Volume reduction was found in 11 of the 12 HS, and most results remained significant when adjusting for global brain volume reduction. Investigations of clinical covariates revealed statistically significant relationships between the whole hippocampus, several HS and scores on depression and anxiety scales in AN. Results from this study show that young AN patients exhibit reduced volume in most subfields of the hippocampus, and that this reduction may be more extensive than the observed global cerebral volume loss.

1. Introduction

Anorexia nervosa (AN) is a severe mental health disorder characterized by a disturbance in body image perception and a restriction of nutrient intake resulting in abnormally low body weight (American Psychiatric Association, 2013). Patients with AN have significantly elevated mortality rates compared to other mental health disorders (Arcelus et al., 2011) and the majority have their illness debut during adolescence. Brain imaging studies consistently find that global gray matter (GM) volume is reduced in patients with AN, although there are some discrepancies regarding the degree of atrophy and affected areas (Gaudio et al., 2011; King et al., 2015; Seitz et al., 2016). A recent meta-analysis concluded that GM reduction is significantly greater in adolescent patients with AN compared to adults (Seitz et al., 2016). Findings regarding white matter (WM) are inconsistent, but recent studies suggest that WM volume and integrity are better preserved in young patients with AN compared to adults (Pfuhl et al., 2016; Seitz et al., 2016). Longitudinal studies indicate that total brain volume mostly normalizes as patients recover (Bernardoni et al., 2016; Mainz et al., 2012), but it is yet unclear whether regeneration is total and if it applies to all cerebral regions.

Volume reduction of the hippocampus formation has been reported in several studies in both adults and adolescents with AN (Burkert et al., 2015; Chui et al., 2008; Connan et al., 2006; King et al., 2015; Mainz et al., 2012). The formation of the hippocampus is well known for its involvement in learning and memory, but also plays an important role in emotional regulation (Fanselow and Dong, 2010). Hippocampal atrophy is evident in other severe mental health disorders, such as major depression (Treadway et al., 2015), schizophrenia (Wright et al., 2000), bipolar disorder (Haukvik et al., 2015), post-traumatic stress disorder (PTSD) (Hayes et al., 2017) and borderline personality disorder (Driessen et al., 2000) and a common underlying mechanism driven by stress and elevated glucocorticoid levels has been proposed (Sapolsky, 2000). Patients with AN often experience comorbid symptoms of depression and anxiety (Kaye et al., 2004; O'Brien and Vincent, 2003). The link between hippocampal volume reduction and comorbid

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symptoms has not been extensively investigated. One study found no relationship between depression and coping and hippocampus volume in adult AN (Burkert et al., 2015).

The hippocampus is a heterogeneous structure with multiple cell layers and several distinct "hippocampal subfields" (HS) that are structurally and functionally different from one another (Duncan et al., 2012; Leutgeb et al., 2007; Zeineh et al., 2000; Zhu et al., 2017). Advanced new methods for segmentation of the hippocampus enable examination of the HS separately. The FreeSurfer v6.0 hippocampal subfields atlas was built from ultra-high resolution (0.13 mm), combined *ex vivo* and *in vivo* images. The fully automated algorithm can model 13 segments, and has been shown to perform well in neurodegenerative disease populations (Iglesias et al., 2015).

A number of neuroimaging studies have investigated HS separately in disease populations and found that neuropathology can affect these regions differently. Among patients with severe mental health disorders, the most frequently reported findings are volume reduction in the CA structures, the subiculum and dentate gyri (Haukvik et al., 2015; Hayes et al., 2017; Ho et al., 2017; Ota et al., 2017; Treadway et al., 2015). A recent study found that Cornu Ammonis 1 (CA1) volume was reduced in early stages of schizophrenia, but that atrophy spread to other subfields as the illness progressed (Ho et al., 2017), indicating that duration of illness may be an important factor to consider when studying volume reduction in the hippocampus in mental health disorders.

To our knowledge, only one previous study has investigated HS in AN patients (Burkert et al., 2015). Adult AN patients who had been ill for several years were found to have a significant reduction in the fimbria – a white matter bundle projecting along the anterior-posterior axis of the hippocampus (Burkert et al., 2015), and an increase in the size of the hippocampal fissure – the "ventricle" of the hippocampus. Recent studies suggest that variability in duration of AN, which typically debuts in adolescents, may lead to different findings in neuroimaging studies of adults and adolescents (Pfuhl et al., 2016; Seitz et al., 2016). It is therefore of interest to investigate the hippocampus and HS volumes in the early stages of AN.

The studies that have reported hippocampal atrophy in AN (Burkert et al., 2015; Connan et al., 2006; Giordano et al., 2001; Mainz et al., 2012) vary in their methods of correction for individual differences in brain volume. None of the reported studies have aimed to investigate the selective effect of AN on the hippocampus by adjusting for the observed global brain volume reduction. It remains unclear whether the hippocampus is particularly affected in AN, or if the volume reduction in the hippocampus is a consequence of the observed global volume reduction. Furthermore, methods of segmentation vary and results from the manual delineation of HS can be particularly difficult to replicate (Van Leemput et al., 2009). Further investigation is needed to reveal the relationship between AN and the hippocampus and its subfields.

The aim of the current study was to examine HS in young patients in an early stage of AN. We investigated 12 subfields segmented by the hippocampal subfields segmentation tool in the FreeSurfer software package (Iglesias et al., 2015) – a fully automated algorithm. We expected to find that adolescent AN patients had volume reduction in total cerebral GM and the whole hippocampus compared to healthy age-matched controls. We expected to find a selective HS volume reduction and an increased fissure, similar to what has been found previously in adult AN patients (Burkert et al., 2015). Furthermore, we investigated if HS volumes were significantly smaller in AN patients when adjusting for total brain volume - which we expected to be reduced in AN. As HS volume reduction is also found in mental health disorders that often occur as comorbid conditions in AN patients, we wished to further explore the association between HS volume, AN symptoms and symptoms of anxiety and depression. We expected to find a negative relative relationship between HS volumes and symptoms of depression and anxiety.

2. Methods

2.1. Study design and sample

Inpatients with AN were recruited from the Regional Center for Eating Disorders at the University Hospital of North Norway (RSS) and Oslo University Hospital (RASP). In total, 33 female patients with AN (Age: M = 15.8, SD = 1.7) and 30 female healthy age-matched controls (Age: M = 16.2, SD = 1.9) were recruited for the study (10 patients and 10 controls were tested and scanned at RASP). Healthy controls (HC) were recruited from local high schools. Neuropsychological testing and scanning was conducted less than two weeks apart. All participants were scanned in the evening between 3 pm and 8 pm.

Inclusion criteria for AN patients were the DSM-V criteria for restrictive AN (no history of binge-purge episodes), diagnosis set by a clinical specialist in psychology or medicine. Age-adjusted, standardized body mass indexvalues (BMI-SDS) were calculated using Norwegian normative data from the Bergen Growth Study (Júlíusson et al., 2013). A measure of body mass index increase between admission and scanning (BMI-increase) was calculated by subtracting body mass index (BMI) at admission from BMI at the day of scanning. Exclusion criteria for all participants were neurological disorders and organic brain injury, history of bulimia nervosa, schizophrenia, psychotic episodes and the use of antipsychotic medication. Additional exclusion criteria for HC were lifetime or current eating disorders or obesity (BMI $>$ 30).

2.2. Ethics

The Norwegian Committee for Medical and Health Research Ethics (REC), North region approved the study, under protocol number 302969. Informed, written consent was obtained from all participants. Parents also gave written consent for participants $<$16 years of age.

2.3. Image acquisition

MR scanning was performed with a 3T Siemens Magnetom Skyra Syngo MR D13C at the University Hospital of Tromsø and with a Phillips Achieva 3T scanner at the University Hospital of Oslo. At both sites, high resolution 3D T1-wheighted images were acquired. In Tromsø, we used a magnetization-prepared rapid gradient-echo (MPRAGE) sequence with the following parameters: Orientation = Sagittal; No. of slices = 176; Voxel size = 1 × 1 × 1; Slice thickness = 1 mm; repetition time (TR) = 2300ms; echo time (TE) = 2.98ms; field of view (FOV) = 256 × 256; Flip angle = 9°; and inversion time (TI) = 900ms. In Oslo, a 3D sequence was used for acquisition with the following parameters: Orientation = Sagittal; No of slices = 184; Voxel size = 1 × 1 × 1; Slice thickness = 1 mm; TR = 2300ms; TE = 2.98ms; FOV = 256 × 256; Flip angle = 8°; and TI = 900ms.

2.4. Image processing

Surface reconstruction and volumetric segmentation was performed with FreeSurfer v6.0 software (http://surfer.nmr.mgh.harvard.edu) version 6.0; Fischl et al. 2002, Fischl et al., 2004) with the recon-all processing pipeline and the hippocampal subfields module (Iglesias et al., 2015). The pipeline includes motion correction, normalization to Talairach space, intensity bias correction, skull-stripping, surface registration and segmentation. Two of the authors (TRV and ADM) visually inspected image registration results.

2.4.1. Selected brain volumes

The following 12 HS are modeled by the FreeSurfer hippocampal subfields atlas (Iglesias et al., 2015) and were investigated in this study: The CA1, CA2/3, CA4, the molecular layer of the CA regions (ML), the

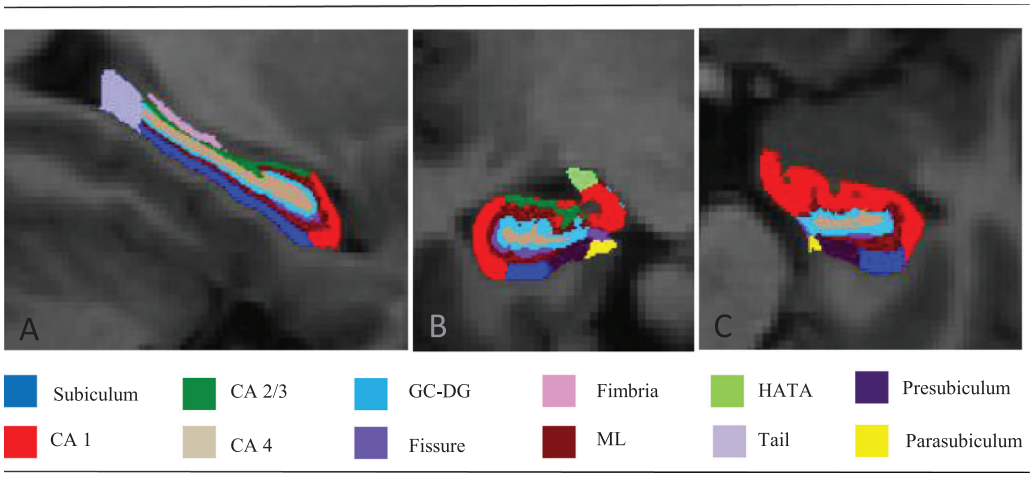

Fig. 1. Hippocampal subfields in healthy control participant.

Granule Cell layer of the Dentate Gyrus (GCDG), the pre-, parasubiculum, and the subiculum, the hippocampus-amygdala transition area (HATA), the fimbria, the hippocampal fissure and the hippocampal tail (Fig. 1). We also investigated total GM and WM volumes, estimated total intracranial volume (eTIV) and whole brain volume (ventricles excluded).

2.5. Mental health

The Norwegian versions of the Beck's Depression Inventory (BDI-II) (Beck et al., 1988), and the State-Trait Anxiety Inventory (STAI) forms Y1 (state anxiety) and Y2 (trait anxiety) (Spielberger et al., 1970) was used to measure symptoms of depression and anxiety, respectively. The Eating Disorder Examination Questionnaire (EDE-Q) (Fairburn and Beglin, 2008) was used to measure eating disorder symptoms. The EDE-Q consists of four subscales (restriction, concerns about eating, weight and figure) and a global scale. The Mini-International Neuropsychiatric interview (M.I.N.I) 6.0 (Sheehan et al., 1998) was used to screen for comorbid mental health disorders before patients were assessed by a clinical specialist in psychology or medicine. IQ was measured by Wechslers Adult Intelligence Scale IV (WAIS-IV) or Wechslers Intelligence Scale for Children IV (WISC-IV) for participants <16 years of age (Wechsler, 2008,2003).

2.6. Statistical analyses

We performed tests of normality and inspected plots for all variables and found no violations of the assumptions for parametric tests. Group differences in demographic variables and psychometric measures were investigated by one-way analysis of variance. Linear regression analyses were used to investigate group differences on global GM and WM, adjusted for age, drug use and scanner site. Inspections of the cortical surface and subcortical volumes revealed a substantial spread of cortical volume reduction and volume reduction in several subcortical structures. To investigate whether brain volumes were affected by scanner site, we performed linear regression analyses using only HC participants with total GM and the whole hippocampus, adjusted for age and eTIV, as the outcome variables and scanner site as the independent variable. Scanner site, adjusted for age and eTIV, was not associated with total GM ($b = 0.02$, $p = 0.44$) or left hippocampus ($b = -0.27$, $p = 0.21$), but was close to significant in the right hippocampus ($b = -0.39$, $p = 0.05$). We adjusted for site in all further analyses. As an additional measure against the potential confounding effect of site, we re-performed the main analyses of hippocampus and HS in a subsample with participants from one scanner only (Supplement Tables 1,2).

A series of linear regression analyses was performed to investigate group differences in the whole hippocampus and HS volumes, averaged across hemispheres. All analyses were also performed separately for the two hemispheres. To adjust for potential confounding effect of age dispersion, depressive symptoms, individual differences in intracranial volume, psychopharmacological treatment and the two different scanners, the variables age, BDI-II score, eTIV, drug use and scanner site were entered as covariates. In a secondary series of analyses, we replaced eTIV with whole brain volume as a covariate to investigate whether volume reduction in the whole hippocampus and HS was affected by total brain volume. All analyses were also repeated with STAI-Y1 (measuring state anxiety symptoms) score replacing the depression score to adjust for potential confounding effect of anxiety symptoms. To further investigate the relationship between brain volumes and clinical measures in AN, we conducted group stratified linear regression analyses of all HS volumes that were significantly smaller in the AN group and the following variables: BMI, BMI-SDS, BMI-increase, Weeks since admission (to inpatient care), Years since first GP consultation (regarding eating disorder symptoms), EDE-Q (four subscales and global scale) BDI-II, STAI Y1. In all models we added age, scanner site, drug use and eTIV as covariates to adjust for potential confounding effects. All results were corrected for errors of multiple comparisons with the false discovery rate (FDR) method using a syntax for SPSS (http://www-01.ibm.com/support/docview.wss?uid=swg21476447) and a false discovery rate with $q=0.05$. All statistical analyses were performed using IBM SPSS 24.

3. Results

The AN group had significantly higher scores on self-report measures of mental illness and significantly lower BMI and BMI-SDS (Table 1). Linear regression analysis of global GM and WM volumes showed that AN patients had significantly reduced volume in cerebral GM and total brain volume. No group differences were found in cerebral WM and eTIV (Table 2).

All HS volumes except for the hippocampal fissure were significantly explained by group affiliation adjusted for site, age, depression score (BDI-II), drug use and eTIV, and remained significant after FDR correction (Tables 3 and 4). In the secondary analysis, where eTIV was replaced by brain volume as a covariate, the fimbria and the hippocampal tail where no longer significantly explained by group affiliations after correction for multiple comparisons (Table 4). When adjusting for anxiety, results were similar for the eTIV adjusted analyses, but none of the HS remained significant when adjusting for total brain volume (Supplement Table 3). We conducted the same analyses on a subgroup collected from one single scanner ($N=41$) to avoid the

Table 1
Clinical measures in adolescent AN and HC.

Clinical measures	AN Mean (SD)	HC Mean (SD)	F-value	*p*
N	30	28		
Age	15.8 (1.7)	16.2 (1.9)	0.9	.343
BMI	16.3 (1.6)	21.8 (3.1)	73.9	<.001
BMI admission	15.2 (1.4)	–		
BMI-increase	0.9 (0.6)	–		
BMI-SDS	−2.4 (1.2)	0.3 (1.1)	73.2	<.001
Drugs (SSRI/GH)[a]	7	0		
Left hand dominant	2	2		
Weeks since admission*	4.5 (4.0)	–		
Years since first GP consult.**	1.6 (1.4)	–		
FSIQ*	101.1 (12.0)	104.0 (8.2)	292.0	.068
BDI II***	22.8 (11.8)	4.3 (5.1)	56.7	<.001
STAI Y1***	49.8 (14.1)	30.8 (9.7)	32.9	<.001
STAI Y2***	52.0 (15.2)	33.9 (10.9)	27.1	<.001
EDE-Q restriction**	3.0 (2.0)	0.4 (0.5)	44.2	<.001
EDE-Q eating**	2.3 (1.7)	0.2 (0.5)	37.1	<.001
EDE-Q weight**	3.0 (1.8)	0.7 (0.8)	36.3	<.001
EDE-Q figure**	3.9 (1.9)	0.9 (1.2)	50.1	<.001
EDE-Q global**	3.0 (1.7)	0.6 (0.6)	53.7	<.001
Mini sum*	1.0 (1.2)	0.1 (0.3)	17.8	<.001

Note: Statistics: One-way ANOVA. BMI = Body mass index. BMI-SDS = Standardized BMI values based on Norwegian norms for children.

[a] 5 subjects used Serotonin reuptake inhibitor (SSRI), 2 used growth hormones (GH). Years since first GP consult = Consultation concerning eating disorder symptoms. FSIQ = Full Scale Intelligence Quotient. BDI = Becks Depression Inventory II. STAI 1 & 2 = State Trait Anxiety questionnaire form Y1 (State anxiety) and Y2 (Trait anxiety). EDE-Q = Eating Disorder Examination Questionnaire. MINI sum = Sum of diagnoses from MINI except Anorexia nervosa.

* AN N = 29.

** AN N = 27.

*** AN N = 25.

Table 2
Total brain volumes in adolescent AN and HC.

Brain volumes	AN Mean (SD)	HC Mean (SD)	Beta	*p*	R-square
Total gray matter	**662812.9 (56607.4)**	**717920.5 (59586.9)**	**−.426**	**<.001**	**.776**
Cerebral white matter	417027.1 (47223.0)	436765.0 (46027.6)	−.100	.246	.681
eTIV	1452452.6 (139298.6)	1485015.9 (121664.0)	−.118	.360	.142
Total brain volume[a]	**1107935.4 (91540.3)**	**1184735.0 (94086.8)**	**−.409**	**.001**	**.247**

Note: Statistics: Linear regression adjusting for age, drug use and site. eTIV = estimated total intracranial volume. Total gray and white matter was also adjusted for eTIV. Group variable was coded AN = 0 and HC = 1. Mean values are mm^3.

[a] Ventricles were excluded from total brain volume.

potential confound of scanner variability and results showed similar results for the eTIV adjusted analyses, but none of the HS were significantly explained by group affiliation when adjusting for whole brain volume (Supplement Tables 1 and 2). Because we did not have a hypothesis about lateralization of volume reduction and because the results for the two hemispheres were highly similar, only results from analyses performed on volumes averaged across hemispheres are presented.

In the group stratified regression analyses of HS of interest and clinical measures (BMI, BMI increase, duration of inpatient care, AN symptom duration, scores from EDE-Q, BDI-II and STAI measuring AN symptoms, depression and anxiety) results revealed significant relationships between BDI, STAI Y1 and several HS (Table 5). No significant associations were found regarding BMI and EDE-Q scores (Table 5), or any of the other AN-related measures. We did not find any

Table 3
Hippocampus volumes in mm^3 for adolescent AN and HC.

Brain volumes	AN Mean (SD)	HC Mean (SD)	% difference
Whole hippocampus	3327.7 (299.8)	3566.7 (242.3)	6.7%
HS:			
Tail	517.7 (55.8)	550.4 (54.3)	5.9%
Subiculum	422.2 (39.4)	441.1 (34.8)	4.3%
Presubiculum	307.4 (29.7)	326.7 (26.6)	5.9 %
Parasubiculum	62.8 (8.1)	68.1 (6.3)	7.8 %
Fissure	144.8 (18.6)	145.9 (18.6)	0.8 %
CA1	610.3 (69.4)	661.7 (60.2)	7.8%
CA2-3	187.2 (27.1)	206.0 (26.4)	9.1%
CA4	241.0 (26.2)	258.0 (22.7)	6.6%
Molecular layer	545.1 (51.3)	588.2 (43.7)	7.3%
GCDG	280.7 (29.9)	301.4 (25.5)	6.9%
HATA	61.2 (8.9)	67.1 (6.5)	8.8%
Fimbria	92.0 (12.7)	98.1 (13.2)	6.2%

Note: Values are mean mm^3 and standard deviations, averaged across hemispheres. HS = Hippocampal subfields. CA = Cornu Ammonis. GCDG = Granule cell layer of the dentate gyrus. HATA = Hippocampus-amygdala transition area. % difference was calculated from mean volumes in mm^3 (HC – AN).

Table 4
Hippocampus volumes in adolescent AN vs. HC.

	Adjusted for eTIV			*Adjusted for total brain volume*		
Brain volumes	Beta	*p*	R-square	Beta	*p*	R-square
Whole hippocampus	−.769	**<.001**	.525	−.542	**.002**	.588
Tail	−.483	**.014**	.359	−.306	.138	.400
Subiculum	−.651	**.001**	.442	−.511	**.012**	.444
Presubiculum	−.684	**<.001**	.461	−.526	**.007**	.495
Parasubiculum	−.645	**.001**	.422	−.432	**.027**	.482
Fissure	−.190	.353	.269	−.267	.263	.190
CA1	−.649	**<.001**	.488	−.423	**.021**	.541
CA2-3	−.611	**.003**	.345	−.470	**.028**	.371
CA4	−.670	**.001**	.351	−.469	**.024**	.415
ML	−.776	**<.001**	.502	−.557	**.002**	.566
GCDG	−.687	**.001**	.402	−.475	**.017**	.469
HATA	−.667	**<.001**	.46	−.441	**.018**	.528
Fimbria	−.462	**.031**	.24	−.335	.148	.247

Note: Statistics: Linear regression analyses of group affiliation (AN vs. HC) and HS with two different adjustments for brain size: eTIV (estimated total intracranial volume) and total brain volume without ventricles. Group variable was coded AN = 0 and HC = 1. For both sets of analyses, covariates were age, depression score (BDI-II), scanner site and drug use. Variables presented in bold are significant after FDR correction for multiple comparisons. HS = Hippocampal subfields. CA = Cornu Ammonis. GCDG = Granule cell layer of the dentate gyrus. HATA = Hippocampus-amygdala transition area.

statistically significant associations between HS volumes and clinical measures in the HC group.

4. Discussion

The aim of the present study was to investigate hippocampal subfields in adolescents with restrictive AN compared to healthy age-matched controls. We found statistically significant volume reductions in all but one of the investigated HS volumes when adjusting for age, depression score (BDI-II), scanner site and eTIV. Results showed that the AN group had smaller CA areas and less volume in the presubiculum, the molecular layers of the CA areas, the HATA and the GCDG. Most results remained significant also when adjusting for global brain volume which was expectedly reduced in the AN sample. This might indicate that the volume reduction in the hippocampus is more extensive than the general brain volume reduction, and that this structure is particularly vulnerable in AN. The fissure was not increased in the AN group as found in a previous study of adult AN patients

Table 5
The association between hippocampal subfields and clinical measures in AN.

	BMI-SDS		*EDE-Q*		*BDI-II*		*STAI-Y1*	
Brain volumes	Beta	*p*	Beta	*p*	Beta	*p*	Beta	*p*
Total GM	.136	.293	.180	.232	.242	.107	.066	.677
Whole hippocampus	−.270	.115	.124	.494	.565	**<.001**	.567	**.001**
Tail	.137	.489	.025	.906	.346	.084	.334	.105
Subiculum	−.321	.058	.152	.405	.612	**<.001**	.619	**<.001**
Presubiculum	−.177	.334	.109	.591	.595	**.001**	.446	**.021**
Parasubiculum	.169	.425	.235	.308	.617	**.003**	.487	**.028**
CA1	−.204	.238	.178	.313	.473	**.004**	.522	**.003**
CA2-3	−.222	.186	.017	.927	.264	.160	.338	.074
CA4	−.352	.049	.047	.811	.477	**.012**	.488	**.011**
ML	−.291	.092	.140	.442	.557	**.001**	.582	**.001**
GCDG	−.341	.050	.055	.775	.486	**.008**	.496	**.007**
HATA	−.140	.436	.094	.629	.521	**.004**	.452	**.016**
Fimbria	−.080	.681	.121	.539	.438	.048	.284	.219

Note: Statistics: Linear regression adjusting for age. site. drug use and eTIV. Variables presented in bold are significant at the 5% level after FDR correction for multiple comparisons. BMI-SDS: Standardized body mass index (BMI) values based on Norwegian norms for children. BDI-II: Becks depression inventory II. EDE-Q: Eating disorder examination questionnaire (global score). STAI: State Trait Anxiety Inventory form Y1 (State anxiety) and Y2 (Trait anxiety). CA = Cornu Ammonis. GCDG = Granule cell layer of the dentate gyrus. HATA = Hippocampus-amygdala transition area.

(Burkert et al., 2015). In their study of adult patients, Burkert et al. found volume reduction only in the fimbria and our results seem to indicate that hippocampus reduction is more extensive in adolescent AN patients and not specific to selected subfields. The reason for the discrepancy might be the young age of our sample and that the developing brain may respond differently to illness debut. Another explanation could be that GM areas normalize after the initial acute phase of AN. Our results are consistent with findings regarding global GM in AN. A recent meta-analyses of volumetric studies in AN found that adolescents had significantly greater GM volume loss compared to adults (Seitz et al., 2016).

The use of different hippocampal segmentation methods complicates the comparison of the results of studies of HS. In their study of adult AN patients, Burkert and colleagues (Burkert et al., 2015) used FreeSurfer version 5.3, which performs a more crude segmentation and does not model all of the subfields. The previous version has been criticized for not agreeing well with volumes from histological studies (Schoene-Bake et al., 2014). The FreeSurfer v6.0 atlas is an improvement to previous atlases in that it is made from higher resolution images and is built from more cases, makes no assumptions about acquisition parameters and can model more subfields than any other atlas (Iglesias et al., 2015).

Stress and excessive glucocorticoid exposure is often reported in severe mental health disorders and is proposed as the driving mechanism of hippocampal atrophy (Mondelli et al., 2010; Sapolsky, 2000; Videbech and Ravnkilde, 2004; Watanabe et al., 2017). Higher self-reported stress levels have been found to be associated with greater hippocampus reduction in major depressive patients (Treadway et al., 2015), and higher serum cortisol levels were found in first-episode depressive patients (Watanabe et al., 2017). Excessive hormone production can lead to volume reduction in the hippocampus, as seen in patients with the hypercorticolism disease Cushing's syndrome (Starkman et al., 1992). Patients with AN often have comorbid depression and anxiety disorders (Kaye et al., 2004), report higher stress levels (Burkert et al., 2015) and have elevated cortisol levels (Mainz et al., 2012) and it is possible that this is also driving volume reduction in AN. In the present study, the potential confound of depression was addressed by adjusting for BDI-II score in the main analyses of HS. The group effect was still present with this adjustment, indicating that depressive symptoms in our sample is not driving volume reduction in the hippocampus. Similar results were found when adding anxiety scores as a covariate, but none of the results from analyses with adjustments for whole brain volume remained significant after correction for multiple comparisons. These results may have been significant in a larger sample.

Group stratified analyses revealed significant, positive relationships between several HS and symptoms of depression and anxiety measured by BDI II and STAI Y1, and Y2, showing that patients with larger HS volumes had higher scores for these measures, indicating more severe symptoms. No such relationships were found in the HC group. These findings were somewhat unexpected since previous studies have found a reduction in hippocampus volume to be associated with depression and PTSD (Hayes et al., 2017; Treadway et al., 2015). However, the relationship between depression and HS volume appear to be a matter of duration and not severity – i.e. more depressive episodes is associated with greater volume loss (Treadway et al., 2015). Depression in AN is found to be highly related to core symptoms of the disorder such as body dissatisfaction, and the assessment of comorbidity between these disorders is challenging (Espelage et al., 2003). Very few patients in our sample received a comorbid diagnosis according to the M.I.N.I interview, in spite of high scores on BDI and STAI. Furthermore, it is possible that patients that experienced less symptoms of depression and anxiety prior to admission will experience more emotional distress from being admitted to inpatient care. The patients in our study were recently admitted and scores on depression and anxiety scales may have been temporarily elevated due to the new imposed weight rehabilitation regimen. The relationship between symptoms of depression and anxiety and HS in our sample may thus be driven by related factors such as stress and coping mechanisms.

The contribution of low BMI and emaciation to hippocampal volume loss in AN is unclear. Findings regarding global GM volume are inconsistent, but some studies have identified significant correlations with BMI (Seitz et al., 2015), lowest lifetime BMI and degree of weight loss prior to admission (Bomba et al., 2013). In addition, the fact that brain volume tends to normalize when body weight is restored (King et al., 2015; Mainz et al., 2012) suggests that weight is a contributing factor in global cerebral volume reduction. One study found regional volume reductions in the ACC but not global GM (Mühlau et al., 2007) suggesting that some regions may be more vulnerable to malnourishment. In line with the previous study on HS (Burkert et al., 2015), we did not find a significant relationship between BMI and hippocampal volume.

A limitation to our study is the use of two different scanners – a probable confounder of the results. To account for this, we re-performed the main analyses on a subgroup from only one scanner. These results were similar to the results from the main analyses, indicating that scanner site did not affect the main outcome in a large extent. However, the subgroup analyses had a low N (AN N = 21) and this may not be sufficient to detect group differences. Although the most recent version of the FreeSurfer HS atlas used in this study is an improvement upon the previous version, there still are limitations regarding the boundaries between some of the subfields, for example the CA-fields. The CA4 and the dentate gyrus also overlap in the atlas, and it might not be possible to distinguish these two subfields practically. The atlas was built from manual delineations in elderly subjects and might not perform as well in younger populations (Iglesias et al., 2015).

Further limitations of our study were that we did not have data available to control for variations in pretest severity of illness, notably periods of marked weight loss (i.e. a BMI < 17) and lowest lifetime BMI or comorbidity prior to admission. The patients in our study had been admitted for a mean duration of 4.5 weeks with a large dispersion (SD = 4.0 weeks) and were likely to have been on weight rehabilitation programs for several weeks. The mean BMI of 16.3 (SD = 1.6) in the AN group suggests that not all of the patients were in the most acute phase of their illness. However, we did not find a significant association between BMI increase score, measured by subtracting the BMI at admission from the BMI at the day of the scan, and the HS, indicating that

hippocampus volumes were not affected by patients' weight gain during the first weeks of inpatient treatment.

The present study is the first to investigate hippocampal subfields selectively in adolescent AN patients in an early stage of illness. The most important finding was that several HS were found to be significantly reduced in adolescent patients with AN compared to healthy controls. The effect was present when adjusting for depression and anxiety, suggesting that the extensive HS volume reduction in AN that is not driven by depression or anxiety. However, no AN characteristic variables were associated with the observed volume reduction. The positive association between depression and anxiety might be a result of associated factors such as stress and coping mechanisms. Future studies should include more elaborate measures of comorbidity and AN symptomatology, particularly measures of stress and coping.

Declaration of interest

All authors declare no conflicts of interest.

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Supplementary materials

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Paper II

Cerebral cortical thickness and surface area in adolescent anorexia nervosa: Separate and joint analyses with a permutation-based nonparametric method

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ORIGINAL ARTICLE

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Cerebral cortical thickness and surface area in adolescent anorexia nervosa: Separate and joint analyses with a permutation-based nonparametric method

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Abstract

Objective: Reduction in cerebral volume is often found in underweight patients with anorexia nervosa (AN), but few studies have investigated other morphological measures. Cortical thickness (CTh) and surface area (CSA), often used to produce the measure of cortical volume, are developmentally distinct measures that may be differentially affected in AN, particularly in the developing brain. In the present study, we investigated CTh and CSA both separately and jointly to gain further insight into structural alterations in adolescent AN patients.

Method: Thirty female AN inpatients 12–18 years of age, and 27 age-matched healthy controls (HC) underwent structural magnetic resonance imaging. Group differences in CTh and CSA were investigated separately and jointly with a permutation-based nonparametric combination method (NPC) which may be more sensitive in detecting group differences compared to traditional volumetric methods.

Results: Results showed significant reduction in in both CTh and CSA in several cortical regions in AN compared to HC and the reduction was related to BMI. Different results for the two morphological measures were found in a small number of cortical regions. The joint NPC analyses showed significant group differences across most of the cortical mantle.

Discussion: Results from this study give novel insight to areal reduction in adolescent AN patients and indicate that both CTh and CSA reduction is related to BMI. The study is the first to use the NPC method to reveal large structural alterations covering most of the brain in adolescent AN.

KEYWORDS

adolescent, anorexia nervosa, cortical surface area, cortical thickness, magnetic resonance imaging, nonparametric combination, permutation testing

1 | INTRODUCTION

Cerebral structural alterations are consistently found in acutely ill patients with anorexia nervosa (AN), and most frequently reported is cerebral volume reduction (Bomba et al., 2013; Fonville, Giampietro, Williams, Simmons, & Tchanturia, 2014; Frank, Shott, Hagman, & Yang, 2013; Gaudio et al., 2011; Mainz, Schulte-Ruther, Fink, Herpertz-Dahlmann, & Konrad, 2012; Seitz et al., 2015). Findings regarding the

dispersion of structural alterations vary; most studies report that clusters scattered across the cortical mantle are reduced in AN compared to healthy control (HC) participants, but the findings only moderately overlap (Seitz, Herpertz-Dahlmann, & Konrad, 2016). A few studies show regionally larger volumes in AN patients (Frank et al., 2013). The inconsistent results may be due to differing methodology and sample characteristics (King, Frank, Thompson, & Ehrlich, 2018), and the question is unsettled as to whether the observed volume reduction is a regionally specific or global phenomenon.

Cortical volume is often measured as the product of cortical surface area (CSA) and cortical thickness (CTh). These measures reflect two genetically and developmentally distinct measures with individual life span trajectories and different association with cognitive development and disorder (Fjell et al., 2015; Winkler et al., 2010). The combination of these two metrics may be imprecise as it does not account for the unique contribution of area and thickness (Panizzon et al., 2009). During childhood and adolescence, the development of CTh follows a linear curve, while CSA seem to follow a curvilinear trajectory with a later peak (Wierenga, Langen, Oranje, & Durston, 2014). Full syndrome AN normally develops after CTh peak, but early-onset AN may develop before CSA peak in some cortical regions. As CTh is steadily decreasing in adolescents, and CSA may still be expanding, the effect of AN may differ for CTh and CSA in adolescents.

As AN patients may be subject to both cerebral volume increase and decrease, the combination of CSA and CTh may result in "cancelling" out effects and thus mask structural alterations. Separately, these measures may be more specific in detecting cortical changes (Winkler et al., 2010). While several studies have investigated cerebral volume in AN, few have studied CTh and CSA separately.

One study of CTh in adolescent AN reports widespread thinning (King et al., 2015). Studies of adult patients with AN have yielded small group differences and conflicting results; One study reported lower CTh in frontal and temporal lobes (Nickel et al., 2018), while another one reported greater thickness in several frontal regions (Lavagnino et al., 2018). A more recent study found widespread CTh reduction in adult patients with acute AN that mostly normalized after weight rehabilitation and was negatively associated with age (Kaufmann et al., 2020).

CSA is associated with cortical volume (Winkler et al., 2010) consistently found to be reduced in AN (Seitz et al., 2016). However, two studies investigating CSA in adult AN patients compared to HC report small (Leppanen, Sedgewick, Cardi, Treasure, & Tchanturia, 2019) and no group difference (Miles, Voineskos, French, & Kaplan, 2018). Results from a meta-analysis of studies investigating cerebral volume indicate that adolescent AN patients have a greater volume loss than adult AN patients (Seitz et al., 2016) giving cause to investigate adolescent and adult AN patients separately. To our knowledge, no studies have examined surface area in adolescent AN patients.

Traditionally, measuring volume is done by voxel-by-voxel classification of tissue or based on surface registration, multiplying area by thickness at each vertex. The first method is known to be sensitive to artifacts (Ashburner & Friston, 2000) and the latter is likely to under- or overestimate volume (Winkler et al., 2018). A recently proposed method allows the combination of thickness and surface area metrics as an alternative to the traditional volume analyses. The permutation-based nonparametric combination (NPC) method is a multivariate statistical method that utilizes permutations, first testing metrics separately and recording results for each permutation. Subsequently, resulting *p*-values are combined into a joint and more powerful statistic. In this manner, very few assumptions are made about the data and over or underestimation is less likely. As the method is nonparametric it does not assume independence between the two metrics studied, which is an advantage as CTh and CSA share the same environment (Winkler et al., 2016). In summary, the NPC method accounts for the combined effect of CTh and CSA, and is less prone to the drawbacks of traditional volumetric methods and may thus give a more precise measurement of structural alterations in adolescent AN.

In the present study, we investigated CTh and CSA separately and jointly. Based on previous findings of cortical thinning and volume reduction, we hypothesize that both surface area and thickness are affected in adolescent patients with AN. As CTh and CSA peak at different ages and in different regions, we expect to find some differences regarding regions affected. The newly proposed NPC method offers an advantage in detecting affected areas that may be subject to alterations in both CTh and CSA. We expect that separate analyses reveal cortical thinning and surface area decrease in adolescent AN patients, and that the combined analyses reveal that more of the cortex is affected than what has been previously found using traditional volume methods.

2 | METHODS

2.1 | Study design and sample

Participants were inpatients at the regional center for eating disorders at the university hospital of North Norway in Tromsø, and Oslo University Hospital. In total, 31 female patients with AN (Age: M = 15.7 SD = 1.8) and 27 female healthy age-matched controls (Age: M = 16.1, SD = 1.9) were included (10 patients and 10 controls were tested and scanned at the Oslo clinic and the remaining were included in Tromsø). HC were recruited from local high schools in Tromsø and Oslo, respectively. Inclusion criteria for AN patients were DSM-5 criteria for restrictive AN (no history of binge-purge episodes). Upon admission, patients were set on a meal plan and started psychotherapy (family-based treatment for eating disorders). Two of the patients were tube fed in the period between admission and inclusion in the study. Exclusion criteria for all participants were history of brain injury, neurological disorder, bulimia nervosa, schizophrenia or psychotic episodes and use of antipsychotic medication. Additional exclusion criteria for HC were lifetime or current eating disorders or obesity (BMI > 30). Most of this sample was also included in our previous study (Myrvang et al., 2018). Norwegian versions of the Beck's Depression Inventory (BDI-II; Beck, Steer, & Carbin, 1988), and the State-Trait Anxiety Inventory (STAI; Spielberger, Gorusuch, & Lushene, 1970) were used to measure symptoms of depression and

anxiety. The Eating Disorder Examination Questionnaire (EDE-Q; Fairburn & Beglin, 2008) was used to measure eating disorder symptoms. The EDE-Q consists of four subscales (restriction, concerns about eating, weight and figure) and a global scale.

2.2 | Ethics

The study was approved by The Norwegian Committee for Medical and Health Research Ethics (REC), under protocol number 302969. Informed, written consent was obtained from all participants. Written consent was also obtained from parents of participants <16 years of age.

2.3 | Image acquisition

A 3T Siemens Magnetom Skyra in Tromsø and a Phillips Achieva 3T scanner in Oslo was used for MR imaging. Scanners were equipped with 64 channel head coils and high-resolution 3D T1-wheighted images were acquired at both sites. Both sites used an ADNI protocol for the 3D T1 sequence (Jack et al., 2008). In Tromsø a magnetization-prepared rapid gradient-echo (MPRAGE) sequence was utilized, with the following parameters: Orientation = Sagittal; No. of slices = 176; Voxel size = 1 × 1 × 1; Slice thickness = 1 mm; repetition time (TR) = 2,300 ms; echo time (TE) = 2.98 ms; field of view (FOV) = 256 × 256; Flip angle = 9°; and inversion time (TI) = 900 ms, parallel excitation factor 2 (GRAPPA). In Oslo, a 3D-TFE sequence was used for acquisition with the following parameters: Orientation = Sagittal; No of slices = 184; Voxel size = 1 × 1 × 1; Slice thickness = 1 mm; TR/TE/T1 = 3000/2.3/853 ms; FOV = 256 × 256; Flip angle = 8°; and, parallel excitation factor 2 (SENSE). To test for the potential effect of scanner site, a group analysis (Oslo > Tromsø) was conducted using only participants from the HC group.

2.4 | Image processing

Surface reconstruction and volumetric segmentation was performed with the FreeSurfer v6.0 software (http://surfer.nmr.mgh.harvard.edu) version 6.0 (FS 6.0); (Fischl et al., 2002; Fischl et al., 2004) with the recon-all processing pipeline. The pipeline includes motion correction, normalization to Talairach space, intensity bias correction, skull-stripping, surface registration and segmentation. Two of the authors (TRV and ADM) visually inspected image registration results and manually corrected when necessary. Minor corrections were performed on about 1/3 of the sample, and the majority of corrections were of skull stripping errors leading to inclusion of dura mater, and in a few cases, parts of the skull.

2.5 | Statistical analyses

Analyses of group differences in descriptive variables were performed with IBM SPSS 24 using analysis of variance (ANOVA).

The statistical analyses of CTh and CSA were performed within the software package Permutation Analysis of Linear Models (PALM; Winkler, Ridgway, Webster, Smith, & Nichols, 2014). The preprocessing of the cortical surfaces was performed in the mris_preproc module in FS 6.0. The design matrixes for the permutation analyses consisted of group (HC vs. AN patients) whereas age was treated as a continuous covariate. The variable "age" was mean centered before the analyses. The permutation analyses were performed with 5,000 iterations, and threshold-free cluster enhancement (Smith & Nichols, 2009) was used to correct for multiple comparisons (Winkler et al., 2016), and a family-wise error rate (FWER) corrected $p < .05$ was considered significant. All contrast were two-tailed. The joint analyses of surface and CTh were performed in PALM with nonparametric combination (NPC; Winkler et al., 2018) using Fisher's method for combining p-values (Fisher, 1934). The Desikan-Killiany atlas incorporated in FS 6.0 (Desikan et al., 2006) was used for annotation of the cortices. All analyses were performed in two stages; a model testing contrast between groups (HC > AN) regressing out the effect of age was firstly performed. Secondly, the effect of body mass index (BMI) was included in the model to test the positive effect of BMI on CTh, CSA, and in the joint analysis of thickness and area. Both BMI and BMI-SDS were investigated, and results were similar. Results from analyses with BMI are displayed. In the patient sample, associations between morphometrics and symptoms of depression, anxiety and eating disorders, measured by BDI-II, STAI (Y1 and Y2), and EDE-Q, were investigated with similar statistical methods as described above.

3 | RESULTS

3.1 | Sample characteristics

The AN group had significantly higher scores on self-report measures of symptoms of depression (BDI-II) and anxiety (STAI, Y1, & Y2) and eating disorder symptoms (EDE-Q). Patients had significantly lower BMI and BMI-SDS compared to HC (Table 1).

3.2 | Group comparisons—Imaging data

3.2.1 | Cortical thickness

Group differences (HC > AN) were found in several anatomical regions (Desikan et al., 2006) in both hemispheres (Figure 1). Clusters of significant group differences ($p < .001$) were located in the pre- and paracentral area, precuneus, superior- and inferiorparietal, superiortemporal and superiorfrontal area. Only a few frontal and inferior frontotemporal areas such as the medial and lateral orbitofrontal and rostral middle frontal, entorhinal and parahippocampal areas were lacking significant group differences.

Effect sizes (Cohen's d) above 0.2 were found in all of the anatomical areas where significant group differences were observed (Figure S1) and ranged from small (Cohen's $d > 0.2$) to high (Cohen's $d > 0.8$).

TABLE 1 Sample characteristics

	AN Mean (SD)	HC Mean (SD)	*F*-value (*p*)
N	31	27	
Age	15.7 (1.8)	16.1 (1.9)	0.78 (.382)
BMI	16.3 (1.6)	22.0 (3.1)	83.0 (<.001)
BMI-SDS	−2.4 (1.3)	0.4 (1.0)	88.2 (<.001)
BMI-gain	0.9 (0.6)	—	—
Drugs (SSRI/GH)	5/2[a]	0/0	—
Weeks inpatient	5.1 (4.2)	—	—
Years since first GP visit	1.6 (1.5)	—	—
Psychiatric symptoms screening			
BDI II[b]	22.4 (11.7)	4.3 (5.2)	53.7 (<.001)
STAI Y1[b]	49.9 (13.8)	30.2 (9.2)	37.7 (<.001)
STAI Y2[b]	52.1 (14.8)	32.2 (10.3)	31.4 (<.001)
EDE-Q restriction[b]	3.0 (2.0)	0.3 (0.5)	43.9 (<.001)
EDE-Q eating[b]	2.3 (1.7)	0.2 (0.5)	36.1 (<.001)
EDE-Q weight[b]	3.0 (1.8)	0.7 (0.8)	37.6 (<.001)
EDE-Q figure[b]	3.9 (1.9)	0.7 (0.9)	61.1 (<.001)
EDE-Q global[b]	3.0 (1.7)	0.5 (0.5)	55.8 (<.001)

Note: One-way ANOVA. BMI: Body mass index. BMI-SDS: Standardized BMI values based on Norwegian norms for children. BMI-gain: Difference between BMI at scan day and BMI at admission.
[a]Five participants used Serotonin reuptake inhibitors (SSRI), two used growth hormones (GH). Weeks inpatient: Weeks between admission and scanning. Years since first GP visit = Consultation concerning eating disorder symptoms. BDI: Becks Depression Inventory II. STAI 1 & 2: State Trait Anxiety questionnaire form Y1 (State anxiety) and Y2 (Trait anxiety). EDE-Q: Eating Disorder Examination Questionnaire (index scores are reported).
[b]AN *N* = 27.

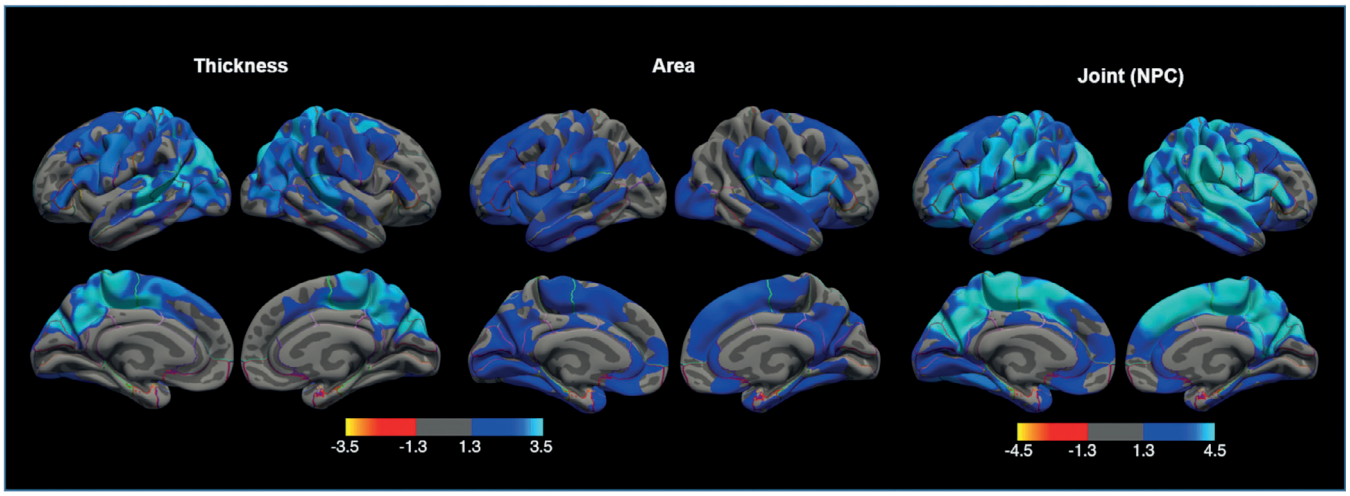

FIGURE 1 Group differences in separate and joint, two-tailed analysis (age adjusted) of cortical thickness and area, showing reduced thickness, area and combination in AN compared to HC. Images are thresholded with $p < .05$ and FWER corrected for multiple contrasts and modalities. Color bar indicate $-\log_{10}(p)$ thresholds for individual results. Thickness: Main body of significant clusters are located in temporal, parietal and superiorfrontal areas. Area: Main body of significant clusters are located in temporal, parietal and frontal areas. NPC: Significant clusters were found in all anatomical regions. AN, anorexia nervosa; FWER, family-wise error rate; HC, healthy controls; NPC, nonparametric combination [Color figure can be viewed at wileyonlinelibrary.com]

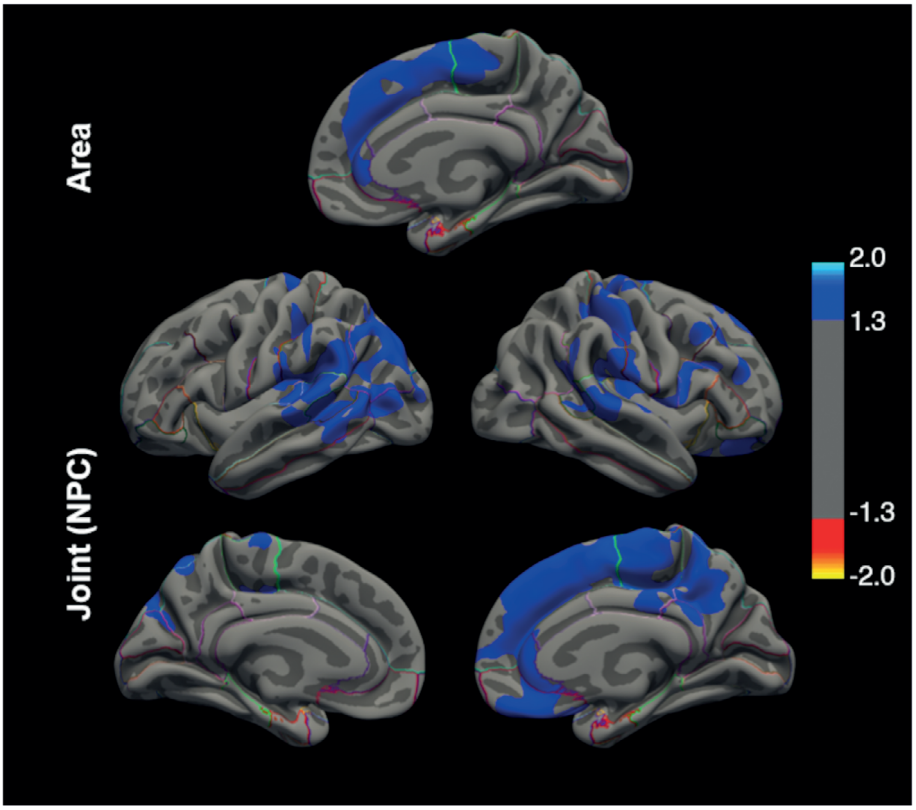

FIGURE 2 Group differences in joint (NPC), two-tailed analysis of cortical thickness and area adjusted for age and BMI, showing smaller combined cortical thickness and surface area in AN compared to HC. Images are thresholded with $p < .05$ and FWER corrected for multiple contrasts and modalities. Color bars indicate $-\log_{10}(p)$ thresholds. AN, anorexia nervosa; FWER, family-wise error rate; HC, healthy controls; NPC, nonparametric combination [Color figure can be viewed at wileyonlinelibrary.com]

When BMI was included as a covariate, none of the observed group differences remained significant.

3.2.2 | Surface area

Significant group differences were found in several anatomical regions (Desikan et al., 2006) in the central brain including all temporal and parietal regions except for the superior parietal region in both hemispheres (Figure 1). Significant clusters mainly encompassed the temporal (superior- and middle temporal), frontal (lateraorbitofrontal, rostral- and caudal middlefrontal and the insula) and parietal (post- and paracentral) lobes. Peak areas of significant group differences ($p < .001$) were found in parietal and prefrontal areas such as the pre- and postcentral gyri, insula and the inferior frontal gyri (pars triangularis and opercularis). The only prominent difference between the two hemispheres was that significant clusters were found in the right lateral occipital area and not the left. When BMI was included as a covariate, only the observed group differences in the right superiorfrontal area remained significant (Figure 2). Medium to high effect sizes (Cohen's *d*) were found in most anatomical labels where there were significant group differences, for both analyses steps (Figures S1 and S2).

3.2.3 | Joint NPC of CTh and CSA

The joint analyses of CTh and CSA (Figure 1) showed a significant effect of group (HC > AN) in most of the cortex. Significant differences ($p < .001$) were observed in all cortical regions (Desikan et al., 2006), the only exception being the frontal pole and three orbitofrontal regions in the right hemisphere. The largest clusters encompassed several regions in the temporal, occipital, parietal lobes. When adjusting for BMI, significant group differences were found in two clusters including temporal and parietal regions with peak coordinates ($p < .05$) in the superiorparietal region in the left hemisphere (Figure 2). In the right hemisphere clusters in the parietal and frontal cortex were significantly reduced in AN patients. Peak coordinates ($p < .001$) were found in the superiorfrontal, and precentral area.

3.2.4 | Scanner effect

To investigate the potential confounding effect of two different scanners, we performed analyses of all three morphometrics in HC only with scan site as group variable. No significant effect of scan site was found.

3.2.5 | Cerebral morphology and psychopathology in patients

In order to test the impact of symptoms of depression and anxiety and eating disorder symptoms on cerebral morphology, we tested the association between CSA and CTh and combination measure and scores on BDI-II, STAI Y1, and EDE-Q global scale. No significant associations with morphometrics and clinical variables were found.

The effect of BMI increase between admission and scan day was also tested, but no significant association between weight gain and morphometrics was found.

4 | DISCUSSION

Both CTh and CSA were independently reduced in AN compared to HC. Cortical thinning and areal reduction were found predominantly in the temporal, parietal and frontal lobes, but affected regions within the lobes differed somewhat between the two morphometrics. No areas were found to be larger in AN patients. When adjusting for BMI, the group effect observed for CTh and CSA was no longer significant, indicating that BMI largely explain the group effect. For the joint NPC analyses, some clusters including parietal and frontal regions remained significant when adjusting for BMI.

4.1 | Cortical thickness

Results showed reduced CTh in adolescent AN covering an extensive area in the posterior brain, bilaterally. Compared to a previous study in the same age group, also uncovering extensive cortical thinning (King et al., 2015), results from our study appear not to include the frontal areas to similar extent. A reason for this discrepancy may be that participants in our study had a higher BMI than participants in the study of King and colleagues. It has been suggested that normalization of anatomical changes starts within 3 months (Bernardoni et al., 2016), and it is plausible that brains of the patients in our study, who had been admitted for several weeks, were in a process of regeneration.

4.2 | Cortical surface area

We found an a real reduction in AN patients in large parts of the frontal cortex, most prominently in the rostral middle frontal, parsorbital, parstriangular and insular areas. Previous findings in adult AN patients have been limited to smaller areas (Leppanen et al., 2019) or no CSA reduction (Miles et al., 2018), and our results may imply that adolescent patients are more affected. A similar discrepancy between adults and adolescents have been reported in major depressive disorder (MDD; Schmaal et al., 2016). As suggested for adolescents with MDD, the reason for the surface area decrease in adolescent AN may be a delay in cortical maturation (Schmaal et al., 2016) as a result of illness debut in a critical period in brain development. The lack of such findings in adults may suggest that the cortex eventually matures in spite of the disturbance in adolescence. However, as in many studies in this field, the methods used differ substantially which may contribute to the discrepancies in findings reported.

As the mechanisms underlying volume reduction in AN are mostly unknown (King et al., 2018), an important first step may be to distinguish the two morphometrics that constitute volume. Results from the present study show that CTh and CSA reduction mostly overlap. However, some significant group differences were found in separate areas. For example, areal reduction, but not thickness reduction was found in the fusiform, entorhinal and parahippocampal areas. Studies have shown volume reduction in these areas (Amianto et al., 2013; Brooks et al., 2011; Fonville et al., 2014), and results from this study suggest that areal reduction is driving this decrease. The discrepancies in results in CTh and CSA may be due to the different developmental trajectory of these two metrics and future studies should investigate in a larger sample where there is possible to examine the relationship between age and morphometrics in AN patients in a more detailed manner, particularly including the youngest patients that may not have reached CTh and CSA peak. A study in adult AN patients found that restoration of CTh during weight rehabilitation was negatively associated with age, indicating that older patients had a lower rate of regeneration, perhaps due to reduced plasticity (Kaufmann et al., 2020). This finding indicates that CTh alterations in AN may be age dependent and future studies should investigate the relationship with age and development in other morphometrics.

4.3 | Nonparametric combination

The combined analyses show widespread cerebral reduction in AN patients, covering most of the cortical mantle. Few studies, independent of metrics studied, have found cortical alterations to this extent. Many of the studies investigating brain volume in adolescent AN have included few participants ($N < 20$), which may explain the discrepancy. However, one comparable study found global alterations, but in smaller clusters (Seitz et al., 2015). Superiority of the NPC method was demonstrated in a study of participants with very low birth weight, where NPC revealed more extensive structural alterations that was not detected using thickness or surface measure alone (Winkler et al., 2018). Results from this study may indicate that the NPC method is more reliable in detecting group differences in patients with AN, but direct comparisons to other methods are necessary to conclude.

Some clusters in the left posterior and the right parietal–frontal brain were still significant when adjusting for BMI suggesting that these areas are associated with other factors than weight. The areas that remained significant when adjusting for BMI in the left occipital temporal brain are associated with somatosensation and imagery. King et al. (2015) found clusters in the same area to be associated with "drive for thinness." It is possible that this area is more closely linked to eating disorder specific symptoms such as body image disturbance. In the present study, the relationship between morphometrics and eating disorder symptoms (measured by global EDE-Q scores) was only tested in the patient group with nonsignificant results, and thus could not explain the mass reduction nonrelated to BMI. Future studies should include other and more precise measures of eating disorder symptoms such as subscales of standardized interviews or questionnaires.

4.4 | Strengths and limitations

Strengths to this study comprises the use of up-to-date software, robust methods and stringent controls for multiple comparisons, a larger sample size than in most other studies in the field, a narrow age range, and sub-analyses of potential confounding variables like scan site, age, drug use and weight gain during inpatient care. As for the latter, the patients were not in the most critical and catabolic phase of their illness at the time of testing. The benefit to this approach was that the effects of extreme malnutrition were reduced. Moreover, the nonsignificant association between BMI increase score (subtracting the BMI at admission from the BMI at the day of the scan) and morphometrics indicate that our results would have been more or less the same had participants been included upon admission at a lower weight.

In the field of structural neuroimaging in AN patients it has been recommended (King et al., 2018) to control for the effects of pubertal stage, oral contraceptives and duration of illness. This was not done in the present study, and our question about the first time the participants visited their general practitioner (year) was a too crude variable to be informative in analyses. The possible effect of two different scan sites was not tested in the AN group, because the mean weight in the Tromsø and Oslo group differed somewhat. Although not statistically significant, this could have a confounding effect on a between site analyses, and therefore this was only conducted in the HC group.

5 | CONCLUSION

This study strengthens previous findings of global cortical thinning, provides novel insight to CSA reduction in adolescent AN, and indicates that both morphometrics are strongly related to BMI reduction. Compared to traditional volume analyses, the more powerful joint NPC analytic strategy shows that mass reduction in adolescent AN may be even more extensive than previously shown. To extend our knowledge on cortical changes in AN, and their relation to BMI, this analytic strategy may be recommended for longitudinal studies in recovering AN patients to investigate potential reductions remaining after weight rehabilitation. Such studies are important to understand for instance why body dissatisfaction tend to be more resilient to treatment compared to more behavioral symptoms of AN.

CONFLICT OF INTEREST

The authors have no conflict to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, ADM, upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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Paper III

Altered functional connectivity in adolescent anorexia nervosa is related to age and cortical thickness

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RESEARCH Open Access

Altered functional connectivity in adolescent anorexia nervosa is related to age and cortical thickness

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Abstract

Introduction: Functional networks develop throughout adolescence when anorexia nervosa (AN) normally debuts. In AN, cerebral structural alterations are found in most brain regions and may be related to the observed functional brain changes. Few studies have investigated the functional networks of the brain in adolescent AN patients.. The aim of this explorative study was to investigate multiple functional networks in adolescent AN patients compared to healthy age-matched controls (HC) and the relationship with age, eating disorder symptoms and structural alterations.

Methods: Included were 29 female inpatients with restrictive AN, and 27 HC. All participants were between the ages of 12 to 18 years. Independent component analysis (ICA) identified 21 functional networks that were analyzed with multivariate and univariate analyses of components and group affiliation (AN vs HC). Age, age × group interaction and AN symptoms were included as covariates. Follow-up correlational analyses of selected components and structural measures (cortical thickness and subcortical volume) were carried out.

Results: Decreased functional connectivity (FC) in AN patients was found in one cortical network, involving mainly the precuneus, and identified as a default mode network (DMN). Cortical thickness in the precuneus was significantly correlated with functional connectivity in this network. Significant group differences were also found in two subcortical networks involving mainly the hippocampus and the amygdala respectively, and a significant interaction effect of age and group was found in both these networks. There were no significant associations between FC and the clinical measures used in the study.

Conclusion: The findings from the present study may imply that functional alterations are related to structural alterations in selected regions and that the restricted food intake in AN patients disrupt normal age-related development of functional networks involving the amygdala and hippocampus.

Keywords: Eating disorders, Anorexia nervosa, Adolescent, RS-fMRI

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Introduction

Structural and functional changes in the brain have frequently been revealed in patients with anorexia nervosa (AN), a severe eating disorder characterized by abnormally low body weight and a body image disturbance. Cerebral structural alterations are found to mainly involve reduction in gray matter (GM) in numerous brain regions, and several studies find that most cortical areas are affected [1–3]. Functional magnetic resonance imaging (fMRI) studies typically utilize stimulus paradigms to uncover brain activity related to AN characteristic traits such as body image disturbance [4] and food and taste aversion [5]. These studies have revealed altered activity in several brain regions and functional networks, improving our understanding of the neurobiological correlate to this disorder.

In recent years, it has become increasingly common to investigate brain activity while subjects are at rest, not responding to any stimuli in the scanner – so called resting state fMRI (RS-fMRI). RS-fMRI can be used to identify resting-state networks (RSNs) – spatially separated areas of the brain where the BOLD-signal is temporally correlated [6]. Several RSNs that are consistent across trials and studies have been identified [7]. The networks are linked to known cognitive domains such as vision, somatosensation and motor function. A much studied network is the default mode network (DMN) [8]. The DMN is found to correlate negatively with task-driven activity in fMRI studies [9].

In RS-fMRI studies conducted in AN patients, several different analytical approaches have been utilized. Many studies have used seed-based approaches, which are useful to investigate areas of interest. However, such approaches rely on a priori hypotheses and can thus fail to detect alterations in unselected brain regions. Some studies have investigated selected RSNs that may be linked to core symptoms of the eating disorder such as visuospatial [10] and executive control networks [11] and suggest that altered connectivity in these networks contribute to disturbance in body image perception and excessive cognitive control, respectively.

AN typically has its debut in adolescence [12], during a period in development where drastic changes occur in the organization of brain networks, both internally within networks and between different RSNs [13]. During adolescence intra-network connectivity appears to increase and inter-network connectivity decreases, suggesting that the networks become more established and that communication between networks becomes more efficient with increasing age [13]. Particularly RSNs involving areas such as the precuneus, the cingulate cortex and the insula were found to gain increasing intra-network connectivity during adolescence. AN often delays normal developmental processes such as the onset of puberty and may also delay structural and functional brain development. To our knowledge, no studies have investigated the relationship between alterations in brain networks and development in adolescent AN patients. Adolescent AN patients are found to have a greater GM volume reduction compared to adults AN patients [2], and there may be considerable spatial overlap between functionally and structurally altered regions. A structure-function relationship is suggested, but not established in adult AN [14]; Scaife et al. (2017) reported that GM morphometrics explained functional connectivity alterations [15], and de la Cruz (2021) found reduced connectivity in regions where cortical thickness was reduced in AN patients [16]. Two other studies did not detect such a relationship [10, 17]. Seidel et al. (2019) reported a decreased structure-function relationship in AN relative to HC [17]

As the structural alterations in AN appear to be occurring across most of the cortex and several subcortical regions [1–3], it is possible that networks in several anatomical areas are affected. A common method for investigating whole-brain connectivity is independent component analysis (ICA). ICA is data-driven and does not require a-priori selection of regions to examine. To our knowledge, only one study has conducted whole brain ICA in adolescent AN patients, examining all the known RSNs detected [18]. The authors found that increased functional connectivity in a fronto-parietal network and DMN were associated with problems with interoceptive awareness.

The aim of this study was to investigate multiple networks detected in our dataset, covering large parts of the cortex and some subcortical regions that may be related to eating disorder symptoms, such as visuospatial-, executive control- and default mode-networks. Furthermore, we investigate the relationship between functional networks and age in adolescent AN-patients compared to healthy controls (HC). As a structure-function link may exist, we also aimed to examine the relationship between functional networks and structural measures (cortical thickness and subcortical volume) in relevant anatomical regions.

Methods

Study design and sample

Acutely ill patients admitted to one of two clinics (Regional Center for Eating Disorders at the University Hospital of North Norway in Tromsø, and Oslo University Hospital). In total, 29 female patients with AN (Age: M = 15.9 SD = 1.7) and 27 gender and healthy age-matched controls (Age: M = 16.1, SD = 1.9) between the ages of 12 to 18 years were recruited for the study (8 patients and 8 controls were tested and scanned at the Oslo clinic and the rest were included in Tromsø). The HC participants

were recruited from local high schools. All participants were scanned in the afternoon. The inpatient AN group were scanned after dinner but before supper at the hospital. However, the exact time between meal to scanning were not recorded for any of the participants but the scanning did not interfere with the meal plan for any of the patients. In the healthy control group, the scanning was performed between 3 and 8 pm. During scanning, all participants were asked to stay awake and keep their eyes open and fixate their gaze on a cross on the in-scanner screen.

Inclusion criteria for AN patients were DSM-5 criteria for restrictive AN (no history of binge-purge episodes), diagnosis set by a clinical specialist in psychology or psychiatry. Age-adjusted, standardized body mass index values (BMI-SDS) were calculated using Norwegian normative data from the Bergen Growth Study [19]. Exclusion criteria for all participants were neurological disorders and organic brain injury, developmental disorder, history of bulimia nervosa, schizophrenia, psychotic episodes, and the use of antipsychotic medication. Additional exclusion criteria for HC were lifetime or current eating disorders, BMI < 17.5 or obesity (BMI > 30). The sample is the same as described in two previously published articles [3, 20].

Image acquisition

MR scanning was performed with a 3 T Siemens Magnetom Skyra Syngo MR D13C in Tromsø and a Phillips Achieva 3 T scanner in Oslo, both equipped with 64 channel head coils. At both sites, high-resolution 3D T1-wheighted images were acquired. In Tromsø, we used a magnetization-prepared rapid gradient-echo (MPRAGE) sequence with the following parameters: Orientation = Sagittal; No. of slices = 176; Voxel size = 1 × 1 × 1; Slice thickness = 1 mm; repetition time (TR) = 2300 ms; echo time (TE) = 2.98 ms; field of view (FOV) = 256 × 256; Flip angle = 9°; and inversion time (TI) = 900 ms. In Oslo, a 3D-TFE sequence was used with the following parameters: Orientation = Sagittal; No of slices = 184; Voxel size = 1 × 1 × 1; Slice thickness = 1 mm; TR = 2300 ms; TE = 2.98 ms; FOV = 256 × 256; Flip angle = 8°; and TI = 900 ms.

The following parameters were used for functional imaging at both sites: Voxel size: 3x3x3, matrix size: 80 × 80, TR: 2500 ms., TE: 30 ms., acquisition order: interleaved (43 slices), no. volumes: 288. Scan-time for fMRI sequence was 12.08 min.

A group analysis of the potential confounding effect of scan site (Oslo > Tromsø) was conducted using participants from the HC group.

Preprocessing and image analyses

The functional and structural images were preprocessed using FSL FEAT (FSL ver. 5.0.11, fsl.fmrib.ox.ac.uk). The functional images were corrected for scan-to-scan motion, coregistered to the high-resolution anatomical image, warped to the MNI152 template and spatially smoothed with an 8 mm FWHM Gaussian filter. No temporal filtering was applied. Next, motion-related independent components were removed with ICA-AROMA [21, 22].

The software Group Independent Component Analyses Toolbox (GIFT) was used to extract functional networks (components) from the dataset and all further analyses [23]. ICA applies blind source separation to extract statistically independent components in the dataset. Group ICA was performed on the preprocessed images with the Infomax algorithm. Based on results from several large sample RSN studies [7, 24–26] a decision was made to set component numbers to 25. The module ICASSO implemented in GIFT was set to run the Infomax algorithm 10 times, as is recommended [27]. ICASSO graphs were inspected and evaluated by their component stability/cluster quality index (Iq > .80), representing the difference between intra and extra-cluster similarity, and visual inspection of component maps. Two of the authors (PMA and ADM) rated the components. This process is further described in the Supplementary material 1. One noise-related component (activation outside the cortex and in the ventricles) was identified by visualization and excluded from further analyses. Two components seemingly representing auditory networks were also excluded from further analyses as we did not hypothesize an impact of AN core symptoms in such networks. One cerebellar network received a low score from the two raters and was also excluded from further analyses. The excluded components are presented in Supplemental Fig. 2.

Statistical analyses

Group difference in sample characteristics were investigated with Mann-Whitney U-Tests using IBM SPSS 26. Shapiro-Wilk tests were used to test normality of the sample characteristics, cortical thickness, and cortical volumes. Furthermore, visual inspection of Q-Q- and Boxplots was performed. Significant deviations from a normal distribution were found for all sample characteristics variables except age.

Multivariate group analyses were conducted on time-courses spectra and spatial maps of the selected 21 components (Supplemental Fig. 1), including age and age*group interaction term as covariates. In subsequent analyses steps, BMI-SDS, the two EDE-Q scales “Restriction” and “Concerns about figure” [28] were included as covariates in separate models. The two subscales were selected because they did not correlate as highly with each other as the remaining subscales and thought to capture different presentations of AN. All analyses were performed with the MANCOVAN toolbox implemented

in GIFT software [26]. MANCOVAN performs backward selection of predictors (factors and covariates) by testing whether each predictor in the model explains variability in the multivariate response using a multivariate analysis of covariance (MANCOVA), and for the reduced model of significant predictors proceeds to perform univariate tests corrected for multiple comparisons [26]. The multivariate results determine the significant covariates used in univariate analyses for timecourses spectra and spatial maps. False discovery rate (FDR) correction is implemented in MANCOVAN for multiple comparison corrections. Results retaining $p < 0.05$ after FDR were considered statistically significant. Estimates of effect sizes are shown by weighted Beta values (group coding: 0 = AN, 1 = HC) for each significant covariate. In the MANCOVAN toolbox, Beta values are averaged using weighted mean activated number of voxels in the groups. Following group analyses, we investigated the relationship between significant components and structural measures (cortical thickness and volume) extracted with FreeSurfer software [29], version 6.0 (FS 6.0) [30, 31];. This procedure has been described previously in [3, 20]. To perform correlations between the significant RSN components and structural measures, the maximum activation (peak) value in the selected RSN networks was extracted from MANCOVAN to SPSS and correlated (Pearson correlations) with the mean thickness data from FreeSurfer corresponding to the anatomical location of the maximum activation in the network. The mean value of thickness from both hemispheres were used. Bonferroni corrections were applied to correct for multiple testing in the correlational analyses of structure – function. Structural data were parcellated with the Desikan-Kiliany atlas [32], and regions overlapping spatially with significant RSN's were selected for analyses.

Results

Sample characteristic

Table 1 shows sample characteristics and tests of group means for AN and HC. AN patients had significantly lower BMI and higher scores on self-report measures of eating disorder and depressive symptoms. Table 2 shows additional characteristics of the AN group only.

Multivariate results

Multivariate analyses of spatial maps showed that there was a significant group effect ($p < .05$) in five networks (Fig. 1), when including age and the interaction term age*group as covariates. Including BMI-SDS in this model did not alter results. A significant effect of age and a significant interaction effect of group and age was found in three of these networks (C6, C15 and C24).

Table 1 Sample characteristics

	AN Mean (SD)	HC Mean (SD)	U-value	*p*
N	29	27		
Age	15.9 (1.7)	16.1 (1.9)	.33	.37
BMI	16.3 (1.7)	22.0 (3.1)	50	<.001
BMI-SDS	−2.4 (1.3)	0.4 (0.9)	49	<.001
Left hand dominant	2	2		
BDI II[a]	24.1 (12.6)	4.3 (5.2)	1166	<.001
EDE-Q restriction[a]	3.3 (1.9)	0.3 (0.5)	1129	<.001
EDE-Q eating[a]	2.5 (1.6)	0.2 (0.5)	1127.5	<.001
EDE-Q weight[a]	3.2 (1.7)	0.7 (0.8)	1117.5	<.001
EDE-Q figure[a]	4.1 (1.7)	0.8 (0.9)	1148.5	<.001
EDE-Q global[a]	3.3 (1.5)	0.5 (0.5)	1155	<.001

Note: Mann-Whitney U-Test. *BMI* Body mass index, *BMI-SDS* Standardized BMI values based on Norwegian norms for children, *BDI* Becks Depression Inventory II, *EDE-Q* Eating Disorder Examination Questionnaire. [a]AN *N* = 27

The multivariate model including EDE-Q restriction scale as a covariate showed similar results with significant effects of group, age and age*group interaction in the same networks and an additional significant effect of EDE-Q on a fifth network (C17). However, the EDE-Q variables were not retained for univariate analyses and are not reported further.

Univariate results

Univariate results of spatial maps showed significant group difference in C4 a default mode network. Figure 2a shows that the group difference (B = – 3.1) is most prominent in the central part of network C4 (peak voxels coordinates: X: -12, Y: -56, Z: 56). Univariate results of group*age showed a significant interaction effect in network C6 (B = – 3.1) and C24 (B = 1.1), the two subcortical networks with peak activation in the amygdala (X: -26, Y: -6, Z: - 20) and hippocampal areas (X: -30, Y: -30, Z: – 16) (Fig. 2b and c). Results for the left amygdala network (C6) indicates that there is a positive relationship with group*age, indicating greater intra-network

Table 2 Characteristics of the AN group

	AN Mean (SD)
N	29
BMI admission	15.0 (1.4)
BMI-increase [a]	0.9 (0.5)
Drugs (SSRI/GH)[b]	4
Weeks admitted	4.6 (4.2)
Time since first GP contact (years)	1.6 (1.5)

Note: [a]BMI increase between admission and scan date. [b]5 subjects on serotonin reuptake inhibitor (SSRI), 2 on growth hormones (GH). Time since first GP contact = Consultation concering eating disorder symptoms.

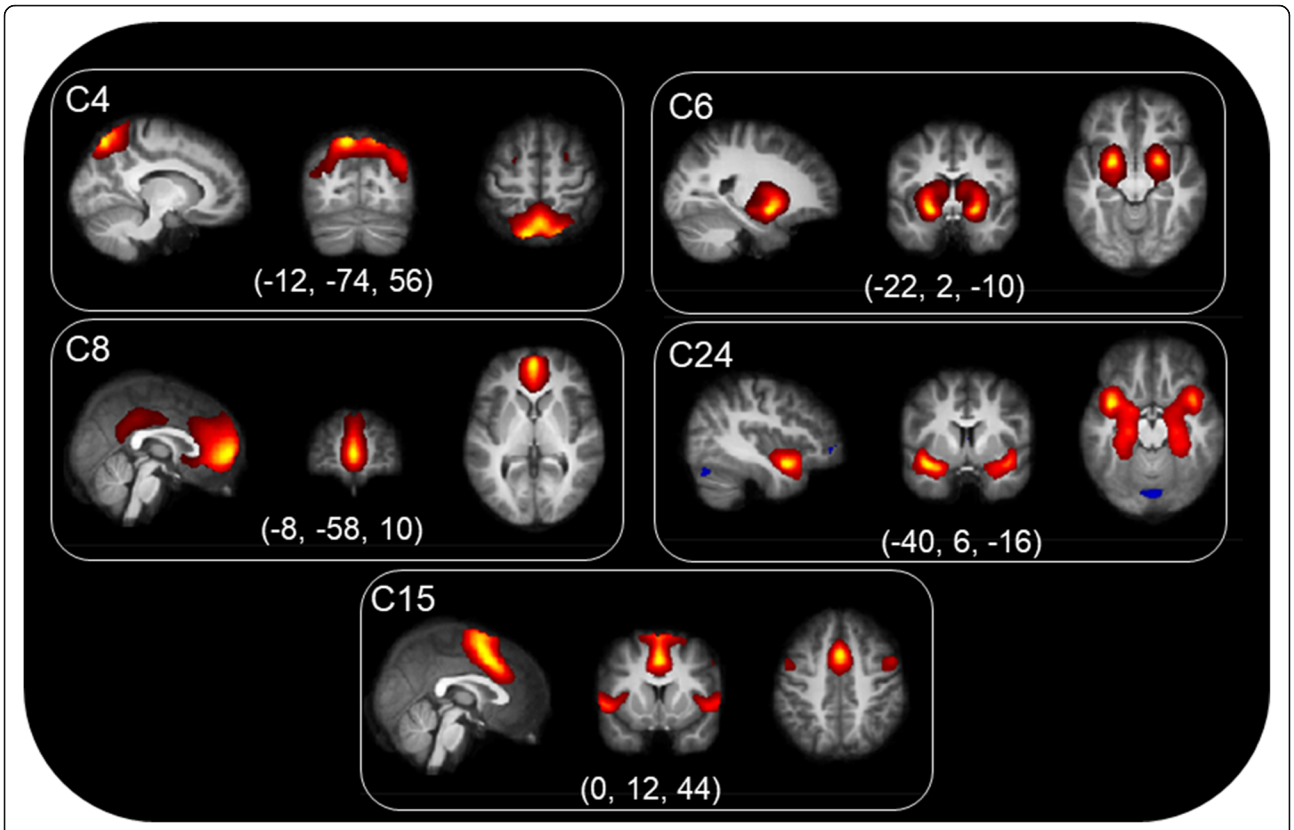

Fig. 1 Spatial maps of components showing significant group effect. The three most informative slices in sagittal, coronal and axial view are presented for each component. Images are thresholded at Z > 2. C4: Posterior default mode network, C6: Subcortical (amygdala) network, C8: Anterior default mode network, C24: Subcortical (hippocampus) network, C15: Sensorimotor network

connectivity with increasing age in AN group (coded 1). Figure 2c shows that the significant interaction effect in C24 is negative, indicating decreasing intra-network connectivity in AN patients with increasing age compared to HC.

Correlation with structural measures

Correlational analyses of structural measures were performed with the network that were significantly different between AN patients and controls, or had a significant interaction of group*age, namely C4, C6, and C24. The correlation analyses showed that precuneus thickness and component C4, the precuneus network, was significantly associated (r = .53, $p < .001$). The overlap between the precuneus area and the C4 component is shown in Fig. 3, whereas the correlation between C4 and precuneus thickness is shown in Fig. 4. Amygdala and hippocampal volumes were not significantly correlated with the components comprising these areas (component number C6 and C24 respectively).

Control variables

We performed a between-site (Oslo vs. Tromsø) analyses of HC participants to test for the effect of scanner site. To test for the effect of drug use, all analyses were re-performed controlling for/excluding the AN patients who were on prescribed drugs at the time of scanning ($N = 5$). We found no significant effect of scanner site or drug use.

Discussion

Compared with HC, AN patients had decreased connectivity in a DMN network involving mainly the precuneus. Age affected two subcortical networks involving the hippocampus and amygdala differently for AN and HC. In AN patients increasing age was associated with increasing connectivity within a network involving the amygdala and decreasing connectivity within a network involving the hippocampus. Precuneus thickness, found in our previously published study [3] to be reduced in AN compared to HC, was significantly associated with connectivity in the DMN (precuneus) network.

The precuneus is a parietal region bordering to the visual cortex and is considered to be a functional core of the DMN [33]. In AN patients functional alterations are found repeatedly in this region, and have been linked to body image perception [4, 34–36] most often in terms of reduced activity or altered connectivity with other

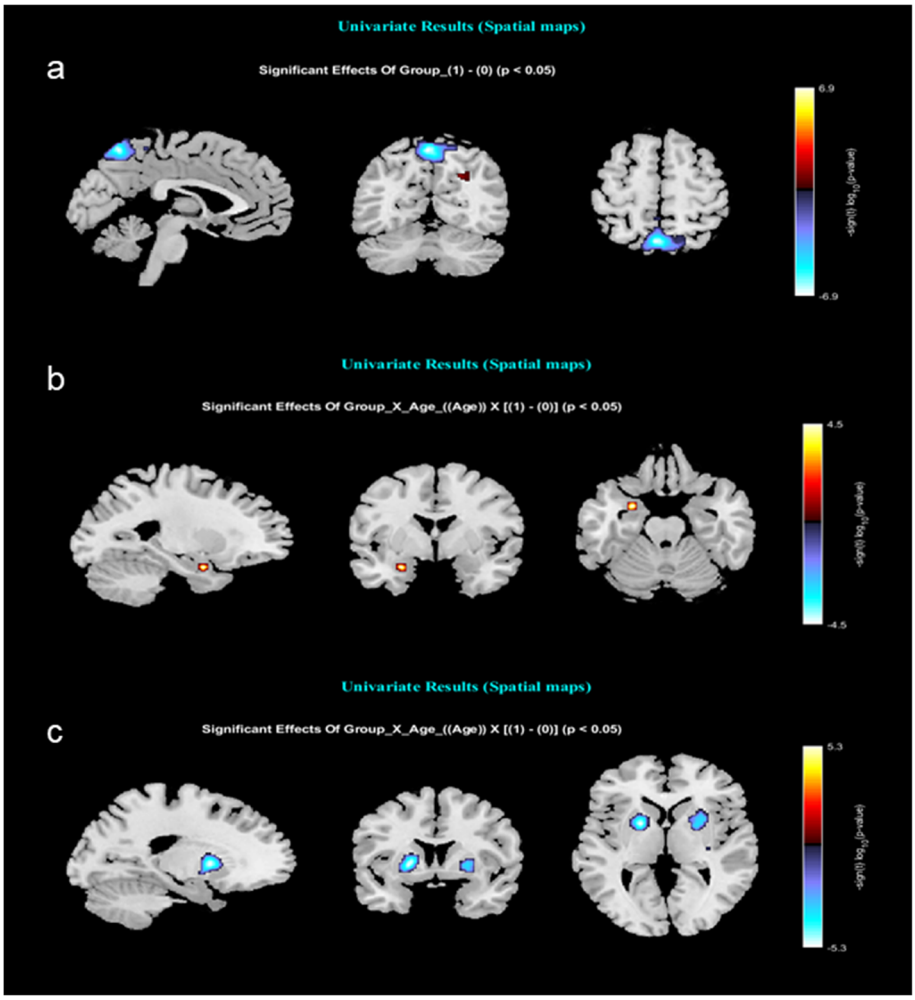

Fig. 2 Univariate results showing **a**) significantly lower intrinsic connectivity in the AN group in component C4, and a significant group x age interaction effect in component C6 (**b**) and C24 (**c**)

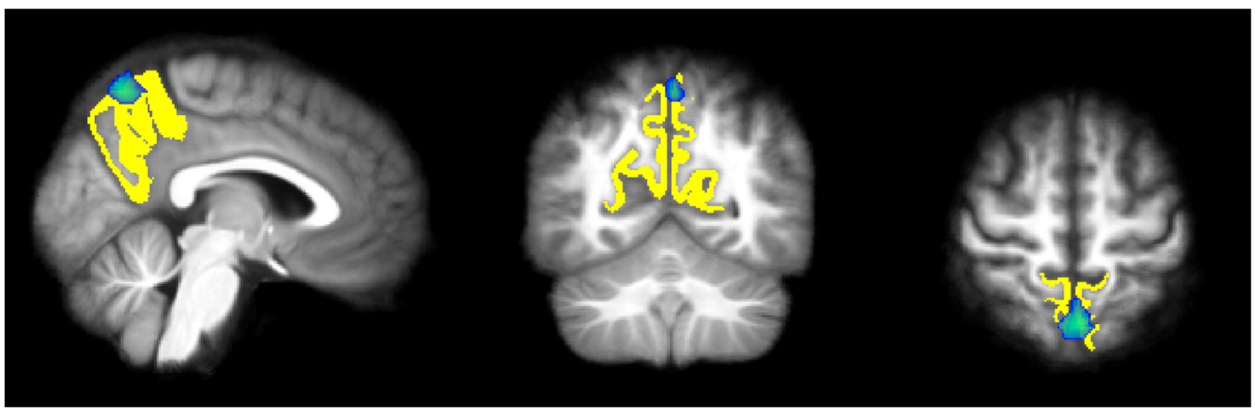

Fig. 3 The overlap between the cortical areas constituting the precuneus (yellow color) from FreeSurfer and the activation found in the resting-state fMRI analysis (blue color)

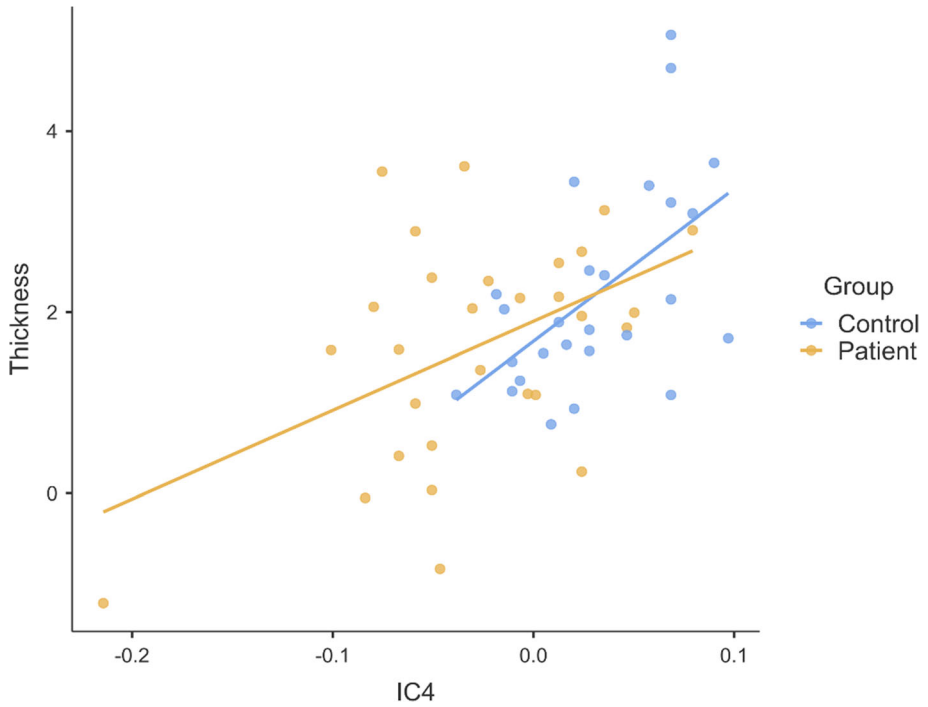

Fig. 4 Pearson correlation between mean precuneus thickness (mean of left + right precuneus) in the precuneus and the peak activation in C4. r = .53, p < .001 Bonferroni adjusted

regions Results from the present study did not show a significant association with the self-reported measure of "concerns about figure", as one might expect in light of previous findings.

Correlational analyses showed that connectivity in the precuneus network was associated with precuneus thickness, suggesting a cerebral structure-function link. Several studies have reported decreased volume or cortical thickness in the precuneus in AN patients [37–42], and a recent study in adult AN patients showed a relationship between precuneus thickness, reduced in their AN sample, and functional connectivity in the DMN and a central executive network [16]. A structure-function link is also found in a somatosensory network [14]. Findings from two recent studies with adolescent samples contradict this link however; Lotter et al. (2021) report global connectivity alterations that are unrelated to global GM volume [51] and Seidel et al. (2019) report a weakened relationship between measures of local characteristics of the BOLD signal and cortical thickness and volume [17]. This discrepancy may be due to the different approaches to investigating functional connectivity. As GM reduction and functional connectivity alterations is not observed in all brain regions, and may not overlap in several anatomical areas, investigating whole brain measures may mask regional relationships. Regional structure-function links may exist, and future studies should aim to investigate areas of decreased cortical volume or thickness and functional connectivity in corresponding anatomical areas.

Results from the present study show that AN patients have decreasing intra-connectivity in a hippocampus network and increasing intra-connectivity in an amygdala network with increasing age compared to HC. These results may suggest that AN disrupts normal age-related development of network intra-connectivity, expected to increase during adolescence [13]. Two studies using graph theoretical metrics to detect functional networks also found decreased connectivity in adolescent AN patients in networks resembling the two subcortical networks found in this study [43, 44]. One of these studies tested the association with age, with no significant findings, however neither investigated the interaction effect of age and group as done in the present study. Future RSN studies should investigate the effect of age in adolescent AN patients, preferably with longitudinal sampling. Development of functional networks have been linked to pubertal status [45]. Delayed or disrupted pubertal onset is commonly found in AN, and a possible delay in network development may be due to this. A recent review of fMRI-studies in adolescent AN suggest that

puberty delay can affect brain maturation and lead to impaired cognitive flexibility that in turn maintains the disorder and makes it difficult to combat [46]. Pubertal status was not recorded in this study and future research should include such measures to investigate if delayed or disrupted puberty affects brain maturations in AN.

In a previous study including the same sample [20], we found that the hippocampus may be more vulnerable to AN in terms of volume decrease compared to brain as a whole. However, correlational analyses of hippocampus volume and the hippocampus network were not significant, indicating that the structural alterations in this region were not associated with the functional alterations in RSNs. Analyses with eating disorder symptoms as covariates did not produce significant results and could thus not shed light on the mechanisms behind the interrupted development of these networks. Variables not included in this study such as hormonal levels and a broader mapping of eating disorder and comorbid symptoms could possibly explain these findings and future studies should include such measures.

Previous RSN studies of adolescent AN patients have found altered connectivity involving visuospatial networks [10], fronto-parietal networks and DMN's [11, 47]. In the present study, we did not find altered functional connectivity in such networks. The previous studies investigated a few selected networks and discrepant findings may be due to the multi-network approach in this study. Another possible explanation for the different findings in the present study may be that patients had higher BMI compared to the samples in previous studies. It is possible that functional changes in the brain vary across the different stages of AN as structural alterations do [48].

Strengths and limitations

There was no a-priori selection of cerebral regions to examine and only two RSNs were excluded from analyses, leaving analyses largely data-driven. By contrast, previous studies have mostly investigated a few selected components, perhaps discarding several relevant networks. On the other hand, it could be argued that the auditory networks excluded in the present study could have an effect on the analyses given the findings in adult patients in Scaife et al. [15] even if auditory dysfunction is not a core symptom in anorexia. Furthermore, we did not assess the effect of the varying durations of treatment preceding the resting-state scan which possibly could have an impact on cerebral network functioning.

Generally, it is difficult to disentangle the effects of starvation on cerebral functioning from the effects of acute AN because the physiological and psychological responses are overlapping [49]. The present study was not designed to answer whether the cerebral changes observed was due to AN or starvation only, and the results should be interpreted according to this. The study sample was larger compared to previous studies in the field, and with a narrow age range. The analyses were conducted with up-to-date software and methods, and we controlled for potential confounding variables like scan site and drug use and multiple comparisons. Patients were not likely to be in a catabolic phase of their illness when included in the study. All patients included were on meal plans and their BMI had been increasing since admission, reducing the confounding effects of extreme malnourishment.

In a previous review, it has been recommended to control for the effects of pubertal stage, oral contraceptives and duration of illness [50]. These types of data were not available in the present study. The use of two different MRI-scanners may confound results as the magnetic fields differ between scanners. Although site effect for AN-participants was not investigated, the non-significant differences across sites among HC participants indicate that scan site did not affect main findings in this study.

Conclusion

This study provides novel findings of age and structure related alterations in functional networks in adolescent AN. Investigating multiple RSNs in a multivariate analysis increases the likelihood of detecting the most affected functional networks in AN, indicated by results from this study to be a DMN (precuneus) network and two subcortical networks (hippocampus and amygdala). These RSNs have been implicated in previous studies in AN but have not previously been linked to structural alterations (precuneus) or age (hippocampus and amygdala). Results from this study indicate that reduced cortical thickness is associated with reduced functional connectivity in the precuneus in our adolescent sample. Furthermore results may indicate that AN disrupts normal development of RSNs involving the hippocampus and amygdala. A disruption of functional network development may contribute to the maintenance of AN, often having a prolonged course of illness and is difficult to treat. Results from this study highlights the importance of investigating multiple networks in relationship with age, brain structure and endocrinological measures in adolescent AN patients whose functional networks are still evolving.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12888-021-03497-4.

Additional file 1: Suppl Fig. 1. Spatial maps of the 29 investigated components. **Suppl. Fig. 2.** Excluded components.

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Authors' contributions

All authors contributed to the development of the study design. ØR, KS, TE, TV, PA and ADM contributed to data collection. PA conducted analyzes and ADM and TV made substantial contributions in this process. ADM drafted the manuscript in close collaboration with PA and TV. All authors gave valuable comments and suggestions to the first manuscript drafts and all authors read and approved the final version.

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Availability of data and materials

The data that support the findings of this study are available from the corresponding author, ADM, upon reasonable request.

Declarations

Ethics approval and consent to participate

The study was approved by the Norwegian Committee for Medical and Health Research Ethics (REC), North region (protocol number 302969). Informed consent was obtained from participants and parents in the case of participants below 16 years of age. All methods used in the present study were performed in accordance with the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

The authors have no conflict to declare.

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