Children at high-familial risk for Eating Disorders: study of psychopathology, neuropsychology and neuroimaging

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'I, Manuela Martinez-Barona Soyer confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.'

'The content of chapters 5 and 6 have been published (see appendix A) and the papers put as appendices. The inclusion of the content of the papers in chapter form adheres to University regulations (http://www.ucl.ac.uk/srs/academic-regulations/docs/rd-section2.pdf; p.6. Section 1.2) and has been authorised by Mr. Toby Whyte, UCL Research Degree Officer, 3rd Sept. 15.'

Abstract

Evidence suggest that a diagnosis of an eating disorder (ED) is associated with differential neurocognitive functioning and neural mechanisms. However, whether differences are present prior to the onset of the disorder (‘*trait*’), possibly affecting risk status for development of an ED; or whether differences are a consequence of secondary features of the disorder such as low nutritional intake (‘*state*’), is not clear. Family studies have established that first-degree relatives of individuals with ED are at higher risk of developing an ED than the general population, therefore, children of mothers with an ED (current or history) are the perfect group to study risk pathways to developing ED.

This is the first study to explore neural alterations as well as neurocognitive functioning in girls at high-familial risk of developing an ED, in comparison to children who are not. High risk status of girls were defined using a maternal clinical interview to confirm lifetime ED diagnosis. Intelligence, social cognition, reward responsiveness, neuropsychological function and brain imaging were investigated in girls at high-familial risk.

Girls at high familial risk demonstrated difficulties in set-shifting (cognitive flexibility) and increased reward responsiveness when compared to girls at low risk. Girls at risk also had overall increased Gray matter (GM) volume, and specifically increased GM in amygdala, caudate, hippocampus and orbitofrontal cortex when compared to girls at low risk. There were no differences in white matter (WM) connectivity from amygdala to areas of the cortex in girls at risk compared to girls at low risk.

Results suggest that differences observed may constitute putative intermediate phenotypes for ED, although this requires further study with larger samples. Findings are important as they support hypothesis of altered set-shifting as an endophenotype for ED. They also provide evidence of alterations in ventral (limbic) neurcircuit that includes the amygdala and caudate, both of which are of importance for identifying emotional stimuli and generation of affective response to these as well as playing a role in reward processes and behaviour regulation.

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Impact Statement

The study undertaken within this Ph.D. has been the first neuroimaging study to be conducted in this cohort. ED have the highest mortality rates of any other psychiatric disorder, great social and economic burden and current treatment options are limited in their effectiveness. Therefore, the understanding of its neurobiological underpinnings, which can help understand risk and maintenance factors, is of great importance.

In the short term, the pilot study will inform a larger study by developing testable hypotheses. A larger study may then be able to consolidate findings and explore further hypotheses that were unable to be explored given the current sample size; such as possible differences in risk stratified by maternal diagnosis and to correct for multiple comparisons clarifying the differences between false positive and false negative findings. Results will then be able to help disentangle a long lasting question in the study of ED, i.e. what neurocognitive and neural alterations found in acute patients are due to undernutrition and should be considered *'state'* and what can be considered *'trait'* and may therefore influence risk for the development of the disorder. This will help further our understanding of the neurobiological underpinnings of these disorders.

In the long term, understanding of altered neural pathways can help develop new treatments for resistant ED patients. With the development of neuromodulation technologies, we may be able to directly target specific brain areas that have been identified as central to ED psychopathology. An understanding of altered neurocognitive functions will help develop treatments aimed at improving cognitive impairments which have may in turn allow patients to engage better in ED focused treatments. Currently Cognitive Remediation Therapy has already shown to improve treatment engagement and recovery and more targeted treatment may improve this further.

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List of abbreviations

AD – axial diffusivity

ADC – apparent diffusion coefficient

ADHD – attention deficit hyperactivity disorder

AN – Anorexia nervosa

APA – American Psychiatric Association

ASD – autism spectrum disorder

BEN – Binge eating disorder

BAS – Behavioural activation system

BIS – Behavioural Inhibition System

BN – Bulimia Nervosa

CANTAB - Cambridge Neuropsychological Test Automated Battery

CC – corpus callosum

CSD – constrained spherical deconvolution

CSF – cerebrospinal fluid

ED – Eating Disorders

EDNOS – Eating Disorder Not Otherwise Specified

DICOM – digital imaging and communications in medicine

DSM – Diagnostic and Statistical Manual of Mental Disorders

DTI – diffusion tensor imaging

EPI – echo planar imaging

FA – fractional anisotropy

FDR – false discovery rate

fMRI – functional magnetic resonance imaging

FOV – field of view

FSL – FMRIB software library

FT – Fourier transformation

FWE – family wise error

GOSH – Great Ormond Street Hospital

ICH – Institute of Child Health

ICD – International Classification of Diseases

IFOF – inferior fronto-occipital fasciculus

ILF – inferior longitudinal fasciculus

IQ – intelligence quotient

MD – mean diffusivity

MNI – Montreal Neurological Institute and Hospital

MR – magnetic resonance

MRI – magnetic resonance imaging

NIfTI – neuroimaging informatics technology initiative

OCD – Obsessive Compulsive Disorder

OSFED - Other Specified Feeding and Eating Disorders

PDD – principal diffusion direction

RD – radial diffusivity

RF – radiofrequency

ROI – region of interest

SCID - Structured Clinical Interview for DSM

SFOF – superior fronto-occipital fasciculus

SLF – superior longitudinal fasciculus

SNR – signal-to-noise ratio

T - Tesla

T1 – spin-lattice relaxation time

T2 – spin-spin relaxation time

TBSS – tract-based spatial statistics

TE – echo time

TFCE – threshold-free cluster enhancement

TR – repetition time

UCL – University College London

UF – uncinate fasciculus

VBM – voxel-based morphometry

WASI – Wechsler abbreviated scale of intelligence

Dissemination

Publications

Barona, M., Nybo Andersen, AM., Micali, N. Childhood pyshcopathology in children of women with eating disorders. *Acta Psychiatrica Scandinavica* 2016: 134, 295-304

Barona, M., Brown, M., Clark, C., Frangou, S., White, T., Micali, N. White matter alterations in anorexia nervosa: evidence from a voxel-based meta-analysis. *Neuroscience and Biobehavioural Reviews* 2019: 285-295

Barona, M., Clark, C., Micali, N. Subcortical and cerebellar volumetric differences in children at high-familial risk for eating disordres. *In preparation*

Conference Presentations

"Childhood psychopathology in children of mothers with eating disorders" 12th *London International Eating Disorders Conference, 2015*

"Eating disorders in pregnancy: the potential for adverse outcomes for mother and infant" *Beat – Eating Disorders International Conference, 2016* (workshop)

"White matter alterations in anorexia nervosa: evidence from voxel-based meta-analysis" *International Conferences in Eating Disorders, 2017*

"Subcortical and cerebellar volumetric differences in children at high-risk for eating disorders" *European Council of Eating Disorders, 2019*

Conference posters

"Childhood psychopathology in children of mothers with eating disorders: findings from the DNBC birth cohort" *Eating Disorders Research Society, 2015*

Chapter 1. Introduction to Eating Disorders

1.1. Chapter overview

The aim of this chapter is to provide an up to date account of our current understanding of eating disorders, to explore the diagnosis, prevalence, mortality rates and aetiology. The chapter will also provide an overview of current neurocognitive findings in eating disorders, which will provide a background for result chapters.

1.2. Diagnoses

Eating disorders are severe psychiatric illnesses characterised by abnormal attitudes towards food and weight that interfere with many areas of a person's life and are associated with numerous negative outcomes, including medical and psychological complications.

In 1952 the American Psychological Association (APA) developed the first edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM), which included diagnostic categories and had a clinical focus. Anorexia Nervosa was first introduced in this first version of the DSM, however, it was then introduced as a psycho physiological reaction (a neurotic illness) and it wasn't until the second edition in 1968 when it was placed under the section of special symptoms – feeding disturbances. It was not until the following version of DSM in 1980 that Bulimia was included as well although it wasn't until 1987 that the term Bulimia Nervosa was coined.

Currently, the Diagnostic and Statistical Manual of Mental Disorders version 5 (DSM-5) (APA, 2013) recognizes three primary diagnoses: Anorexia Nervosa (AN), Bulimia Nervosa (BN), Binge Eating Disorders (BED). ED presentations that do not fit into the above diagnoses are classified as OSFED (Other Specified Feeding or Eating Disorder). It is important to note that one of the biggest changes in the diagnostic categories from DSM-IV to DSM-5 is the change of chapter name, from "Eating Disorders" to "Feeding and Eating Disorders". This has led to an increase in the number of diagnoses under the

category, from three in DSM-IV to eight in DSM-5. These include: Pica, Rumination Disorder, Avoidant/Restrictive Food Intake Disorder, AN, BN and BED. As well as the change from Eating Disorder Not Otherwise Specified (EDNOS) category, to Other Specified Feeding or Eating Disorders (OSFED) and the inclusion of Unspecified Feeding or Eating Disorder (UFED). For the purpose of this chapter, we will only discuss AN, BN, BED and OSFED as they are the disorders of interest for this thesis.

Although in previous years, review articles have showed that groups at higher risk for ED are those who are female, young and Western, ED also occur in older women, men and in non-Western countries (Hoek, 2014).

1.2.1. Anorexia Nervosa

According to DSM-5, Anorexia Nervosa (AN) is characterized by a severe restriction of calorie intake leading to a significant low body weight (in the context of what is minimally expected for age, sex, developmental trajectory and physical health); either an intense fear of gaining weight even when underweight, or persistent behaviour that interferes with weight gain (even when significantly low weight); and a disturbance in the way they perceive their body weight or shape, undue influence of body shape and weight on self-evaluation or a persistent lack of recognition of the seriousness of the current low body weight (APA, 2013). One of the changes incurred in DSM-5, is the change from “body weight less than 85% of that expected” to “significantly low body weight” which allows clinicians to introduce clinical judgment as opposed to adhering to a rigid percentage of ideal or expected body weight as a necessary criteria for diagnosis. Both main classification manuals for mental health disorders (DSM (APA, 2013) and the International Classification of mental Disorders- ICD (Organization, 1992)) have a weight criterion for the diagnosis of AN, with ICD-10 stating 15% below expected weight for age and height.

Accompanying behaviours can include excessive exercise, purging behaviours (e.g. vomiting, excessive use of laxatives, diuretics or other weight suppressants, etc.) and episodes of binge eating (episodes of over eating in a discrete period of time accompanied by a sense of lack of control). Depending on the presence of these accompanying behaviours, individuals with AN can fall into two sub-types: binge-purge subtype (for those that engage in binge eating and/or purging behaviours) and restricting subtype (for those

who reach and maintain low weight through food restriction and behaviours such as excessive exercising).

The onset for AN is typically in mid-adolescence (Attia, 2010), with epidemiologic studies suggesting the highest incidence for AN to be in females aged between 15 to 19 years old (Hoek, van Hoeken, & Katzman, 2003). However, in recent years, there have been more discussions regarding increasing earlier onset for eating disorders (Madden, Morris, Zurynski, Kohn, & Elliot, 2009). Although a common discussion amongst clinicians, data is lacking. A study by Hindler et al. (Hindler, Crisp, McGuigan, & Joughin, 1994) in 1994 examined the pattern of presentation of AN patients who had been referred in a period of 30 years (from 1960 to 1990) and found that during this period there was no change in age of onset. However, a more recent study by Favaro et al. (Favaro, Caregaro, Tenconi, Bosello, & Santonastaso, 2009) conducted in a large sample of patients with AN (N = 1,666) who had been referred to a clinical department between 1985 and 2008, found that the age of onset was indeed decreased in younger generations. Although age of onset might be decreasing, the peak for onset continues to be in late adolescence for AN. It has been proposed that an earlier onset (below the age of 15 years old) is associated with worse outcomes however, early onset AN shows some differences in presentation to later onset AN. For example the ratio of girls to boys for this group is 4:1, in contrast to the 10:1 ratio for later onset (Lask, Waugh, & Gordon, 1997).

1.2.2. Bulimia Nervosa

Bulimia Nervosa (BN) is characterized by recurrent episodes of binge eating, recurrent compensatory behaviours in order to prevent gain weight and self-evaluation influenced by body shape and weight. Due to the combination of both binge eating and behaviours to prevent gain weight, patients with BN are general of normal weight.

For a diagnosis of BN according to the DSM-5, the following criteria must be met:

- Recurrent episodes of binge eating, where an episode of binge eating is characterised by both of the following:
    a. Eating, in a discrete period of time (for example, within a two hour period), an amount of food that is larger than what most people would eat during a similar period of time and under similar circumstances.

b. A sense of lack of control over eating during the binge eating episode (e.g. a feeling that one cannot stop eating or control what or how much one is eating).

- Recurrent inappropriate compensatory behaviour in order to prevent weight gain: such as self-induced vomiting, use of laxatives, enemas, diuretics, excessive exercising, etc.
- The binge eating and inappropriate compensatory behaviours both occur, on average, at least once a week for a period of three months.
- Self-evaluation is unduly influenced by body shape and weight.
- The disturbance does not occur exclusively during episodes of AN.

Onset of BN is usually found to be later than that for AN, with some studies suggesting an onset in the early twenties (Hoek et al., 2003), however, a more recent study by Favaro et al. (Favaro et al., 2009) found that by 20 years of age 65% of patients with BN were already affected. Similarly to AN, Favaro and colleagues (Favaro et al., 2009) found that the age of onset for BN is decreasing.

1.2.4. Binge Eating Disorder

The new diagnostic category of Binge Eating Disorder (BED) was first proposed by Spitzer et al. in 1993(Robert L. Spitzer et al., 1991; R. L. Spitzer et al., 1993) and was first introduced in DSM-IV (as a Not Otherwise Specified category) for further study. During the DSM-IV task force, clinicians and researchers were in agreement regarding the fact that there was a need for a diagnosis for patients who engaged in binge eating but did not engage in the inappropriate compensatory behaviours that are characteristic for BN. In DSM-5, BED was included as a distinct eating disorder outside of the EDNOS category.

According to DSM-5 (APA, 2013), BED is characterized by recurrent episodes of binge eating, associated with at least three of the following symptoms (eating more rapidly than normal, eating until uncomfortably full, eating when not feeling hungry, eating alone because of feelings of embarrassment and feeling disgusted/guilty with one self) and accompanied by a marked distress. Episodes of binge eating must happen at least once a week for three months. Importantly, binge eating cannot be associated with the recurrent use of inappropriate compensatory behaviours as in BN (such as self-induced vomiting) and cannot occur exclusively during the course of either BN or AN.

BED has been found to be associated with obesity, however, not all obese patients have BED and not all BED patients are obese. As with AN and BN, BED is more common among females than males(Robert L. Spitzer et al., 1991).

1.2.5. Other Specified Feeding or Eating Disorders (OSFED) and Unspecified Feeding or Eating Disorder (UFED)

According to DSM-5 in order for an individual to be diagnosed with OSFED, they must present with feeding or eating behaviours that cause clinically significant distress and impairment in areas of functioning, but not meet full criteria for any of the other feeding or eating disorders.

The difference between OSFED and UFED is that in OSFED a diagnosis might be given with a specification of why it does not meet full criteria, whilst in UFED there is no need for specification.

The following are examples of OSFED:

1. Since the revision of the DSM-IV-TR (Association, 2000) individuals can also be classified as “Atypical AN” if they meet all other criteria but maintain a normal weight. Over 40 years ago a study was conducted showing that there is no specific amount of weight loss associated with any other AN symptoms (HALMI, 1974). This change was included in the fifth revision of the manual in order to help reduce the number of patients who were previously diagnosed as Eating Disorder Not Otherwise Specified (EDNOS) (Fairburn & Bohn, 2005) but who only did not meet the weight criteria for AN.

2. If all of the criteria for BN are met, except that the binge eating and inappropriate compensatory behaviours occur at a lower frequency (less than once a week) and/or for less than three months, then the diagnosis is of “OSFED-BN”.

3. Individuals with BED who meet all criteria except for the frequency and/or duration, are classified as “OSFED BED”.

4. Purging Disorder: recurrent purging behaviour to influence weight or shape in the absence of binge eating.

5. Night Eating Syndrome: recurrent episodes of night eating.

1.3. Eating Disorders Prevalence

Prevalence is defined as the proportion of individuals in a population who have a disease or characteristic. It is a statistical concept that refers to the number of cases of a disease that are present in a population at a given time. On the other hand, incidence refers to the number of new cases that develop in a given period of time in a given population.

Making an estimation of lifetime ED prevalence is difficult, not only due to the rarity of the disorder but also to the secrecy associated with it. Patients with ED frequently deny any problems and often only seek treatment due to concerns of those close to them or when their lives might be in danger because of the disorder.

Epidemiological studies use medical and psychiatric records to estimate prevalence, however, these figures are likely to be an underestimate, as not all individuals who suffer from an eating disorder will be diagnosed or treated. In fact, it has been estimated that only one third to one half of the cases of AN, BN and BED in the population seek treatment (James I. Hudson, Hiripi, Pope, & Kessler, 2007; A. Keski-Rahkonen et al., 2007). Most prevalence studies to date have been done using DMS-IV criteria; however, with the changes to the diagnosis in DSM-5, prevalence is likely to be higher. As one of the reasons for the revision of diagnostic criteria in DSM-5 was to reduce the number of patients who received a diagnosis of EDNOS (up to 60% in specialized ED units) (Fairburn & Bohn, 2005), the introduction of the new criteria should show changes in the prevalence to AN and BN categories and reduce the number included in EDNOS (now OSFED). A recent study by Smink et al., (Smink, van Hoeken, Oldehinkel, & Hoek, 2014) found the lifetime diagnosis of ED to increase from DSM-IV (4.4%) to DSM-5 (5.7%), in adolescence, and a study on adult women showed an increase of 50% for AN diagnosis and 40% of BN ones (N. Micali et al., 2017).

1.3.1. Anorexia Nervosa

Most prevalence studies up to 2006 provided a prevalence that ranged between 0% and 0.9%, with an average of 0.29% among young females (Hoek, 1993; Hoek & van Hoeken,

2003; Rosenvinge & Pettersen, 2014). A review by Wade and colleagues in 2011 (Tracey D Wade, Keski-Rahkonen, & Hudson, 2011) gave a lifetime prevalence ranges for AN across studies published in Australia/New Zeland, Europe and North America (using DSM-IV criteria) of 0.9 to 2.2%. As part of the study they also incorporated the prevalence of partial AN syndromes (all criteria met except for amenorrhoea, one of the changes in DSM-5) which ranged between 3.0 and 4.6%. Three large population-based cohorts studies of twins have investigated prevalence of AN, yielding prevalence's for broad AN (all criteria met, except for amenorrhea – DSM-IV diagnosis) of 1.2% - 2.2% and 2.4 – 4.3% (Bulik et al., 2006; A. Keski-Rahkonen et al., 2007; T. D. Wade, Bergin, Tiggemann, Bulik, & Fairburn, 2006). A study by Mustelin et al. in 2016 (Mustelin et al., 2016) examined the impact of the diagnostic criteria changes to the prevalence of ED in a nationwide longitudinal study of Finnish twins and found a 60% increase in the lifetime prevalence of AN using the new diagnostic criteria.

1.3.2. Bulimia Nervosa

Prevalence figures for BN range in from 0.9% to 1.5% (Hoek, 1993; Hoek & van Hoeken, 2003; Rosenvinge & Pettersen, 2014). A review by Wade and colleagues in 2011 (Tracey D Wade et al., 2011) gave a lifetime prevalence ranges for BN across studies published in Australia/New Zeland, Europe and North America (using DSM-IV criteria) of 1.5 to 4.6%. As part of the study they also incorporated the prevalence of partial BN syndromes, which ranged between 4.0 and 6.7%. Keski-Rahkonen and colleagues found a lifetime prevalence of 1.7% for BN in a large Finnish twin cohort (A. Keski-Rahkonen et al., 2009).

1.3.3. Binge Eating Disorder

Two large studies investigated BED prevalence using DSM-IV criteria (James I. Hudson et al., 2007; R. C. Kessler et al., 2013). The first one, the 2001-2003 US National Comorbidity Survey Replication Study(James I. Hudson et al., 2007) reported estimates of lifetime BED of 2.6% and a 12-month prevalence estimate of 1.2% in a population of 9,282. The second large study, studied a population of 24,124 using a series of surveys across 14 countries and reported a 12-month and lifetime BED prevalence estimate of 0.8% and 1.9% respectively (R. C. Kessler et al., 2013). A recent study (Smink et al., 2014) investigating prevalence and severity of DSM-5 ED diagnosis in a community cohort of adolescents reported a lifetime prevalence for BED of 2.3% for women, and 0.7% for men.

1.4. Eating Disorders Mortality

Eating disorders have been identified as an important cause of mortality in young individuals. The most common reported causes of death in patients with ED are electrolyte imbalance, sudden cardiac arrest, and suicide (Cartwright, 2004), however, since ED are not usually mentioned in death certificates, it is possible that the frequency of ED contributing to death is underestimated. In fact, we know that there is an increased risk of suicide amongst patients with ED, however, this will not always be apparent in death certificates. Another limitation is that a large majority of mortality research in ED has focused on AN.

A meta-analysis of 36 studies in 2011 (Arcelus, Mitchell, Wales, & Nielsen, 2011) found that the crude mortality rate for AN was of 5.1 deaths (95% CI: 4.0 – 6.1) per 1000 person-years, of which 1.3 deaths were the results of suicide. Of the thirty six studies, only 12 described mortality rates of patients with BN. The mortality rate for BN was found to be lower than for AN, with a crude mortality rate of 1.74 (95% CI: 1.1 – 2.4) per 100 person-years. Only six studies investigate mortality rates in EDNOS patients and found a crude mortality rate of 3.31 deaths (95% CI, 1.5 – 5.8) per 1000 person-years.

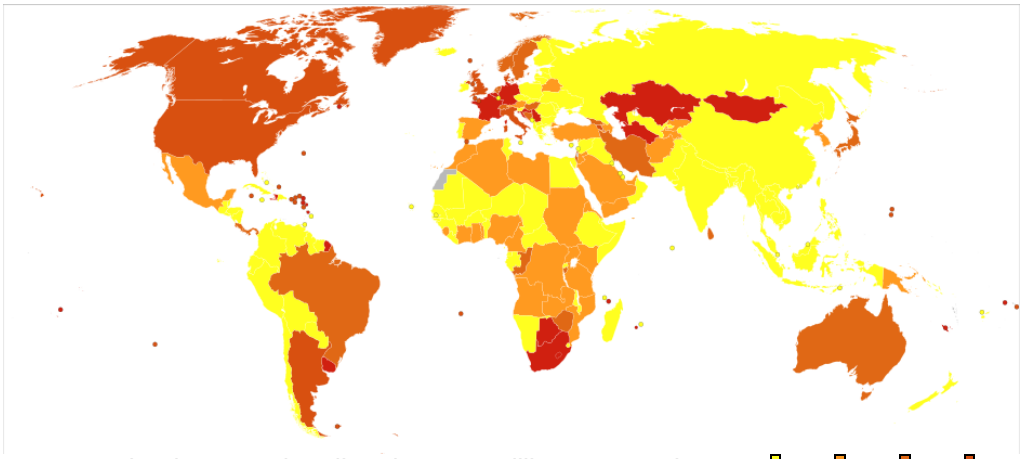
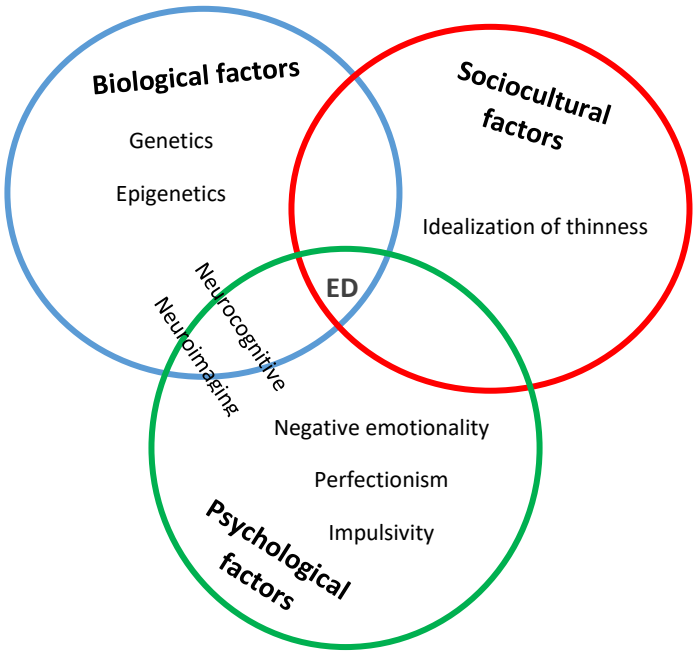


Figure 1.1. Deaths due to eating disorders per million persons in 2012 0-0 1-1 2-2 3-3 4-25 (Lozano et al., 2012; WHO, 2014; Wikipedia)

1.5. Eating Disorders Aetiology

ED are severe psychiatric disorders with devastating consequences for the patient therefore developing an understanding of why someone develops an ED is of central importance. However, the aetiology of ED is very complex, and like other psychiatric disorders, it involves the interplay of several causal factors (biological, psychological and sociocultural factors) rather than just a main causal one. Therefore, the consensus is one for integrating factors that contribute to ED into one model, the "biopsychosocial" model (Figure 1.2). The advantage of the model is that it takes into account factors that range from cultural to biological, including in the way social, familial, cognitive, personality as well as other factors. Decades of research into ED aetiology has seen a search for socio-cultural factors and body image issues as the main causal factors for the development of ED, however, these factors on their own have been unable to explain the underlying reasons for the development of the disorders.

Figure 1.2. Visual depiction of a biopsychosocial model for ED



There has been an increase in the search for biological factors as well as any other neurocognitive and neural factors that can explain the aetiology of ED. It is important to note that just like biological and socio-cultural factors, these factors are not completely distinct and most likely influence each other creating a model of risk. The following sections will briefly summarise research on genetic and social cultural factors. A more in depth overview of the research on neurocognitive and interpersonal risk factors for ED will be given in this chapters as an understanding of these is central for hypotheses development in this thesis.

1.5.1. Genetic Risk

Family studies have established a 10-fold greater lifetime risk of developing an ED in first degree relatives of individuals with AN than those of unaffected individuals (L. R. Lilenfeld et al., 1998; Strober, Freeman, Lampert, Diamond, & Kaye, 2000). However, family studies are unable to discern between what percentage of increased risk is due to genetic factors and what is due to environmental ones.

To determine the genetic risk for ED, twin studies have been performed, consistently pointing towards an influence of genetics in the development of AN. Twin studies support a genetic basis for AN with heritability estimates ranging between 48% to 74% (Yilmaz, Hardaway, & Bulik, 2015). Studies have also revealed that genetic effects also contribute to the risk for BN (Bulik, Sullivan, Wade, & Kendler, 2000).

Over the last 10 years, the development of Genome-wide associations studies (GWAS) has allowed to further our understanding of genetic variants that are associated with ED traits. GWAS studies compare cases with healthy controls and are able to investigate up to 1,000,000 genetic markers in hundreds of thousands of participants. Although initial GWAS studies were underpowered, a recent study by Duncan and colleagues combined existing samples in order to conduct a more powerful GWAS of AN (Duncan et al., 2017). Results were obtained from 10,641,224 single nucleotide polymorhpisms (SNPs), from a total of 3,495 anorexia nervosa cases and 10,982 controls. The study found that the genome-wide common variant heritability of anorexia nervosa was 0.20 (SE=0.02), suggesting that a substantial fraction of the twin-based heritability arises from common genetic variation. More recently, Watson et al (Hunna J. Watson et al., 2019) have published the largest GWAS to date which allowed the authors to characterize in more detail the metabolic contribution to AN. No large GWAS has been performed in either BN or BED.

1.5.2. Socio-cultural Factors

The majority of models of risk investigating socio cultural factors have mainly focused on the idealization of thinness in women as a driving factor in ED. The supporting evidence for this comes from historical records and differences in prevalence across different cultures, with larger prevalence for ED found in Western countries (P. K. Keel & Forney, 2013). Moreover, during the 20th century, the idealization of thinness, specifically in women although in men too, increased in the Western culture and so did the incidence and prevalence of AN and BN (P. K. Keel & Forney, 2013). With this respect, it is important to note that differences exist between ED diagnoses in relation to culture based incidence. For example, although it is true that BN is mostly detected in Western countries, the same cannot be said for AN, which has been found in non-western regions and for which we have historical accounts that take us back hundreds of years (Bemporad, 1996). Gender differences in those affected by the disorder have also been used as supporting evidence for the models of risk investigating socio-cultural factors as most of the idealization of thinness messages are directed towards women. However, data on using idealization of thinness doesn't fully explain gender differences.

Importantly, while idealization of thinness may play a role in the overall model of risk for ED it cannot solely explain it. Body dissatisfaction is high in women and this can be in part attributed to socio cultural factors, which in turn can increase risk for disordered eating and ED. However, these individual risk factors have been shown to have low predictive power (Stice, 2002) suggesting it is important to continue the search for further individual risk factors.

1.6. Neurocognitive Findings

Neurocognitive functions are cognitive functions that are closely linked to the function of a particular brain area, neural pathway or cortical networks in the brain (Posner & DiGirolamo, 2000). Neurocognitive processes have gained an increasing amount of attention in the study of psychiatric disorders and have been proposed as potential transdiagnostic mechanisms contributing to psychopathology. One reason for this was the

introduction of The Research Domain Criteria (RDoC) developed by the US National Institute of Mental Health (NIMH) which offers a framework for investigating mental health disorders across domains of functioning (Cuthbert & Insel, 2013) (a more detailed account of the RDoC framework will be given in Chapter 3, Methodological considerations).

Many neurocognitive functions have been studied in the last 20 years, leading to a picture of difficulties in large constructs of neurocognitive functioning. Measures of intelligence and areas of executive function (set of processes required for planning and goal formation, goal directed behaviour and effective performance) have been largely studied in ED. However there is no real formal definition of executive function and, although widely used and researched, the concept is quite flexible (Friedman & Miyake, 2017; Jurado & Rosselli, 2007). One issue with the measurement of executive function is that it is complex to measure the components individually, as tasks that measure one component in many cases are also tapping into another. Therefore, most tasks rely upon more than one aspect of the overall construct. This makes research exploring executive functioning in ED not always consistent, with many different measures being used and different components being explored. It is therefore not clear if there is a lack of evidence for deficits in executive functioning in ED, or if variability in results is highlighting the difficulties in understanding executive functioning. However, to date, a large amount of the research in ED has focused on executive functions that tap onto the construct of central coherence and set shifting (or cognitive flexibility), which will be described below.

It is important to note that most studies have investigated cognitive markers primarily in ill patients. An increasing amount of studies are also assessing recovered patients which has helped elucidate differences between characteristics considered scars (or state) of the disorder and longstanding characteristics, considered traits. More recently, research has also been conducted in unaffected relatives and children at high-familial risk for ED to help clarify these differences and help develop models of risk, which can in turn inform early intervention and treatments.

The following section will provide a review of neurocognitive processes studied in ED and which are relevant for this Ph.D. Later in the thesis (Chapter 3) an up to date overview of findings in the children at risk literature will be provided.

1.6.1. Intelligence

Although intelligence is not the focus of this thesis, it is worth noting that in the past it has been an area of interest in the study of ED. As the study of neuropsychological profiles has become more popular in mental health disorders, there are several studies which have aimed to explore Intelligence Quotient (IQ) in patients with ED.

The IQ is a composite score from a range of tests which have been developed in order to measure general ability or intelligence (Lezak, 2007). One of the most popular instruments used to measure IQ are the Wechsler Intelligence Scales, first released in the mid 1950's. The scales are formed of subtests which alternate verbal and visual-perceptual tests which form the verbal and performance subscales and which together assess general intelligence.

It has been hypothesized, that patients with AN have a higher IQ level in comparison to the general population which could be partly driving or contributing to the manipulation and planning that allows the disorder to go undetected as well as better school performance (Lopez, Stahl, & Tchanturia, 2010). However, another hypothesis is that the perfectionistic style attributed to AN could explain this too (Dura & Bornstein, 1989). Although higher IQ has been hypothesized for patients in AN, the literature has shown a large amount of variability and it has been hypothesized that the differences in samples studied (clinical vs. community) might be playing a role in findings.

1.6.2. Set-shifting (Cognitive flexibility)

Cognitive flexibility has been described as an executive function that involves the ability to shift attention from one task or operation to another, usually in response to a change in rules or demands (Dajani & Uddin, 2015). It has been largely studied as a construct, as well as in relation to other psychiatric disorders (such as OCD (Gruner & Pittenger, 2017) and ASD (Demetriou et al., 2018; H. Westwood, Stahl, Mandy, & Tchanturia, 2016)). Cognitive flexibility can also be more broadly thought of as the ability to overcome responses that have become habitual in order to create more adaptable ones. It requires several executive functions, such as salience detention and attention, inhibition, working memory and switching that encompass the larger construct (Dajani & Uddin, 2015) located

primarily in the prefrontal cortex (Armbruster, Ueltzhoffer, Basten, & Fiebach, 2012). Cognitive flexibility is typically measured using set-shifting or task-switching behavioural tasks (such as the Wisconsin Card Sort Task, Go/no-go tasks, Attention switching tasks).

One of the features of ED is compulsivity and rigidity to which cognitive inflexibility may contribute (Holliday, Tchanturia, Landau, Collier, & Treasure, 2005). Patients with ED present with rigid behavioural features related to eating behaviours (such as counting calories, exercising, in some cases rigid purging behaviours), they have also been shown to present with rigid rituals in their daily routine (e.g. cleaning, housekeeping, homework; possibly mediated by perfectionistic behaviours) and can experience difficulties in seeing alternative ways of coping with problems.

Deficits in cognitive flexibility (or set shifting) have been largely studied in ED (Galimberti et al., 2013; N. Kanakam, Raoult, Collier, & Treasure, 2013; Naor-Ziv & Glicksohn, 2016; Roberts, Tchanturia, Stahl, Southgate, & Treasure, 2007; J. E. Steinglass, Walsh, & Stern, 2006; K. Tchanturia, Davies, Roberts, et al., 2012; H. Westwood et al., 2016; Wu et al., 2014), and have been found to be present in recovered patients (I. C. Gillberg et al., 2010; Lindner, Fichter, & Quadflieg, 2014; K. Tchanturia et al., 2004) as well as in first degree relatives of patients with ED (Natalie Kanakam & Treasure, 2013; Lang, Treasure, & Tchanturia, 2016). These findings have led to the hypothesis of set shifting deficits as proposed endophenotypes for ED (Holliday et al., 2005; N. Kanakam et al., 2013; Natalie Kanakam & Treasure, 2013; Roberts et al., 2007). Tchanturia and colleagues in 2011 (Kate Tchanturia et al., 2011) found that severity of illness (measured by the length of the illness) could not fully explain the set shifting deficit findings in AN and EDNOS thus supporting the nature of these deficits as endophenotypes.

As with previous executive function constructs, different tasks have been used to assess set shifting, making comparison between studies complex. However, unlike with other smaller constructs of executive function, a large number of studies have investigated set shifting leading to several reviews and meta-analysis to obtain a better picture of the literature in the last few years. Tasks such as the Trail making task (Kravariti, Morris, Rabe-Hesketh, Murray, & Frangou, 2003), the Brixton Task (Burgess & Shallice, 1997) and the Wisconsin Card Sorting Tests among others have been used to assess this construct. Roberts and colleagues performed a systematic review and meta-analysis of studies

investigating set shifting in adults with ED (Roberts et al., 2007). In total 15 studies were included and the authors found a consistent deficit in set shifting ability across ED diagnoses, stages of illness and different set shifting measures. Meta analyses were performed separately for the most widely used tasks with effect sizes varying between small (Trail making task), to medium (Wisconsin Card Sorting Task) to large (Haptic task). A more recent meta-analysis (N = 22 studies) on set-shifting profiles of AN found a medium effect size on studies using the Wisconsin Sorting Card Task, however, they did not find a significant effect in studies that investigated children/adolescents. In 2013, Lang and colleagues performed a meta-analysis of set shifting in children and adolescents with AN and found that set shifting inefficiencies apparent in adults are not as pronounced in children and adolescents with AN (Lang, Stahl, Espie, Treasure, & Tchanturia, 2014). The authors suggest that a possible hypothesis for the differences between adults and children/adolescents is that set shifting difficulties in the adult AN population are a result of starvation or of a longer duration of the illness. However, this meta-analysis only included 7 studies, therefore, more studies are needed in order to understand set shifting in children and adolescents with AN. Recently, Smith and colleagues (K. E. Smith, Mason, Johnson, Lavender, & Wonderlich, 2018) published a systematic review of reviews investigating the neurocognitive profile of eating disorders in order to systemize a large body of work in the last decades. They included a total of 28 review articles, of which 12 reviewed set shifting (6 of which were meta-analysis) and a total of 13 meta-analysis were included in the review. In 2014 Wu and colleagues published the most recent meta-analysis (Wu et al., 2014) and found evidence of poor set-shifting in patients with ED with medium effect sizes in AN-R, BN, and BED, but not in AN-BP. Wu compared set-shifting across DSM5 ED diagnoses and concluded that set-shifting did not vary across them. Effect sizes of findings also varied across tasks used but those of the most used tasks such as the Wisconsin Sorting Tasks were medium in size (WCST g = -0.53).

Importantly, set shifting deficits have been found to persist in recovery (Nakazato, 2008, 2010; Tchanturia, 2004; Tenconi, 2010). A meta-analysis found that patients recovered from AN differed from controls with an overall small effect size (Natalie Kanakam & Treasure, 2013). Furthermore, more recently there has been an interest in understanding if set shifting is a trait rather than an effect of the disorder and therefore, first degree relatives have been studied in order to understand this difference. Holliday *et al* in 2005 found that set-shifting difficulties in women with AN were also shared by their healthy sisters. They found that in particular, both women with AN and their unaffected sisters took a significant increased amount of time to shift between tasks in the CatBag cognitive set-

shifting task and the Haptic illusion task, both of which assess perceptual rigidity. Similar results were also showed by Tenconi *et al* (Tenconi et al., 2010) were both patients with AN and their healthy sisters showed poorer performance in tasks investigating set shifting and central coherence. The meta-analysis by Kanakam and Treasure showed that overall, first degree relatives of people with ED differ from controls with a medium effect size (Holliday et al., 2005; Natalie Kanakam & Treasure, 2013). These results provide further evidence for the hypothesis that cognitive inflexibility may constitute a biological marker or heritable endophenotype in AN.

There are limitations to the study of cognitive flexibility as the definition of the construct is variable depending on studies and tasks used which leads to inconsistent findings in the literature. Therefore, there is a need for defining and operationalizing the construct and further studies using the same definition and paradigms in order to gain a better understanding of cognitive flexibility in ED.

1.6.3. Central Coherence

Weak central coherence refers to a bias towards detailed or local focus over processing the information in a more global manner. It can also be explained as a limited ability to see the "big picture" or understand the context of something and it was originally proposed as a central disturbance in Autistic Spectrum Disorder (ASD) (Uta Frith, 1989; F. Happe & Frith, 2006). More recently, it has been suggested that weak central coherence could be considered as part of the neuropsychological profile of ED and possibly play a role in the development and maintenance (I. C. Gillberg et al., 2010). Gillberg and colleagues raised the possibility that there could be some overlap between ASD and AN after finding high prevalence of ASD and ASD traits in a cohort of patients with AN who had been followed for over 20 years (C. Gillberg & Råstam, 1992; I. C. Gillberg, Råstam, & Gillberg, 1994; Wentz, Gillberg, Gillberg, & Rastam, 2001). As with other neurocognitive functions there is a need to study not only the overall construct but the different domains that load onto the construct (such as visuospatial, verbal, auditory). In 2008 Happe and Booth (F. G. Happe & Booth, 2008) re-examined the concept of central coherence and concluded that it consisted of two dimensions: (1) global integration and (2) detailed or local information processing. Various tasks have been used in order to investigate the construct such as the Rey-Osterrieth Complex Figure Test (ROFT) (Rey, 1941), Group/Embedded Figures

Test (EFT/GEFT) (Witkin, Oltman, Raskin, & Karp, 1971) and Object Assembly (OA, Wechsler 1974 , 1981).

Unlike set-shifting the study of central coherence in ED has not been as developed. In 2008 Lopez and colleagues (Lopez, Tchanturia, Stahl, & Treasure, 2008) published a systematic review that found consistent evidence of difficulties with global integration but not a bias towards local processing. Since then, further studies have been conducted. Lopez and colleagues (Lopez, Tchanturia, Stahl, Booth, et al., 2008) found that patients with AN show a superior detail focused processing at the expense of global processing, a similar pattern to those of patients with high functioning ASD and Asperger's. They also found that OCD traits were also associated with poorer central coherence but did not find an association between performance in tasks and measures of anxiety or depression. Importantly, they did not find any correlation with Body Mass Index (BMI) suggesting the central coherence could be a trait independent of starvation processes. In 2014, Lang and colleagues (Lang, Lopez, Stahl, Tchanturia, & Treasure, 2014) published a meta-analysis which included 12 studies using three different tasks to measure central coherence (ROFT, G/EFT and OA) confirming weak central coherence in patients with ED. The strongest evidence came from the studies using G/EFT with a medium effect of (d = -0.51) and all studies included finding a bias towards detailed processing across all ED subtypes. Inefficient global processing was demonstrated by studies using the ROFT with a medium effect size (d = -0.62). A recent large study (N = 984) by Lang and colleagues (Lang, Roberts, et al., 2016) (with data drawn from previous studies from the same group) found poorer performance in global processing across all ED subtypes, however, they did not find any differences in performance between recovered AN and HC subjects. Interestingly, the study did yield significant results for poorer central coherence in unaffected sisters of patients with AN which is surprising given that this meant that recovered AN patients performed better than unaffected sisters. One possible explanation is that some of the recovered patients would have received Cognitive Remediation Therapy (CRT) as a treatment package which involved cognitive training as well as behavioural strategies for weight gain. Although this is a hypothesis, it is supported by studies showing cognitive improvement after CRT (K. Tchanturia, Giombini, Leppanen, & Kinnaird, 2017; K. Tchanturia, Lounes, & Holttum, 2014).

Studies have also found similar results after recovery (A. Harrison, Tchanturia, & Treasure, 2011; Lopez, Tchanturia, Stahl, & Treasure, 2009; Roberts, Tchanturia, & Treasure, 2010;

Tenconi et al., 2010) and in first degree relatives of patients with AN (N. Kanakam et al., 2013; Roberts, Tchanturia, & Treasure, 2013; Tenconi et al., 2010). Thus supporting the hypothesis of weak central coherence as an endophenotype for ED.

1.6.4. Social and emotional processing

Difficulties in emotional processing have been proposed to underpin many psychological disorders (Berenbaum, Raghavan, Le, Vernon, & Gomez, 2003), and together with social functioning they have been theorised to play an important role in the development and maintenance of ED (A. Oldershaw et al., 2011; Anna Oldershaw, Lavender, & Schmidt, 2018; Schmidt & Treasure, 2006). Poorer social and emotional processing have also been shown to be associated with greater illness chronicity (A. Harrison, Tchanturia, Naumann, & Treasure, 2012) in these patients. Both emotion generation and regulation as well as social functioning have been studied in patients with ED and found to be altered.

In recent years, there has been an interest in understanding the role that emotional regulation plays in development of ED. One hypothesis is that patients with ED may have deficits in emotion regulation and therefore lack the skills to cope with negative affective states, which can lead to the use of strategies for weight control (excessive exercise, binge or purge behaviours) (Peñas-Lledó, Vaz Leal, & Waller, 2002; Smyth et al., 2007). While this model may have been developed focusing on binge/purge disorders, it has been suggested that problems with emotion regulation might be present across all diagnoses. In Fairburn, Cooper and Shafran's 2003 transdiagnostic model (Fairburn, Cooper, & Shafran, 2003), they suggested that mood intolerance could be a process involved in maintenance of ED in general.

Evidence for difficulties in emotional regulation in ED comes from self-reported studies, several of which have focused on alexithymia (difficulty identifying and describing one's own emotions). The majority of studies have found higher levels of alexithymia in patients across ED diagnoses when compared to healthy controls (Brockmeyer et al., 2014; Eizaguirre, Saenz de Cabezón, Alda, Olariaga, & Juaniz, 2004; Nowakowski, McFarlane, & Cassin, 2013). In fact, the review by Nowakowski and colleagues (Nowakowski et al., 2013) suggests that there is a strong evidence that alexithymia is not simply a product of ED symptomatology and may play a role in its development.

Research in emotion recognition (the ability to recognise facial emotions) has yielded inconsistent findings in ED. While several authors have reported deficits in patients with ED (A. Harrison, Sullivan, Tchanturia, & Treasure, 2009; Kucharska-Pietura, Nikolaou, Masiak, & Treasure, 2004; Legenbauer, Vocks, & Ruddel, 2008; Pollatos, Herbert, Schandry, & Gramann, 2008), others have not (H. Kessler, Schwarze, Filipic, Traue, & von Wietersheim, 2006; Mendlewicz, Linkowski, Bazelmans, & Philippot, 2005). In recent years, two meta-analyses of emotion recognition have highlighted these discrepancies in the literature and showed that differences are often small. Evidence with regards to difficulties in emotion recognition in patients with ED, comes from studies using a range of tasks, including experimental paradigms where patients have to recognise emotional state in pictures of faces presented to them (Kucharska-Pietura et al., 2004). As shown in the previous paragraph, patients with ED have higher levels of alexithymia which has been proposed to also explain difficulties in emotion recognition (Brewer, Cook, Cardi, Treasure, & Bird, 2015). This hypothesis could shed some light into inconsistencies in findings in emotion recognition literature in ED, suggesting that difficulties in emotion recognition might not be explained by the ED, but by co-occurring alexithymia. Further investigation is needed in order to explain the differences in the literature. Interesting differences in patterns of recognition depending on the type of emotion have been identified in studies, although this needs further exploration. Furthermore, dysfunctional aspects such as misinterpretation might be important in this group of patients (Wyssen et al., 2019). Research into Event Related Potentials (ERPs) suggests that patients with BN may use increased cognitive effort in order to evaluate facial expressions (Kuhnpast, Gramann, & Pollatos, 2012), which they hypothesise could lead to difficulties in social interaction (as they require more effort) and in turn lead to increase social withdrawal.

Differences in emotion recognition have not consistently been found in recovered AN patients leading to the hypothesis that difficulties in emotion recognition might be an effect of the acute stage of the disorder (it is well known the severe cognitive consequences that starvation can play) rather than play a role in its development (A. Oldershaw, Hambrook, Tchanturia, Treasure, & Schmidt, 2010). However, there are still reasons to hypothesise that social cognitive deficits that require not just recognition of emotions but more complex cognitive processes required for social interactions (similar to those present in ASD) may be trait like features in AN. Evidence for this comes from premorbid social deficits and social phobia in ED (Godart, Flament, Lecrubier, & Jeammet, 2000; W. H. Kaye, Bulik, Thornton, Barbarich, & Masters, 2004; Schaumberg et al., 2019; J. Swinbourne et al., 2012), and studies using tasks assessing theory of mind (ability to attribute mental states

to oneself and others and that this can be different from one's own), as well as increased prevalence of ASD diagnoses/traits in patients with AN. A recent meta-analysis and systematic review (N = 30 studies) of emotion generation and regulation in AN, concluded that patients with AN have difficulties in their ability to regulate their own emotional state and understand the emotional states of others (A. Oldershaw et al., 2011). Oldershaw and colleagues investigated 5 constructs of social emotional processing (first developed by Ochsner (Ochsner, 2008)) and found alterations in patients with AN in affective values (heightened sensitivity and bias or avoidance of threatening disorder specific emotional stimuli), in recognition of emotions in others, poor theory of mind (high level mental interference) and lastly some difficulty in regulation of emotional responses. A meta-analysis of theory of mind in ED (n=15 studies) concluded that AN was associated with significant deficits in theory of mind while small sized deficits were also present in patients with BN and in those recovered from AN (E. Bora & Köse, 2016). The majority of studies used reading the mind in the eyes test (Baron-Cohen, Wheelwright, Hill, Raste, & Plumb, 2001) to assess theory of mind. The test assesses decoding of affective stimuli, which is not only important for emotion recognition but also the first step of inferring complex social emotions. Meta-regression analyses showed a significant relationship between low BMI and theory of mind scores suggesting that difficulties in theory of mind may be a consequence of starvation effects (E. Bora & Köse, 2016), this however did not explain the difference found in recovered patients. This poses a recurrent question regarding state or trait related characteristics of ED. While the relationship between BMI and theory of mind points to an effect of starvation it can also represent a worsening of an existing deficit as deficits in theory of mind were also found in recovered patients and patients with normal BMI. Interestingly, Oldershaw and colleagues found that even though recovered patients overall performed similarly to HC in a theory of mind task, when examining by task, recovered patients performed equal to currently ill patients in a task that required recognizing positive emotions (A. Oldershaw et al., 2010).

Some alterations in social emotional processing have in fact been demonstrated to be present both premorbidly as well as during the acute phase of the disorder leading to questions with regards to the influence that these social emotional aspects may have in the development of ED. In recent years aspects of social cognitive functioning in patients with ED has been largely studied, leading to findings of an over representation of Autism Spectrum Disorder (ASD) traits in patients with AN (Heather Westwood & Tchanturia, 2017). Both group of patients shared underlying difficulties with both central coherence and set-shifting tasks (H. Westwood et al., 2016) as well as increased levels of anxiety

and difficulties in aspects of social cognition (Zhou, McAdam, & Donnelly, 2018) (further details on the overlap between ASD and AN will be discussed in Chapter 3).

Overall there is some evidence of altered social and emotional processing deficits in patients with ED although the role that starvation plays in these deficits is not clear. Of course, little evidence clarifies if social emotional difficulties are present before the onset of the disorder and therefore could contribute to its development. Different strategies have been used to support the hypothesis that social functioning alterations in ED are premorbid difficulties rather than an artefact of the ill state or a manifestation of malnutrition. Some studies have looked at retrospective reports of childhood social functioning and comorbidity with childhood diagnoses defined by social deficits (such as separation anxiety, social phobia or ASD) and have found that difficulties predated the onset of ED (W. H. Kaye et al., 2004; Råstam, 2008; Silberg & Bulik, 2005).

1.7. Interim conclusion

ED are complex mental health disorders with the highest mortality rate of any other mental health disorder despite having a relatively low prevalence. They have an early onset in early adolescence (childhood in some cases) and if not treated promptly, can become chronic and last over 20 years. ED have a devastating effect for both the patient and the family. Furthermore, there is a stigma associated with the development of the disorder, which has an effect on treatment seeking and hinders patients' chances to recover. Although, as said previously, the aetiology is not well understood, we know that bio-psycho-social factors play a role in the development of ED. In more recent years, the exploration of the neurocognitive and socio-emotional profile of patients with ED has led to hypotheses of how alterations in cognitive processing can play a role in the maintenance and/or development of the disorder.

Chapter 2. Neuroimaging in Eating Disorders

2.1. Chapter overview

Magnetic Resonance Imaging (MRI) is a safe and non-invasive technique developed in the 1970s and 1980s. The following section (2.1.) describes the basic principles of MRI physics and has been referenced from selected textbooks. After introducing the principles of MRI this Chapter will provide an overview of neuroimaging findings in patients with ED.

While the current thesis will also explore white matter alterations in children at risk, to date most studies have been conducted using small samples and using at least two different approaches to analysing the findings resulting in mixed results. No meta-analysis of white matter alterations in ED has been published and therefore Chapter 7 will focus on meta-analysing these data and providing an overview on the current literature. Thus this chapter will only cover findings in volumetric and functional findings in ED.

2.2. Basics of MRI

MRI stands for Magnetic Resonance Imaging and is an imaging method which uses a powerful magnetic field (B_0) to align the magnetization of hydrogen protons found in water content of tissue and radio frequency fields to systematically alter the alignment of this magnetization.

An MR machine consists of the following components:

- Magnet: a large magnet that generates the magnetic field. The strength of the magnet is measures in Teslas (T), and with a higher strength the image resolution improves.

- Shim coils: in order to make the magnetic field as homogenous as possible.

- Gradient coil: provide spatial localization of the signals. Three sets of coils are used in the x, y and z directions.

- Radiofrequency (RF) coil: which transmits a radiofrequency signal into the body part being imaged.

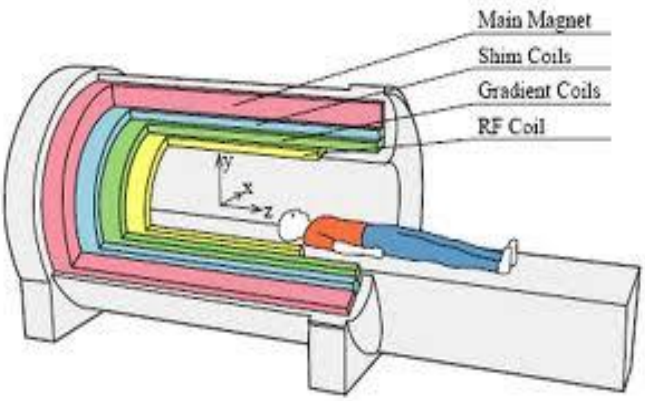


Figure 2.1. MRI components diagram

2.2.1. Origin of MR signal

On average, a human body is formed by 75% water. Water contains hydrogen protons containing an electron which rotates (precess) in its own axes and is positively charged. These hydrogen protons get aligned with the main magnetic field in the MRI in two possible ways: in the direction of the magnet (spin-up or parallel) or against it (spin-down or anti-parallel). The direction in which the proton aligns itself is dependent on how much energy it has, with the anti-parallel direction requiring more energy than the parallel one. The rate of precession is directly proportional to the strength of the local magnetic field (B_0) and can be determined by the Larmor equation:

$$\omega_0 = \gamma B_0$$

Where frequency (ω) is the frequency of the spins, Ɣ is the gyro-magnetic ratio (a property of the nucleus, for hydrogen protons the value is 42.56 $mHzT^{-1}$) and B_0 is the magnitude of the magnetic field strength. At different T, the Larmor frequency of the hydrogen protons changes: at 3T, protons precess around the main magnetic field at 127 MHz. See figure 2.2.

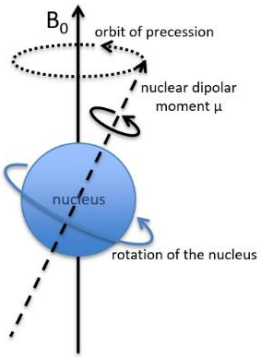


Figure 2.2. Proton precession. Protons precess around the axis of the main magnetic field (B_0) at the Larmor Frequency, which at 3T scanner is 127MHz.

The MR images are produced using pulse sequences which contain radiofrequency (RF) pulses and gradient pulses. When an RF pulse is introduced (using a coil which produces an oscillating magnetic field) the protons absorb the energy and spin together, which tips the magnetization out of equilibrium into the transverse plan. By doing this we are then able to record the signal.

In order to get the maximum signal, the oscillating magnetic field applied needs to tip the magnetisation into the transverse plane. The RF pulse creates a magnetic field which oscillates at the Larmor frequency and is perpendicular to B_0. The necessary flip angle (α) is given by the following equation:

$$\alpha = \gamma B_1 t_p$$

Where B_1 is the strength of the RF magnetic field and t_p is the duration of the pulse. Therefore, the strength of the pulse can be changed to create different flip angles.

When the RF pulse is removed, the transverse magnetization decays away exponentially as the spins dephase with respect to each other and return to equilibrium (this is called relaxation).

2.2.2. Relaxation time and contrast

There are two main features of relaxation: a dephasing of the spins following the RF pulse and the realignment of the spins along the transverse plan as they lose their energy which they had absorbed by the RF pulse.

T1 relaxation (spin-lattice relaxation or longitudinal relaxation) refers to the exponantial growth of the longitudinal magnetization. During T1 relaxation, the spins return back into their low energy state, back to equilibrium in the main magnetic field B_0.

T2 relaxation (spin-spin relaxation or transverse relaxation) refers to the process in which the protons get out of phase with each other, they stop spinning together, causing a loss of magnetisation in the transverse plan.

Relaxation are important parameters that reflect the tissue environment. Protons in our body have different local environments, some associated with free water molecules and some fixed in position. Different protons have characteristic differences in their T1 and T2 relaxation. Therefore, in order to accentuate and measure these differences, we can change how quickly we place the RF pulses (T_R or Repetition time) and how quickly we choose to listen to the return signal after the first excitation RF pulse (T_E or Echo time). These two parameters are going to determine the contrast in the image. Changing the T_R is going to affect the contrast between tissues with different T1 relaxation times, whilst changing the T_E will affect contrast between tissues with different T2 relaxation times.

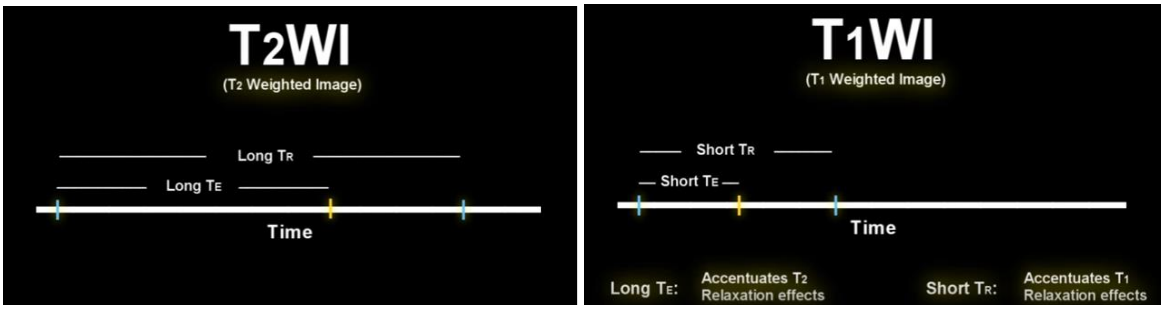


Figure 2.3. Differences in T_R and T_E for different weighted images.

Proton density (PD) represents the number of hydrogen atoms in a particular volume, for example, fluids such as cerebral spinal fluid (CSF) and blood have higher PD than tendon and bone. Each tissue is going to have a different PD, T1 and T2, and these three factors are going to determine the signal (Figure 2.4).

	T1 (ms)	T2 (ms)	PD
White matter	1080	70	0.61
Grey matter	1820	100	0.69
CSF	3817	1442	1.0

	TE	TR
PD	Short	Long
T1	Short	Short
T2	Long	long

Figure 2.4. Relaxation times of various tissues at 3T. Combinations of T_E and T_R to acquire different weighted images(Macintosh & Graham, 2013).

2.2.2.1. T1- weighted image

The contrast in these scans is provided by variations in T_1 relaxation time (transfer of absorbed RF energy from proton spins ot the lattice which results in the protons returnin to their relaxed energy state). Tissue with densley-packed protons have short T_1 relaxtion times, hwile areas with less densley-packed protons have much longer T_1 relaxation times. In order to enhance the differences between tissues, both T_R and T_E are kept short. A short T_R means that not all of the protons have relaxed back to the z-axis before the next T_R is initiatite. Therefore, only protons in tissues with short T_1 relaxation times will have had time to fully relax ans will form a strong signal in the following T_R; tissues with long T_1 relaxation times will forma weak signal in the next T_R. Fluids in T1-weighted images will appear dark, while water-based tissues will apear mid-gray and fat-based tissues will appear very bright (Figure 2.5.). T1-weighted scans are therefore usually used for anatomy scans as they show the boundries bewteen tissues very well.

2.2.2.2. T_2–weighted image

These scans are generated by differences in T_2 relaxation times (transfer of absorbed RF energy from one proton spin ot the neighbouring spins which causes dephasing of the proton spins). This occurs faster in tissues which are densely packed with protons. The MR signal is weaker in short T_2 tissues and stronger in long T_2 (which haven't undergone as much T_2 relaxation time). T_2-weighted scans are generated using long T_E and long T_R. In these scans, fluids appear bright (CSF), whilst water and fat-based tissues apear mid-gray (Figure 2.5.).

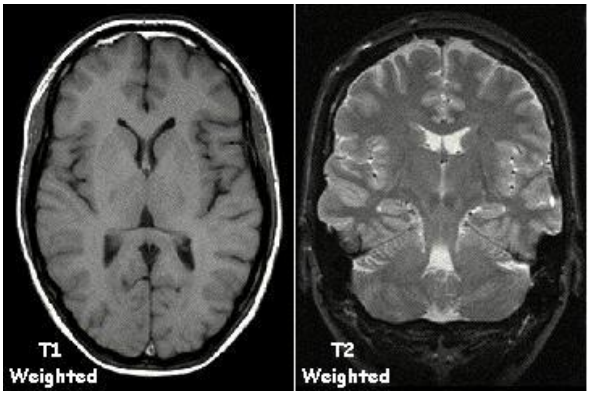


Figure 2.5. T1 and T2 – weighted images.

2.2.3. Spin echo and Gradient echo

There are two principle types of pulse sequences, spin echo (SE) and gradient echo (GE). Both SE and GE start with a 90° RF pulse that produces the magnetisation in the transverse plan. After this, both sequences differ in that for a SE sequence, after the initial RF pulse and subsequent magnetisation decay, a 180° RF pulse is then applied, whilst for the GE sequence, a negative field gradient is applied immediately after the initial RF pulse, causing a rapid dephasing of the spins. SE sequences use two RF pulses and are used to create T1, T2 and PD-weighted images depending on the choice of T_E and T_R (see Figure 2.4). These sequences generally produce better images but take a longer time to run.

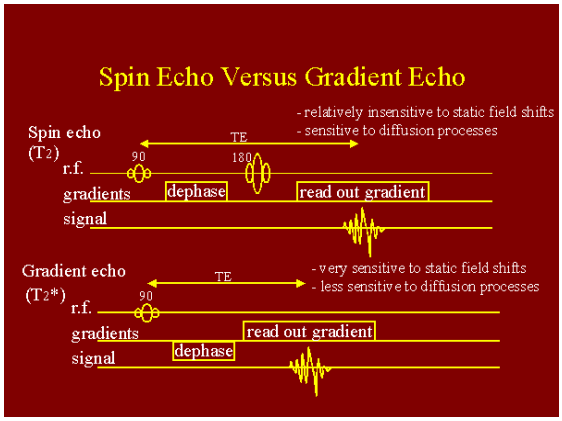


Figure 2.6. Spin Echo versus Gradient

2.2.4. Generating MR scans and quality

The MR signals which are spatially-encoded are obtained from each readout of an MRI scan and digitised. They are then stored in a matrix form (K-space) which is then subjected to a Fourier Transformation which produced the final image.

MRI scans are saved by the scanner in digital imaging and communications in medicine (DICOM) format. In this format, the image is stored in conjunction with the details of the scan, such as the date and time. DICOM data is converted to neuroimaging informatics technology initiative (NIfTI) format prior to analysis in order to maintain privacy and facilitated blinded data analysis.

The quality of each MRI scan must be considered and will be important in order to obtain useful structural and functional information. There are several factors to take into consideration:

2.2.4.1. Signal-to-noise ratio

The signal to noise ratio (SNR) si the strength of the MR signal in each voxel relative to any noise confounding the signal. This noise can be induced by two main factors: molecular movement (motion of electrically-conducting tissues within a magnetic field generate electrical currents with an associated fluctuating magnetic field which is picked by the RF coils) and electrical resistance (fluctuations in electrical current caused by RF coils). Low SNR will result in grainy MR images limiting it's usefulness.

To increase SNR we can either reduce the level of noise or increase the MR signal. One way of reducing noise is to use small coils or an array coil that focuses on an area of interest. The magnet strength can also be increased (e.g. from a 1.5T to 3T) however this is expensive.

2.2.4.2. Spatial resolution

Spatial resolution is the number of voxels that are utilised in the creation of the MRI image (a higher number of voxels means higher resolution). The field of view (FOV) is the total area scanned and is fixed for a study. A FOV that is large enough to cover a multitude of patients (with different body masses) has to be selected. The spatial resolution is defined by the size of the voxels in the image: smaller voxel size means that a greater number of voxels can fill the FOV and therefore will incur in greater spatial resolution.

2.2.4.3. Artefacts

Artefacts are unwanted features on an MRI. A large number of different artefacts can occur during a scan acquisition; however, the most common ones are due to patient motion, magnetic field inhomogeneity or image processing anomalies.

2.3. Diffusion MRI

In the last thirty years Diffusion MRI has become an important modality in radiography to help in-vivo imaging of ischemia and has more recently been used as a research tool to understand white matter microstructure and directionality. Diffusion MRI has become a tool to help understand both cognition and disease.

2.3.1. Basics of Diffusion MRI

The thermal energy carried by molecules results in a random movement of molecules, also called Brownian motion, which move on average over distances around 10 µm (Le Bihan et al., 2001). Molecules travel randomly in space over a distance that is described by the diffusion coefficient (D). This coefficient is dependent on the size of the molecules, the temperature and the nature of the medium.

$$X^2 = 2DT_d$$

Where X^2 is the average mean-squared diffusion distance along one direction and T_d is diffusion time. Free water molecules diffuse in water at 37°C and the diffusion coefficient

for them is 3.10^{-3} $mm^2.s^{-1}$. However, in biological tissues compared to free water, the diffusion coefficient is no longer Gaussian and water molecules move in a different way, bouncing and interacting with the tissue. Therefore, whilst a short diffusion time would reflect the local intrinsic viscosity, a longer diffusion time reflects the presence of obstacles. Therefore, the observation of the diffusion distribution in tissue provides an insight into the microstructure of the neural tissues in which molecules move.

2.3.2. Diffusion-Weighted Imaging (DWI)

Since the human brain does not consist of free flowing water, but also has structures such as axons, myelin and glial cells, the mobility of the water molecules is affected and varies depending of the tissue in which they are in.

The apparent diffusion coefficient (ADC) measures the diffusion of water molecules within each voxel. In areas where there is little hindrance to the movement, water has an isotropic diffusion. The water molecules movement is unrestricted and they can move in any direction, diffusion is isotropic in CSF (where there is high diffusivity). Therefore, the directionality of water diffusion in a tissue arises from its microstructural organisation.

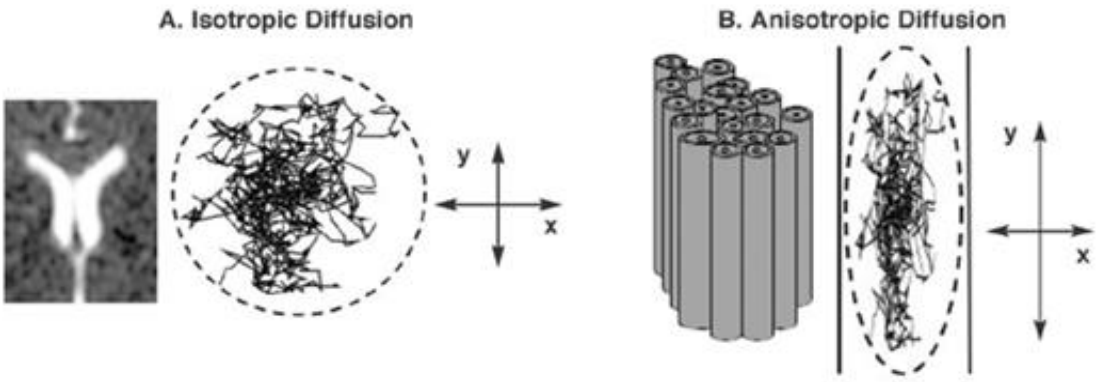


Figure 2.7. Diffusion characteristics in different tissues. Diffusion is influenced by the structure of the tissues, restricting mobility of water molecules. In isotropic diffusion such as in the CSF (A), molecules can move in all directions; in anisotropic diffusion, such as white matter fibre tracts (B), axonal membranes and myelin restrict the diffusion of the water molecules (anisotropic).

For diffusion weighted images we start with a T2 weighted spin-echo sequence where the sequences are made sensitive to diffusion by adding diffusion gradients in certain

directions after the first 90° RF pulse and after the 180° rephasing pulse. The spins of the water molecules are dephased by the first gradient and rephrased by the second. However, the spins of the water molecules that have moved in the direction of the gradients during the interval (between gradient applications), will not be rephased by the second gradient. Therefore, the faster the water molecules move, the more dephased and less signal will be recorded.

2.3.3. Diffusion Tensor Imaging (DTI)

The diffusion measurement only detects water motion along the applied gradient axis, therefore fibre orientations are estimated from diffusion measurements along the X, Y and Z axes. However, fibre orientation is not always along this axes and therefore these measurements are not enough. In order to solve this issue, the concept of Diffusion Tensor Imaging (DTI) was introduced in 1990s (Basser, Mattiello, & LeBihan, 1994). The measurements along the axes are fitted into a 3D ellipsoid whose properties: length of the longest, middle and shortest axes (called eigenvalues λ_1, λ_2 and λ_3) as well as their orientations (called eigenvectors V_1, V_2 and V_3) can be defined by six parameters. Therefore, ADC measurements along these six axes are enough to calculate the ellipsoid. A 3x3 symmetric matrix called tensor is used to convert the measurements to these six parameters, hence the name “diffusion tensor imaging”.

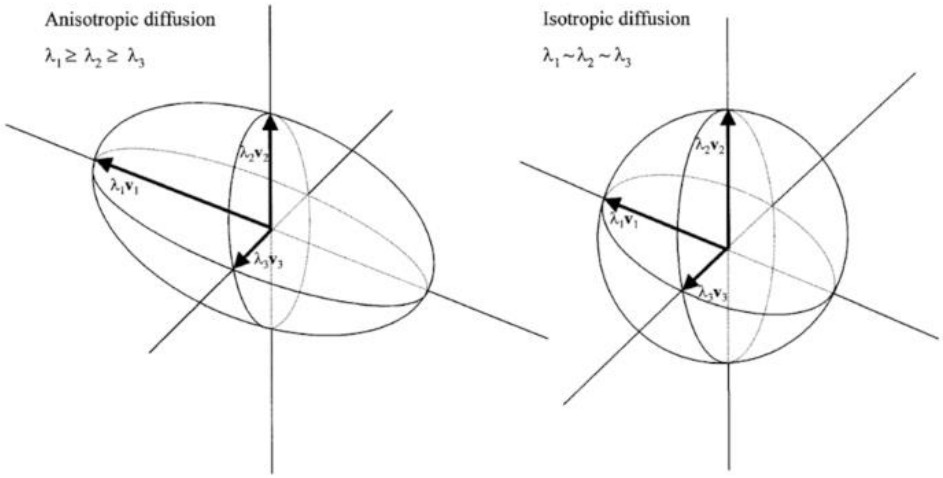


Figure 2.8. Anisotropic vs Isotropic diffusion

From the diffusion tensor model we can calculate various quantitative metrics. The mean diffusivity (MD) can be calculated as a mean of the three eigenvalues and it represents the average water molecular displacement.

2.3.3.1. Fractional anisotropy (FA)

The degree of anisotropy (extent of the directionality of the diffusion tensor) can be qualified using the above measure FA. FA is a scalar value calculated from the tensor eigenvalues.

$$\mathrm{FA} = \sqrt{\frac{3}{2}} \frac{\sqrt{(\lambda_1 - \hat{\lambda})^2 + (\lambda_2 - \hat{\lambda})^2 + (\lambda_3 - \hat{\lambda})^2}}{\sqrt{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}}$$

FA is one of the most used measures of diffusion and its values range from 0 (entirely isotropic) to 1 (entirely anisotropic). FA is therefore used as a measure of white matter microstructure. The white matter part of the brain is anisotropic because of the long thin structures of the axon which align and form tract bundles with a directed structure. FA values in WM therefore tend to range between 0.2 and 0.8, with a higher value reflecting better coherence of the structure. Healthy WM tissue has a large number of axons which helps diffusion of water in one direction and therefore contributes to a high FA. However, damaged WM is likely to have fewer axons. This reduced number contributes to water diffusing less restrictively and therefore FA is reduced. Damaged axons also permit greater perpendicular diffusion and therefore can lead to lower FA. This means that FA is a good measure of health or coherence of WM.

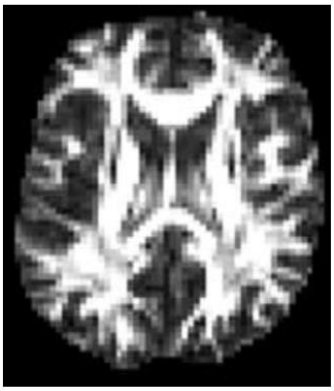


Figure 2.9. FA map. FA values for the diffusion in each voxel can be seen in an FA map, with voxels with higher FA value showing greater intensity in the map, and those with lower FA showing a low signal intensity in the map.

2.3.3.2. Mean Diffusivity (MD)

Mean diffusivity provides a measure of the amount of water diffusion happening in a specified area. MD is independent of the direction of the water. Low values for this measure reflect low movement of water in a voxel, whilst high values reflect high movement of water within a voxel. Low movement is due to water being restricted by tightly packed cells or myelinated membranes, whilst increased movement can be due less cell density or larger axon diameter. Conversely to FA values, a higher MD is therefore indicative of less packed WM structure.

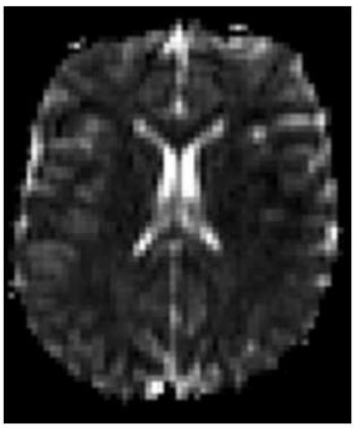


Figure 2.10. Mean diffusivity map. MD values for diffusion in each voxel can be seen in the map, with voxels with higher MD showing a greater intensity in the map and voxels with lower MD less signal intensity.

2.3.4. Analysis methods of diffusion data

DTI scans can be used to obtain information regarding the microstructure of tissues with an inherent directionality, such as the white matter of the brain. There are two common ways of analysing DTI data: a region-of-interest (ROI) or voxel-based approach across the whole brain and tractography.

2.3.4.1. Region-of-interest/voxel-based analysis

Both approaches look at measures of FA and MD within each voxel of a DTI scan and compares them between groups. Methods such as voxel-based morphometry (VBM) (Wright et al., 1995) and tract based spatial statistics (TBSS) (S. M. Smith et al., 2006) compare voxel by voxel between groups and provide precise localisation and identification

of abnormalities in the microstructure of WM. However, both methods are limited by the need of scans from multiple participants, which must be registered and aligned perfectly.

A collection of voxels can also be grouped together to form a ROI from which we can average their FA and MD values and then compare this between subjects. ROI's can be defined manually or using automated software. The ROI approach is not free from limitations and is dependent on the accuracy of the segmentation and restrict analysis to specific regions on the basis of a priori predictions.

2.3.4.2. Tractography

The WM axons in the brain form tracts which structurally connect regions of grey matter to one another. Information is transferred between brain regions along the WM tracts using action potentials which enable different areas of the brain to interact and operate coherently. The restrictions placed by the directionally-restrictive structure of the WM tracts means that we can assume that the path of greatest diffusion occurs parallel to the tract (where diffusion is less hindered). Tractography aims to reconstruct the tracts connection ROIs along the principal diffusion direction. During the DTI data processing the PDD within each voxel is recoded and this represents the path of greatest diffusion. Figure 2.11. shows a colour coded representation of the principal diffusion direction of each voxel as well as the colour label definition.

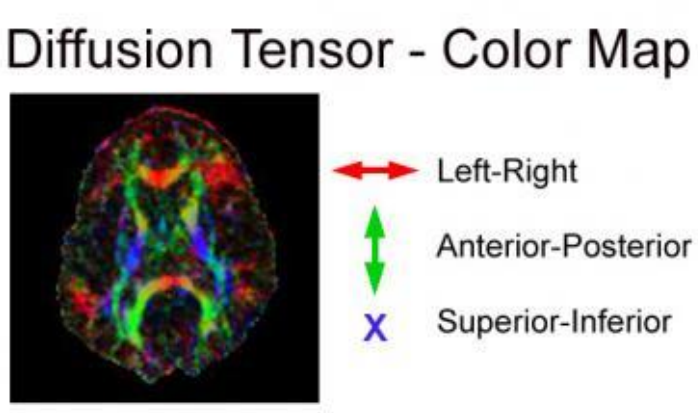


Figure 2.11. Diffusion Tensor colour map.

The figure shows a diffusion tensor map including the principal diffusion direction of each voxel and the colour representation.

There are two tractography methodologies: deterministic and probabilistic. In the first one, the principal diffusion direction of each voxel is used to determine the lines of connections

across voxels which form the pathway of the greatest diffusion. DTI tractography uses the orientation of water diffusion (diffusion orientation density function, dODF) as a proxy for the true WM fibre orientation known as the fibre ODF. Therefore, it estimates the most likely fibre orientation, which carries an intrinsic grade of uncertainty. In order to reduce some of the uncertainty, probabilistic tractography uses repeated sample in order to obtain the probability of different white matter pathway and weighing each of them based on its probability.

There are limitations to both methodologies, false positives can arise in probabilistic tractography due to the inclusion of a range of likely pathways while deterministic tractography is often associated with false negatives because it doesn't take uncertainty into account.

2.3.5. Limitation of the diffusion tensor model

There are limitations to the use of the diffusion tensor model which were identified early on, the main one being how strongly it is affected by crossing fibres (which are present in 60% – 90% of WM) (Farquharson et al., 2013; Jbabdi, Behrens, & Smith, 2010; Jeurissen, Leemans, Tournier, Jones, & Sijbers, 2013; Jones, Knösche, & Turner, 2013). Basser and Pierpaoli, identified how their proposed measure of anisotropy was highly dependent on the degree of coherence of fibre tract directions (Basser & Pierpaoli, 2011). Nonetheless, the ability to study orientation information from living biological tissues in a non-invasive way has made DTI one of the main tools in modern neuroimaging and has been successfully used for both clinical and research purposes (Horsfield & Jones, 2002). However, with the development of new techniques the limitations of the tensor model have become more apparent and ways of overcoming some of the tensor model limitations have been developed in the last few years. A number of techniques have been proposed: multi-tensor fitting (Tuch et al., 2002), Q-ball imaging (Tuch, 2004), ball and sticks (Behrens, Berg, Jbabdi, Rushworth, & Woolrich, 2007) and constrained spherical deconvolution (Dell'Acqua et al., 2007; Dell'acqua et al., 2010; Dell'Acqua & Tournier, 2019; J. D. Tournier, Calamante, & Connelly, 2007; J. D. Tournier et al., 2008).

There are however limitations to the use of these new methods and many studies remain reliant on diffusion tensor model to provide microstructural measure (e.g. fractional

anisotropy, raid and axial diffusivities). This is due to the new fibre estimations methods relied on the assumption that white matter fibres do not vary in their diffusion properties between different tracts. The assumption is that any observed differences in the tensor-derived measures are a result by partial volume effects (e.g. crossing fibres).

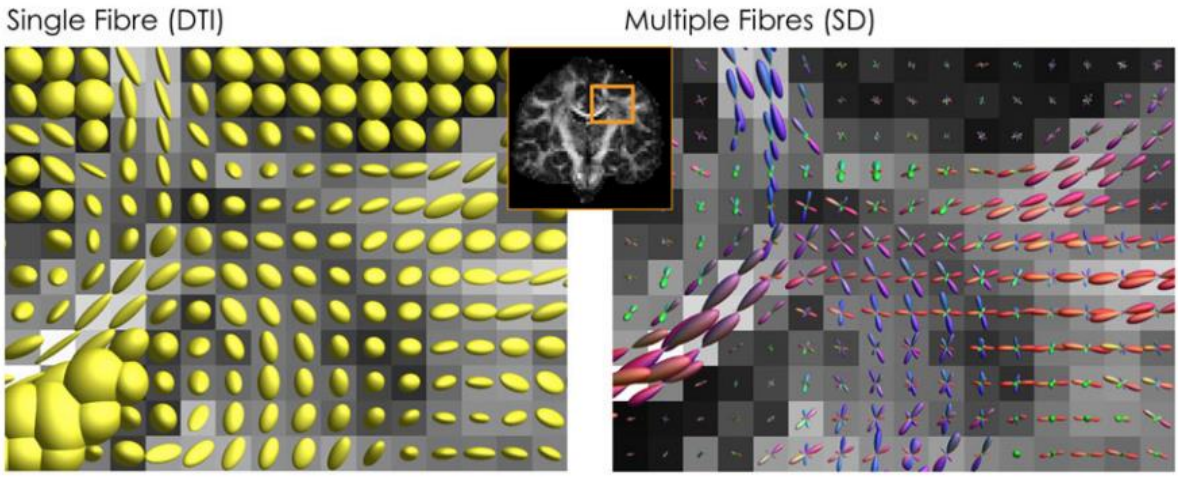


Figure 2.12. Example of modelling of the diffusion signal. Left: diffusion tensor model provides information for a single fibre orientation. Right: multiple fibre orientations can be visualised using a multi-fibre approach.

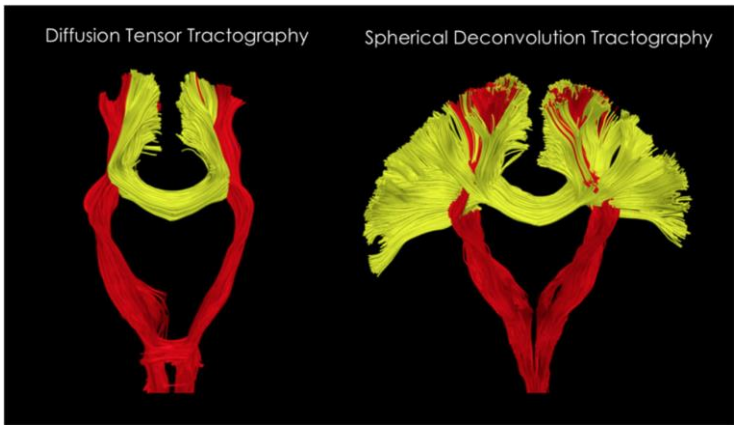


Figure 2.13. Example of DTI and spherical deconvolution tractography of the corpus callosum. In the example, the lateral projections of the corpus callosum are lost using the DTI model because they are interrupted by more dominant components of the corticospinal tract.

For the purpose of this thesis only constrained spherical deconvolution will be described as it is the method used in this thesis.

Constrained Spherical Deconvolution (CSD) (J. D. Tournier et al., 2007) estimates a white matter fibre Orientation Distribution Function (fODF) based on an estimate of the signal expected for a single-fibre white matter population (response function). This is used as the kernel in a deconvolution operation to extract a white matter fODF from dMRI signal measured within each voxel. For the white matter, the response function models the signal expected for a voxel containing a single, coherently oriented bundle of axons (J. D. Tournier et al., 2007; J. D. Tournier, Calamante, Gadian, & Connelly, 2004). In case of multi-tissue variants of spherical deconvolution, response functions for other tissue types are introduced as well; typically to represent grey matter(-like) and/or CSF(-like) signals (Jeurissen, Tournier, Dhollander, Connelly, & Sijbers, 2014).

2.4. Neuroimaging findings in Eating Disorders

Brain imaging provides a way of understanding the living human brain and may help elucidate neural mechanisms that are related to ED pathophysiology. Given the complex interplay of psychosocial and neurobiological abnormalities in ED, the study of neural mechanisms relevant to ED has been a complex one. Over the last few decades, a number of neuroimaging tools have been developed allowing us to further our understanding of neural mechanisms in ED and other psychiatric disorders. As seen in earlier sections, MRI is commonly used to study brain gray and white matter volumes. With the development of neuroimaging tools, we are now able to study measures of cortical thickness, surface area and with the development of DTI we are also able to study white matter integrity. While the above provide an understanding of the 'make up' of the brain, functional magnetic resonance imaging (fMRI) (which measures changes in local blood flow and resulting deoxyhemoglobin levels during brain activation) allows us to understand 'function' in the brain both during tasks and while resting.

Imaging can further our knowledge of altered areas or networks based on ED phenotypes and neurocognitive research. Hypotheses have been based on wondering if disturbances of brain pathways are related to the modulation of feeding behaviours and reward? Or in

those that modulate factors related to body proprioception and thus may result in body image distortion? Or could there be alterations in pathways related to cognitive control?

This chapter will focus on summarising neuroimaging findings in ED that can guide the investigation in children at high risk for ED. While the methods used in this thesis focus on volumetric and white matter integrity, functional imaging research provides information on altered function that can help elucidate differences in structure. Therefore, this chapter will review the current state of functional research in ED as well as volumetric and white matter findings.

2.4.1. Structural/volumetric brain differences in patients with ED

A large number of studies investigating structural differences in patients with ED have been undertaken since the mid – 90's and structural changes have been observed in the majority of studies investigating patients with AN. However, very little research has been conducted in patients with BN and in general differences are not found between these patients and healthy controls. Recently, there has been a wider interest in studying both adolescents with a shorter illness duration and patients who are recovered with the aim of further understanding whether the differences observed in acute patients are a consequence of the (semi-) starvation during acute stages of the disorder or could reflect a possible endophenotype for the disorder.

A few systematic reviews and meta-analysis of structural differences in individuals with AN have been conducted. In 2013 a systematic review investigating the neurobiology of AN was published by Phillipou and colleagues (Phillipou, Rossell, & Castle, 2014). The review concluded that structural differences (reduced gray matter (GM) volumes and larger total CSF volumes) are observed in most studies of patients with AN, and that these differences are thought to reflect the effects of malnutrition. The review also included studies investigating specific brain structures and found reduced GM in several regions, particularly areas of the limbic system, including the amygdala, hippocampus and cingulate cortex (areas that have been proposed to be involved in the processing of emotions) (J. E. LeDoux, 2000).

A meta-analysis by Titova and colleagues (Titova, Hjorth, Schioth, & Brooks, 2013) including 7 studies and a total of 126 patients with AN and 120 healthy controls, was conducted in 2013 and found that acute patients with AN showed a global reduction in GM (effect size = -0.66) and WM (effect size = -0.74) as well as increased cerebrospinal fluid (effect size = 0.98). They also found a regional decrease in GM in left hypothalamus, left inferior parietal lobe, right lentiform nucleus and right caudate. The meta-analysis only included studies investigating structural differences using MRI and using a voxel-by-voxel technique known as voxel-based morphometry (VBM) (Titova et al., 2013) The authors used a meta-analysis technique called the activation likelihood estimation (ALE) used to estimate consistent regional brain changes across different imaging studies (Laird et al., 2005).

More recent meta-analyses of both acutely ill and weight recovered patients with AN have been conducted by Seitz and colleagues (Seitz, Buhren, Von Polier, et al., 2014; Seitz, Herpertz-Dahlmann, & Konrad, 2016). In the 2014 meta-analysis the authors calculated standardised global changes in GM, WM and CSF and compared results separately for acutely ill patients, weight-recovered patients (directly after weight-restoration, short term recovery) and long term weight-recovered patients. In total, 13 studies were included that reported changes in whole-brain volumes in acute AN patients (both adolescents and adults) with a total of 214 patients and 213 healthy controls. In these patients, the meta-analysis found that GM volume was on average reduced by 5.6% compared with HC; WM volumes were on average reduced by 3.7% and CSF was increased by 12.9% compared to HC. All results for acutely ill patients were significant. In total, 8 studies were identified that reported changes in whole-brain volumes in long term recovered patients with AN with a total of 177 patients and 195 controls. On average, the studies had a duration of weight recovery of 4.4 years. The meta-analysis found that GM tended to remain reduced by 1.0%, as well as WM by 0.7% and CSF tended to remain increased by 1.3%. The differences in long term weight recovered patients were lower than those found in the acutely ill group and did not reach significance. In the group of studies that investigated differences on patients with a short-term recovery (weight restoration), initial reductions in GM volumes were found to be restored by 43%, WM volumes were completely restored to normality and CSF increase was restored by 50%, when compared to initial changes in acutely ill patients. Both WM and CSF differences between acutely ill and weight restored were significant, however, GM deficit reduction did not reach significance. Interestingly, this meta-analysis showed that studies on adolescent patients with acute AN showed larger effects for changes in GM and CSF when compared to those of adults.

In their 2016 meta-analysis, Seitz and colleagues aimed to expand earlier findings as well as differences between adult and adolescents patients. This second meta-analysis included a larger number of studies, hence the power to detect differences was higher. In total, 22 studies calculating whole brain volumetric differences in acutely ill AN patients were found, of which 9 studied an adolescent population, with a total of 463 AN patients (297 adults and 166 adolescents) and 450 HC (304 adults and 146 adolescents). Global GM was found to be reduced by 4.6% (1% less than in their earlier meta-analysis), global WM was found to be reduced by 2.7% (1% less that in their earlier meta-analysis) and CSF was found to be increased by 14.2% (1.3% more than in their earlier meta-analysis). All were found to be significant. As in their previous meta-analysis, adolescents showed to have a more significant reduction of GM than adults (adolescents: -8.4% vs. adults: 3.1%, $p = 0.02$), both WM decrease and CSF increase was lower and higher respectively between adolescents and adults but the differences were not significant. Six studies in total were found reporting volumetric differences in short-term recovered AN patients, four of which studied an adolescent sample, with a total of 121 AN patients and 87 HC. Global GM was found to remain significantly reduced (3.6%, $p = 0.02$) and CSF significantly increased (9.3%, $p < 0.001$). Ten studies were found reporting volumetric differences in long-term recovered AN patients, only one of which studied an adolescent sample, with a total of 255 patients and 257 HC. At this stage, the meta-analysis did not find any significant volume changes, however, volumetric differences were still found between patients and controls in the same direction as before. (GM: -0.4%, $p = 0.34$; WM: -0.7%, $p = 0.33$; CSF: 1.0%, $p = 0.29$).

Overall, it has been well established that malnutrition plays a role in early findings of decreased volume in gray and white matter in patients with ED, with the reduction being mostly reversible with weight restoration (although small deficits cannot be ruled out as all volumes remain partially changed even after long term recovery) (Seitz, Buhren, von Polier, et al., 2014; Seitz et al., 2016; Wagner et al., 2006). However, although meta-analyses are powerful statistical ways of analysing published data, it is important to note specific findings as well. Although overall gray and white matter volumes have been found to be reversed after recovery it is important to note that during acute illness stages these volume alterations have been found to be correlated with neurocognitive deficits (McCormick et al., 2008; Seitz et al., 2016; Seitz et al., 2015), with increased desire for thinness (Joos et al., 2010) and linked to worse outcomes after one year (Seitz et al.,

2015). Therefore, we can't rule out the role that reduced volume plays at different stages of the illness or effects in chronic patients.

Differences in the reversibility of white vs. gray matter volume as well as differences between adults and adolescents are also important to consider. Although more research is being completed in adolescents, there is still less data available on long term recovered brain volumetric differences (Seitz et al., 2016). Preliminary analyses showed good reversibility of cortical gray matter reductions with remaining subcortical deficits (King et al., 2015) in adolescents. Adolescents also showed a greater initial deficit compared to adult patients. This might not be surprising given that the brain is continuing to mature during adolescence and therefore may be more susceptible to adverse effects of AN. While most studies have found a correlation between GM loss and low BMI, hypothesising that GM loss is then likely due to starvation.

The underlying mechanisms for the reduction in brain volume during acute stages remains unclear although various hypotheses have been studied such as fluid shifting from the intra- to the extracellular space (Artmann, Grau, Adelmann, & Schleiffer, 1985), loss of glial cells, or hormone alterations such as decreased leptin. A recent study by King et al showed that a volume reduction solely based on fluid shifts may be unlikely after analysing measures of serum and urine osmolarity (King et al., 2015). A recent study by Frintrop et al (Frintrop et al., 2019) explored whether brain volume reduction in AN is associated with astrocyte loss using an activity-based anorexia (ABA) model in rodents which includes food reduction and exercise (running wheels) in female rats. The research found a loss of GFAP-positive astrocytes (after a 3 week starvation period) which was then reversible upon refeeding. Specifically, the authors found that cell reduction was derived from reduced proliferation (rather than increased apopotosis). Interestingly, the authors found that only white matter in the corpus callosum was still reduced after refeeding. Astrocytes have been shown to play a role in synapse formation influencing learning and brain plasticity (Molofsky et al., 2012; Panatier & Robitaille, 2012) and have been shown to be implicated in mood and sleep disturbances. Given the role that astrocytes play in synapse formation, it is possible that they could be responsible for the decrease in cognitive flexibility and increased attention to detail found in patients with AN in the acute stage. In chronic patients with long-term starvation, astrocyte loss could be in part responsible for maintenance of symptomatology.

As established above, studies have found associations between clinical parameters (such as BMI) and brain volumetric differences, however, the studied parameters are largely heterogeneous making it difficult to draw any overall conclusions. Furthermore, most studies to date have been conducted with relatively small samples. One of the most reported associations is between GM and WM volume reductions and BMI, with mostly studies in adolescents finding a negative correlation (Bomba et al., 2013; Fuglset, Endestad, Landro, & Ro, 2015; Muhlau et al., 2007; Seitz et al., 2015).

Associations with other neuropsychological measures have also been studied. Castro-Fornieles in 2009 found that GM volume decrease was associated with visuospatial functioning in a sample of acutely ill adolescents (Castro-Fornieles et al., 2009). McCormick and colleagues also found that reductions in Anterior Cingulate Cortex (ACC) were associated with perceptual organization and reasoning skills in a sample of acutely ill (McCormick et al., 2008) and Bodell and colleagues found that reduction in orbitofrontal volume was negatively associated with reduced decision making skills (Bodell et al., 2014) in acutely ill adults.

While brain gray matter deficits have been shown to largely recover, small deficits cannot be ruled out as all volumes remain partially changed even after long term recovery (Seitz et al., 2016). Some well-controlled studies have found larger frontal and subcortical volumes (Samantha J. Brooks et al., 2011; G. K. Frank, Shott, Hagman, & Mittal, 2013; G. K. Frank, M. E. Shott, J. O. Hagman, & T. T. Yang, 2013). Regional differences in GM and WM have been established. A review by Van den Eynde and colleagues (F Van den Eynde et al., 2012) suggested reduced GM volume in patients with AN in the insula, frontal operculum, occipital and cingulate cortex, however, they highlighted a large level of heterogeneity and inconsistencies across studies. In a well-controlled study for the effects of malnutrition, Frank and colleagues (G. K. Frank, M. E. Shott, J. O. Hagman, & T. T. Yang, 2013) found increased GM volume of the medial orbitofrontal cortex and increased insula volumes (AN patients – ill and recovered- increased right side while BN patients had increased left side) compared to healthy controls. Interestingly, dorsal striatum volumes were reduced in BN and recovered AN and predicted sensitivity to reward in all groups. Both the orbitofrontal cortex and the insula have been hypothesised to be central for the development and/or maintenance of ED. The orbitofrontal cortex is of importance for food intake control and could therefore be associated with food avoidance and be a key structure in ED. In a recent publication, Frank (G. K. Frank, 2015) proposes that the

alterations in either side of the insula related to phenotypic presentation (restricting vs binges), could play a role in either mediating feelings of fullness in BN (left side) and with self-recognition and self awareness in AN (right insula). The cingulate cortex is another commonly reported area of interest in ED (S. Gaudio et al., 2011; McCormick et al., 2008; Muhlau et al., 2007) as well as the basal ganglia, both of which may play a role in emotion regulation and reward.

Not many studies have investigated volumetric brain differences in patients with BN (Joos et al., 2010; Schafer, Vaitl, & Schienle, 2010), however, the few that have, did not find consistent differences between patients with BN and healthy controls. A recent review (Donnelly et al., 2018) found a small number of consistent findings in patients with BN and BED during the acute stage of the illness. The review included sixteen studies on participants with BN, eleven studies on participants with BN and AN, three on participants with BN and BED and two on participants with BED. These studies included a variety of methods: MRI, fMRI, SPECT and/or PET. Only seven studies were conducted on participants using MRI and the findings were mixed.

2.4.2. fMRI findings in patients with ED

As established earlier in the section, while this thesis will not study functional differences in children at-risk, the functional literature allows us to explore hypothesis for altered mechanisms in the development of ED and therefore this section will provide a brief summary of findings and theories based on functional brain findings. Functional Magnetic Resonance Imaging (fMRI) is a neuroimaging technique used to assess brain activity associated with temporal changes in regional cerebral blood flow. This type of imaging can be performed in conjunction with a task designed to elucidate neural areas correlated with specific ED pathophysiology (e.g. decision making food-or other and body-image perception). Resting-state fMRI (rsfMRI) has also become a widely-used tool that allows to study brain function when at rest and have provided information of temporal correlations between brain areas, based on spontaneous fluctuations of signals in the brain (Ugurbil, 2016). Through rsfMRI a number of resting-state networks (sets of brain areas that show strong temporal coherence in the resting brain) have been identified and studied.

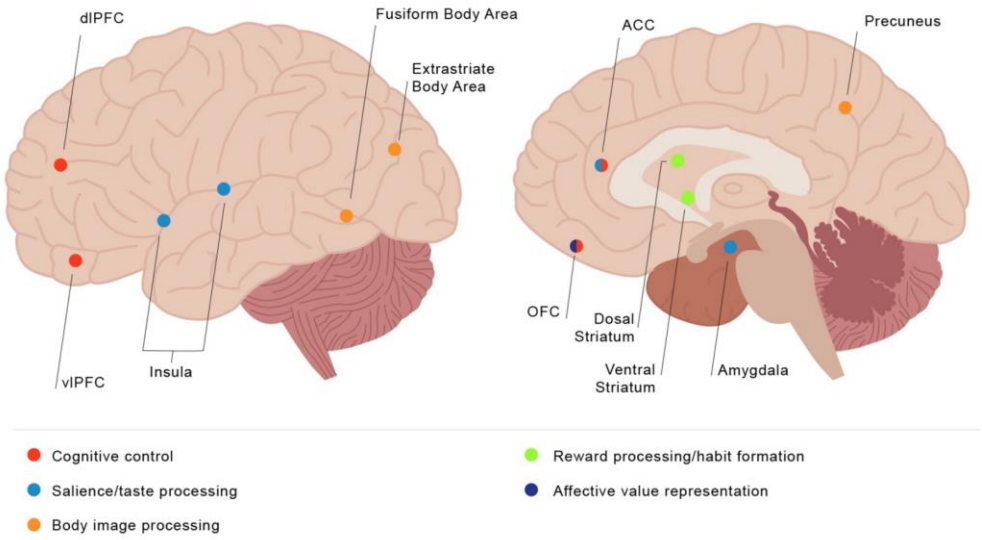


Fig. 2.14. Visual guide of brain regions implicated in ED symptomatology: based on fMRI findings. Picture from Review Article: Steward et al. Neural Networks Alterations Across Eating Disorders: a narrative review of fMRI studies (Steward, Menchon, Jimenez-Murcia, Soriano-Mas, & Fernandez-Aranda, 2018).

Given the central role that food intake plays in ED, the study of mechanisms involved in food intake has been of great importance in ED research. Food intake is driven by a complex interplay of cognitive, emotional and energy homeostasis processes, part of which are regulated by the brain reward system. Important brain regions that regulate these processes are the insula, higher order brain centres such as prefrontal and cingulate cortex (integrate cognition and emotions), orbitofrontal cortex (determines when to stop) and the amygdala (associates stimuli with emotional experience) (Kent C. Berridge, 2009; Kringelbach & Rolls, 2004; O'Reilly, 2006). Patients with ED have consistently shown altered pathways when processing food images or tasting food. Most consistently research has found altered responses to food images in the ventral striatum (in both AN and BN) (W. H. Kaye, Wagner, Fudge, & Paulus, 2011; Phillipou et al., 2014). Studies have used other stimuli to assess reward (such as monetary rewards and more recently reward in the context of social stimuli) (Esther Via et al., 2015; Wagner et al., 2007) in order to help understand if reward response is altered in stimuli unrelated to food.

Another area of interest in fMRI ED research has been cognitive control. It has been long established that patients with AN show cognitive characteristics such as weak central coherence and difficulties set-shifting (K. E. Smith et al., 2018) and personality traits such

as perfectionism (Fairburn et al., 2003) and obsessive compulsive traits (Cederlöf et al., 2015; C. A. Levinson et al., 2019). In contrast, patients with binge-type ED display difficulties in impulse control behaviours. Overall, patients with ED show alterations in cognitive control which refers to processes related to mindful deployment of attention and cognitive resources, including domains such as working memory, set shifting, decision making and emotion regulation, all of which have been shown to be altered in some degree in ED. Not many studies have directly studied specific aspects of cognitive control in ED, although more research is being conducted.

Body image distortions have been of great interest in the study of ED given the central role they play in its presentation. In fact, body image distortion has been hypothesised to precede the onset of AN (Jacobi, Hayward, de Zwaan, Kraemer, & Agras, 2004) and its persistence may determine poor outcome and relapses (P. K. Keel, Dorer, Franko, Jackson, & Herzog, 2005). The behavioural components of the body image construct have been outlined as covering three dimensions: perceptive (relative to detection of one's body size), affective (feelings that the individual develops towards their bodies' appearance) and cognitive (beliefs concerning body shape and appearance) (Thomas F. Cash & Deagle Iii, 1997). Alterations in self-perception in AN are hypothesised to be driven by cognitive distortions regarding the patient's own weight and shape and therefore their neurological underpinnings have received a lot of attention, although mainly based on visual stimuli. A number of regions have been found to show altered activation (either hyper – or hypo) when presenting a patient with images of body shape, such as the fusiform gyrus, precuneus, insula and areas of the prefrontal cortex (PFC) (Esposito, Cieri, di Giannantonio, & Tartaro, 2018; S. Gaudio & Quattrocchi, 2012). Specifically, the precuenus has been of great interest, although some fMRI studies have not obtained differences in parietal lobe activations when comparing patients and HC (S. Gaudio & Quattrocchi, 2012; Santino Gaudio & Riva, 2013; Nico et al., 2010; E. Via et al., 2018). Gaudio and Quattrocchi in 2012 published a review of fMRI studies on body image in AN including a total of 12 studies and proposed a model which conceptualized body image distortion as consisting of three main components: perceptive, affective and cognitive. They concluded that with regards to the perceptive component, the literature supported that patients with AN may suffer from processing bias of self-body image identification with associated neural alterations in the Inferior Parietal Lobule and the precuneus. Interestingly, studies have shown that, when presented with images of other women patients with ED showed increased activation in the amygdala (as well as increased activation of parietal areas and thalamus). Higher activity in amygdala was suggested as

a neural correlate of negative emotional activation. Studies showing distorted images of patients' body were hypothesised to tap into the affective component and showed involvement of the amygdala (although this has also been shown to be true for healthy controls (Yoshie Miyake et al., 2010)). Significant activation of the prefrontal cortex and insula has also been found during presentation of distorted body images in patients with ED (Yoshie Miyake et al., 2010; Mohr et al., 2010; Wagner, Ruf, Braus, & Schmidt, 2003).

Resting-state fMRI analyses results have been consistent over studies (although methodologies used varies greatly) identifying cortico-limbic network abnormalities in patients with ED (S. Gaudio, Wiemerslage, Brooks, & Schioth, 2016). Gaudio and colleagues recently published a systematic review including 15 published papers using seed-based, whole-brain independent component analysis (ICA), network of interest ICA and graph analysis approaches. Importantly the review identified that studies have reported functional alterations in networks and/or areas that are relevant to AN phenotypic presentation and in line with neurocognitive findings (impaired cognitive control and flexibility, impaired visual and somatosensory integration).

Overall findings from volumetric and fMRI studies may offer some insight into the development of more targeted treatment strategies focused on symptoms of impaired cognitive flexibility and integration of visual/body signals.

2.4.3. Diffusion imaging findings in patients with ED

The majority of the research in neuroimaging in eating disorders has been structural and functional, however, in the last few years, a growing number of studies have capitalized on advances in MR technology with the introduction of DTI. To date, 15 studies have been published using this method, the majority of which have been conducted in patients with AN, with only two studies focused on BN. Before reporting results from the studies, it is worth noting that to date most studies have been conducted using small samples and using at least two different approaches to analysing the findings (voxel-based approach and Tractography) resulting in mixed results. Studies have been conducted in adult and adolescent samples as well as in acute and recovered. Initial findings from adult studies show involvement of white matter microstructure in several areas of the brain including the

fornix, fronto-occipital fasciculus, cingulum, posterior thalamic radiation, thalamus, superior longitudinal fasciculus, and cerebellum.

To date, no meta-analysis have been published, therefore Chapter 7 will be dedicated to present the findings from the first meta-analysis I conducted with DTI findings from patients with AN.

2.6. Interim conclusion

Overall, volumetric and functional differences in ED have been found in several brain regions linked to overt symptoms of ED. However, the role that starvation plays in these alteratios continues to be unclear, both during acute stages of the illness, particularly for AN, and as a possible scar of the disorder once patients are recovered. Given the complexity of ED it is clear that a complex interplay of neural mechanisms play a role in its development and/or maintenance.

Chapter 3. Ph.D. Aims and methodological considerations (familial high-risk study)

Sections of this chapter have been published in a book Chapter and the studies summaries in the final table has been included in a systematic review currently under review for publication.

3.1. Chapter overview

The aim of this chapter is to provide an overview of the methodological considerations for the thesis. The previous chapters have provided an overview of the current neurocognitive and neural profile findings in patients with ED. It is clear from gaps in the research that there is a need to help understand what are scars and what could be traits for the disorder and given the effects that ED have on both the brain and neurocognitive markers, it is difficult to know what is considered 'state' (mainly due to undernutrition) and what could constitute of possible 'traits'. Findings from recovered patients have elucidated some of the possible differences. One other way of disentangling these differences is to study a group young enough to not have developed the disorder but with an increased risk for it developing in the future. This chapter will introduce the potential of high-risk research exploring endophenotypes and translational research and will give an overview of findings from children at risk for ED.

3.2. The potential of high risk research

3.2.1. Heritability and genetic liability

There is indeed now a great deal of evidence indicating considerable genetic liability for ED (Chapter 1); however, it is important that high risk studies in the field of ED are conducted with a sample young enough not to have developed any serious ED concerns or behaviours (Lee et al., 2007), making the children of women with a lifetime ED an ideal high risk population for investigation.

3.22. Endophenotypes and the Research Domain Criteria (RDoC) initiative

In the last 10 years, the way in which we think of psychiatric diagnoses has started to change, this was helped the introduction of a new initiative (Research Domain Criteria – RdoC) developed by US National Institute of Mental Health. This initiative aims to be a new framework that can add to the way we currently diagnose and think of psychiatric disorders. The "RDoC is an attempt to create a new kind of taxonomy for mental disorders by bringing the power of modern research approaches in genetics, neuroscience, and behavioural science to the problem of mental illness" (Thomas R. & Jeffrey A., 2013).

Currently, the diagnosis of psychiatric disorders is based on overt symptoms that can be observed through a clinical assessment. The Diagnostic Statistical Manual of Mental Disorders (DSM) (APA, 2013), currently on version 5, and the International Classification of Diseases (ICD), currently on version 10 offer a standard criteria for the classification of psychiatric disorders based on a series of symptom criteria, including symptoms, duration and impact on day-to-day life. However, in the last decades, research into psychiatric disorders has seen a search for something to add to overt symptoms that can help elucidate the development of disorders and risk makers, this has been aided with the development of the study of biological and neuroscientific approaches.

A phenotype is defined as an observable characteristic, which can be the product of both genetic and environmental influences. A large number of medical diseases have a genetic base and therefore phenotypes are an expression of the genotype. However, in psychiatry, where the causes are complex and where the genetics are not certain the relation between phenotypes and genotypes is not simple. Epigenetics, involves genetic control by factors other than the individual’s DNA sequence. Changes that interact with the genetic material and, whilst they do not change the underlying sequence, they can change its expression by indicating when genes should be turned on or expressed (Slatkin, 2009). These epigenetic factors are also of great importance in modifying the development of phenotypes. Although there have been great advances in the understanding of the human genome, within the discipline of psychiatry there has been little success in identifying underlying genes based on the current diagnostic criteria (Cloninger, 2002; Cowan, Kopnisky, & Hyman, 2002).

Taking into consideration the complex genetic underpinning of psychiatric disorders, it has become clear that their classification based on overt phenotypes might not be enough in the search for the biological/neurobiological underpinnings of the disorders. Gottesman and Shileds (Gottesman & Shields, 1967) were the first to describe “endophenotypes” almost 40 years ago when summarizing their genetic theories for the development of schizophrenia. Endophenotypes are trait-like markers (internal phenotypes) that have been proposed to be closer to biological (or genetic) processes than to overt phenotypes (Gottesman & Gould, 2003). They are associated with the disorder but independent from the clinical diagnosis, which is based on overt symptoms, can be measured before the explicit onset of the illness and should be heritable, therefore, can also be found in family members who do not have the disorder (D. Skuse, 2001). The identification of endophenotypes in psychiatric disorders is ideal to help develop our understanding of the aetiological models for psychiatric disorders. This will also help further understand the genetics as the genes involved in producing changes in specific endophenotypes will be less than those involved in producing changes in a whole psychiatric diagnosis (Figure 3.1).

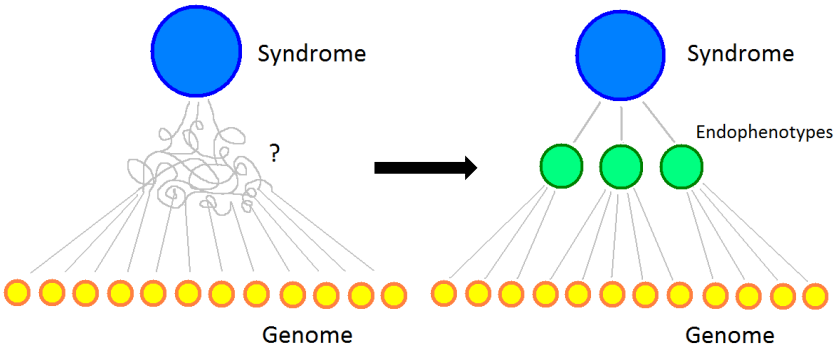


Figure 3.1. Visual graph of endophenotypes

The methods used to further our understanding on what are possible endophenotypes have advanced and are being used in research since the term was introduced. However, most of the advancement has only been seen in the last 15 years. The current fields of investigation as endophenotypes include not only more biochemical measures but also neuroanatomical, cognitive and neurocognitive ones.

The study of endophenotypes in psychiatry has the potential to change the way we think about mental health disorders. As an example, many potential cognitive endophenotypes are shared between different psychiatric disorders making research into each of them much more generalisable than that based on current diagnoses (Figure 3.2).

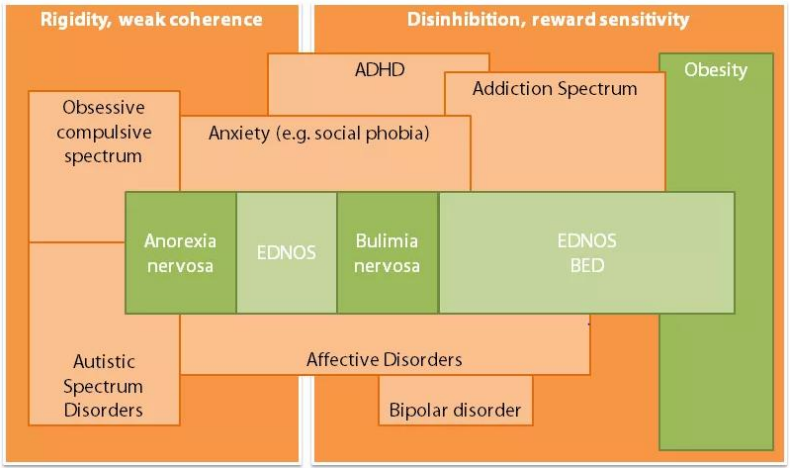


Figure 3.2. Potential endophenotype overlap between DSM-IV ED diagnoses and other psychiatric disorders. Note: the figure was adapted from "Biomarkers and endophenotype in ED" (Lopez, Roberts, & Treasure, 2009).

The search for possible endophenotypes is of special importance in ED due to the instability of current diagnoses (although this has improved since the introduction of the last DSM manual). The term ED encompasses a wide variety of disordered eating and compensatory behaviours that go on to create the different possible diagnoses within the ED umbrella. Diagnostic crossover possibly suggests that the diagnoses are not entirely independent. This can make both treatment and research of these disorders difficult. Longitudinal studies have found that crossover between ED diagnosis is substantial, with studies suggesting that 20%-50% of individuals with AN will go on to develop BN and 10%-30% of individuals with BN will go on to develop AN (Anderluh, Tchanturia, Rabe-Hesketh, Collier, & Treasure, 2009; Castellini et al., 2011; Eddy et al., 2002; Milos, Spindler, Schnyder, & Fairburn, 2005; Tozzi et al., 2005).

The diagnostic revisions (from DSM-4 to DSM-5) have indeed incurred in changes to the prevalence of different ED diagnoses. In fact, a study by Castellini and colleagues in 2011 (Castellini et al., 2011), showed that changes in diagnostic criteria have indeed succeeded in reducing the number of EDNOS categories. However, the diagnostic instability has

remained a crucial point for ED and therefore, the search for endophenotypes in ED has become of more importance.

3.2. Comorbidities with EDs and the use of transdiagnostic research

The overlap of neurocognitive markers between a number of psychiatric disorders has also motivated the search for endophenotypes. While these overlaps with other disorders have been researched, and considered for a long time in ED, it is more recently that comorbidities with other Axis I diagnoses have been considered in the search for endophenotypes, specifically within neurocognitive and neural profiles. This section will provide an overview of the current state of research in ED and overlap with other psychiatric disorders as well as their potential for transdiagnostic research.

Comorbidities with other psychiatric disorders such as OCD (Cederlöf et al., 2015; W. H. Kaye et al., 2004; C. A. Levinson et al., 2019; N. Micali, Hilton, et al., 2011; J. M. Swinbourne & Touyz, 2007), other anxiety based disorders (Goddard & Treasure, 2013; Cheri A Levinson & Rodebaugh, 2016; Schaumberg et al., 2019; J. Swinbourne et al., 2012) and more recently autistic spectrum disorders (Heather Westwood & Tchanturia, 2017; Zhou et al., 2018) have been researched in ED and informed translational models for the disorder.

Approaches within cognitive, behavioural and interpersonal frameworks have informed psycho-social models for both the development and the maintenance of ED. Fairburn and colleagues developed a transdiagnostic model in which they described perfectionism, low self-esteem, low mood and interpersonal difficulties as possible factors maintaining ED (Fairburn et al., 2003). Schmidt and Treasure have described a cognitive interpersonal model, empathizing perfectionism, experiential avoidance, beliefs about the value of the illness and interpersonal difficulties as maintaining factors, highlighting traits underpinned by poor set-shifting and central coherence as well as social-emotional processing difficulties which produce anxiety and encourage avoidance (Schmidt & Treasure, 2006; Janet Treasure & Ulrike Schmidt, 2013).

The development of the study of neural and neurocognitive markers in psychiatry has led to a better understanding of the overlap between ED and other disorders such as OCD

and ASD. This in turn has allowed for the investigation of underlying mechanisms that may be common to these disorders. Both obsessive thinking, impulsivity and perfectionism have been considered as intermediate traits, that could arise from cognitive processes such as weak central coherence and cognitive inflexibility.

3.2.1. Anxiety based disorders

There is evidence in research of high comorbidity between anxiety based disorders and ED (Godart et al., 2000; Godart, Flament, Perdereau, & Jeammet, 2002; J. I. Hudson, Pope, Yurgelun-Todd, Jonas, & Frankenburg, 1987; W. H. Kaye et al., 2004; Perdereau, Faucher, Jeammet, & Godart, 2007; J. Swinbourne et al., 2012). Several hypothesis have been proposed, such as anxiety being a risk factor for ED; anxiety being secondary to the development of an ED or a shared vulnerability between both disorders (Godart et al., 2003b). In order to help clarify these hypothesis retrospective studies have been done although they have their limitations given the way data was collected. Prospective studies would help clarify the temporal relationship between both disorders, however these data is difficult to obtain as it would require following patients up for a long period of time before the develop the disorder. A large number of retrospective studies have reported onset of social phobias (or elevated social anxiety traits) when comparing patients to the general population prior to the onset of the eating disorder (Godart et al., 2002; W. H. Kaye et al., 2004; Schaumberg et al., 2019). For example, Godart *et al.* (Godart et al., 2003b) studied a group of women with ED and comorbid anxiety disorders and found that 47% had at least one anxiety disorder prior to the onset of their ED. They did also find differences based on the type of anxiety disorder (OCD, Generalise Anxiety Disorders (GAD), specific phobias and social phobias). The authors found higher incidences of social phobia (between 51.2% and 71.4% depending of ED type). In another study, Kaye *et al* found that agoraphobia and panic were more commonly developed after the ED onset (W. H. Kaye et al., 2004). Thinking back to the initial hypothesis, it is possible that ED could exacerbate specific anxiety based disorders such as GAD and panic (possible due to starvations effects) (Serpell, Livingstone, Neiderman, & Lask, 2002). It is however possible, that other anxiety based disorders such as social phobia are present prior to the onset of ED and play a role in its development.

An alternative explanation for the frequent comorbidity between anxiety based disorders and ED is that rather than one causing the other, they both result from a common

vulnerability factor (Godart et al., 2003a). For example, rigid, obsessional and impulsive behaviours are seen across ED types. Both impulsivity (trait leading to actions which are not well conceived and prematurely expressed, or risky or inappropriate including elements of dyscontrol, sensitivity to reward and poor planning) and compulsivity (characterised by similar actions to impulsivity but leading to actions that may be inappropriate for the given situation which persist and have no obvious relationship to the goal) are also present in other psychiatric disorders such as OCD. The exploration of these characteristics across psychiatric disorders has led to its transdiagnostic use across OCD and ED. It has been found that first degree relatives of patients with ED, exhibit deficits in cognitive flexibility and inhibition processes (Boisseau et al., 2012; Chamberlain et al., 2007; Gruner & Pittenger, 2017; Shott et al., 2012; Talbot, Hay, Buckett, & Touyz, 2015; K. Tchanturia, Davies, Roberts, et al., 2012; K. Tchanturia et al., 2014). In Treasure and Schmidt's cognitive interpersonal model for AN (Janet Treasure & Ulrike Schmidt, 2013), they suggest that both obsessive compulsive and perfectionistic traits can increase vulnerability towards developing AN, both of which are suggested to arise from cognitive processing styles such as cognitive inflexibility and weak central coherence.

Whilst it is not clear if anxiety disorders are a risk factor for developing an ED, it is clear that some traits in anxiety based disorders are present in ED and therefore studying features that are common to both disorders can help further our understanding the aetiology of these psychiatric disorders. Both impulsivity and compulsivity are features shared between ED and disorders such as OCD. Compulsivity has been formulated as an imbalance between behaviour that is guided by goals and behaviour that has become habit. Both of which have been proposed to be mediated by the ventral medial prefrontral cortex and the dorsal striatum respectively (Gillan & Robbins, 2014; Robbins, Gillan, Smith, de Wit, & Ersche, 2012). The habit system uses information from past behaviours and links the stimulus with a specific response which leads to repetition of behaviour, leading to automatization of these behaviours (Graybiel, 2008). On the other hand, the system guided by goals predicts outcomes based on their values and bases behaviours on this (Dolan & Dayan, 2013). It has been suggested that compulsive behaviour results from from an override of the influence of the habit system over the goal directed system (Gillan, Robbins, Sahakian, van den Heuvel, & van Wingen, 2016). Based on this, it has been hypothesised that food restriction may be a behaviour which is driven by the habit system and could therefore be considered a compulsive behaviour (Godier & Park, 2014). More recently, *et al* (Lloyd, Frampton, Verplanken, & Haase, 2017) extended Godier et

al's model by suggesting a central role of anxiety which may contribute to the formation of habit (Godier & Park, 2014).

3.2.2. ASD

Similarities between ED and ASD were first suggested in the 1980s (Christopher Gillberg, 1983), and since then, their temporal relationship has been studied, suggesting that ASD may precede the onset of ED (Mandy & Tchanturia, 2015; Rastam & Gillberg, 1992). A possible overlap between both behavioural and neurocognitive features has led to new areas of research (Zucker et al., 2007), investigating specific neurocognitive functions impaired in both such as cognitive flexibility and central coherence. As established earlier, there is also a great overlap between social anxiety and ED, with social anxiety having been suggested to precede ED (Godart et al., 2003b). Aspects of theory of mind (ability to attribute mental states to oneself and others) which are a central characteristic of ASD, have also been suggested to be altered in AN (Russell, Schmidt, Doherty, Young, & Tchanturia, 2009). A recent meta-analysis found that patients with AN and those with ASD showed a similar theory of mind profile, with the latter showing greater difficulties (Leppanen, Sedgewick, Treasure, & Tchanturia, 2018). It has also been recently shown that patients with AN may have greater difficulties with the implicit response (bias in the interpretation) rather than the explicit labelling of the emotion (Cardi et al., 2017; Leppanen et al., 2017), while people with ASD may have difficulties in the recognition of the emotion (Uljarevic & Hamilton, 2013). Anhedonia (reduced feeling of pleasure from social stimulation) and alexithymia (inability to identify and describe emotions in the self) have both been found in ED and ASD and support difficulties in social interactions (Chevallier, Grèzes, Molesworth, Berthoz, & Happé, 2012; K. Tchanturia, Davies, Harrison, et al., 2012).

Zucker and colleagues (Zucker et al., 2007) investigated social cognitive endophenotypes in AN using the roadmap of investigation followed into social cognitive processes in ASD to guide their review (Figure 4.3). They proposed that exploring transdiagnostic similarities with Autistic Spectrum Disorder (ASD) (which has social cognition deficits at its core) can help draw a roadmap to understand social cognition differences in ED. Furthermore, relative to AN, social information processing in ASD has been largely studied and therefore we can draw from this knowledge to guide investigation in ED and provide a framework of study. The authors identified premorbid traits of anxiety as well as comorbidities with ASD

(social withdrawal and rigidity as well as impaired social information processing), social phobia and OCD (excessive rigidity).

Conclusions

Overall, it is clear that certain neurocognitive profiles, involving cognitive inflexibility, central coherence, rigidity and aspects of social cognition difficulties are shared between several psychiatric disorders and therefore could be considered shared vulnerabilities. The study of these neurocognitive constructs and it's correlated neural pathways can shed more light into potential mechanisms involved in development and/or maintenance of ED.

3.3. Familial high-risk design in children at risk for Eating Disorders

One of the criteria for a measure to become an endophenotype is for it to heritable, and therefore could be found in family members who does not have the disorder (D. Skuse, 2001). High-risk research refers to a method of studying the aetiology of a specific disorder by investigating individuals who have an increased risk of developing that disorder. Specifically in psychiatry, this method has been used to investigate the development or possible development of psychopathology in children of parents with psychopathology against children of parents without psychopathology. Since familial history of the disorder has been found to be a consistent predictor for the development of the disorder, studies of high-risk children provide an ideal method to identify premorbid vulnerability factors and potential endophenotypes.

The high-risk design has been used to investigate a large number of possible endophenotypes including cognitive function and brain volumetric differences as well as structural and functional connectivity differences in groups at high risk for schizophrenia (Chan, Di, McAlonan, & Gong, 2011; Eack, 2010; Gilmore, 2010; Snitz, MacDonald, & Carter, 2006), autism (Bosl, 2011; Luyster, 2011; Tierney, Gabard-Durnam, Vogel-Farley, Tager-Flusberg, & Nelson, 2012), and depression (Peterson et al., 2009), and progress is being made in identifying markers of risk and therefore potentiating early diagnosis of these disorders.

Although this method has been largely used in other psychiatric disorders, the same cannot be said for the field of ED, where only recently more research has been undertaken using this methodology. One of the reasons for this comes from the difficulty in studying this group (ED) prospectively. Since the prevalence for the disorder is low, large samples are needed in order to have enough people who will develop the disorder. However, it could be considered that since there is a need for prospective studies in the field of ED in order to better understand the poorly understood aetiology, this is in fact the field that would benefit from studying a group of children who are more likely to develop the disorder. By studying a group who has a higher risk of developing the disorder we would increase the incidence of the disorder in the population studied.

As previously said, this methodology has been largely used in other psychiatric disorders but only recently used to investigate potential risk mechanisms for ED. In the next section I will provide a systematic review of the findings of any studies focused on understanding the development of children at familial high-risk for ED and therefore potential risk mechanisms for ED. It is worth noting that a large number of the studies published have been conducted by our team.

As a summary, all studies reviewed were included when the exposure studied was mothers diagnosed with any form of ED (AN, BN or BED) either active or past; and where the outcome was a measure of the child's development assessed from birth until adolescence. Overall a large number of studies have assessed child's development in relation to feeding/eating, as this is at the core of the ED. However, recently more studies have focused on studying general development, temperament, cognitive development and psychopathology in this group.

For a simpler overview of these studies, below is a summary of findings for each outcome studied and a table summarising aspects of these studies as well as main findings for each of them.

3.2.1. Feeding

Classified as feeding, this category includes studies investigating feeding, diet patterns, nutritional intake and interaction during mealtimes (N. Micali, Simonoff, Stahl, & Treasure, 2011; Nadia Micali, Simonoff, & Treasure, 2009; Reba-Harrelson et al., 2010; Alan Stein, Woolley, Cooper, & Fairburn, 1994; Whelan & Cooper, 2000). Overall, the studies found that feeding problems appear to be present amongst infants of mothers with ED, however,

it is important to note that the use of different measures to assess feeding practices makes it difficult to generalise.

Two cohort studies conducted by Micali and colleagues (N. Micali, Simonoff, et al., 2011; Nadia Micali et al., 2009) studied infants of mothers with a past or present ED during the first few months and up to a year after birth and found that mothers reported more difficulties in their child's feeding practices. Stein and colleagues also reported that mother-infant interactions during mealtimes and play was affected, with mothers with current or past ED being more negative and less facilitating in their observational study (Alan Stein et al., 1994). Amongst three to four year olds (Reba-Harrelson et al., 2010; Whelan & Cooper, 2000) several studies found strong associations between feeding difficulties and maternal ED. The remaining two papers measured the nutritional intake and dietary patterns in infants of mothers with ED (Easter et al., 2013). When compared to controls Easter's et al. study showed that children of mothers with ED had a more 'health conscious/vegetarian' diet.

In conclusion, the studies showed that maternal ED is a predictor of eating difficulties in their offspring. However, it is important to note that most of the studies relied on maternal report to measure child feeding difficulties and therefore, we cannot discard the possibility that the children have no problems in their feeding practices but that the mothers who might be more concerned with feeding are in fact perceiving their child as more difficult when he or she is not. Although this is a possibility, it is an important fact to note as this in the long turn could affect the child's feeding practices.

3.2.2. Psychological/emotional development and psychopathology

Classified as psychological and psychopathology development, this section includes five studies (Barbin et al., 2002; Cimino, Cerniglia, Paciello, & Sinesi, 2013; S. Cimino, Cerniglia, & Paciello, 2015; Nadia Micali, Bianca De Stavola, George B. Ploubidis, Emily Simonoff, & Janet Treasure, 2014; N. Micali, Stahl, Treasure, & Simonoff, 2013). Overall, not many studies have focused on this, however, of those that have done, most found significantly higher psychological and emotional difficulties as well as higher indices of psychopathology in children of mothers with ED.

Both Cimino and Barbin used the children behaviour checklist (CBCL) to assess internalising and externalising behavioural/emotional characteristics in children. Cimino found higher scores of emotional-adaptive, internalising and externalising problems in children of mothers with ED, whilst Barbin only found significantly higher levels in children of depressed mothers. However, he did find that mothers with ED reported more significant pregnancy and birth complications, parenting stress, and symptoms of clinical depression when compared to the control group. Cimino found that externalising problems in children of mothers with ED increased with age.

Further studies have investigated psychopathology in children of mothers with ED, and the results support earlier results found in the previous studies, pointing to an effect of maternal ED in children's emotional and psychopathology development. Two large cohort studies by Micali and colleagues (Nadia Micali et al., 2014; N. Micali et al., 2013) found that maternal ED strongly predicted emotional and anxiety disorders in children at ages seven, ten and thirteen. Micali and colleagues used the Strengths and Difficulties Questionnaire (SDQ) (Goodman, 1997) and the Developmental and Wellbeing Assessment (DAWBA) (Goodman, Ford, Richards, Gatward, & Meltzer, 2000) to assess psychopathology at the different ages. Specifically, they found gender was predictive of conduct problems, hyperactivity/inattention but not emotional problems. They also found maternal disorder and gender specific results, where girls of mother with AN were more likely to have emotional, conduct and hyperactivity problems, whilst girls of mothers with BN were more likely to have hyperactivity and inattention problems. Boys of mothers with AN were at higher risk for emotional problems whereas boys of mothers with BN were also at higher risk for conduct problems. Importantly, higher odds of psychopathology were found in all cases in children of mothers with ED.

3.2.3. Cognitive development

More recent studies have focused on cognitive development in children of mothers with ED (R. Kothari, Barona, Treasure, & Micali, 2015; Radha Kothari, Rosinska, Treasure, & Micali, 2013; R. Kothari, Solmi, Treasure, & Micali, 2012). Although only few studies have focused on cognitive and neuropsychological development, they have yielded interesting results with regards to possible endophenotypes for the disorder. Kothari and colleagues (R. Kothari et al., 2012) found that children of mothers with AN had higher full-scale and performance IQ, increased working memory capacity and decreased attentional control

when compared to children of control mothers. Children of mothers with BN were found to have lower scores in object assembly (WISC) which suggests poor visual organisational ability and poor visual motor coordination. Social cognition was also studied in children of mothers with ED (R. Kothari et al., 2015) showing differential patters of cognition for children born to mothers with different ED diagnoses. The Social Communication Disorders Checklist (SCDC) (D. H. Skuse, Mandy, & Scourfield, 2005), the Diagnostic Analysis of Non Verbal Accuracy (DANVA) and the Emotional Triangles (ET) were used to assess social cognition. The SCDC is a questionnaire that assessed social trait characteristics where higher scores above a cut-off have been found to be predictive of ASD. In the study it was children of mothers with bingeing and purging disorders who were found to have higher scores in the SCDC. The DANVA was used to assess facial emotion recognition and ET was used to assess the ability to attribute an emotional state to an inanimate object (a little triangle in a screen). For both tests, children of mothers with bingeing and purging disorders had higher odds of misattributing emotion in faces and showed poorer recognition of fear in ET. Overall, the study found that children of mothers with bingeing and purging subtypes were at higher risk for social cognition difficulties. The findings are in line with previous research showing social cognitive style characteristics as being specifically associated with binge-purge subtypes of ED vs. restrictive subtypes (Troop & Bifulco, 2002). The authors concluded that social cognition difficulties could contribute to the development of binge/purge disorders. Interestingly, there is a large number of studies that have observed deficits in social communication and interpersonal function in patients with AN, and therefore suggest a possible link between the disorders. Kothari and colleagues did not find any social communication difficulties associated with a restrictive (AN type) pattern (R. Kothari et al., 2015). One possible explanation is that social communication difficulties in patients with AN could be a result of low weight in these patients which therefore only develops after the onset of AN. However, more studies are needed in order to better understand the associations between ED and social communication difficulties.

Table 3.1. Summary of findings from studies investigating children at familial high-risk for ED

Study, design	**Participants: n, age (SD), diagnoses and recruitment**	**Results**
Stein *et al*; Case-control	Total n = 58. Cases recruited from community (any ED disorder)	▪ *Negative expressed emotion* was more frequent among the case mothers compared to controls during mealtimes but *not* during play ▪ Mothers with ED were less facilitating during both mealtimes and play, had significantly more conflict with infants. ▪ Case infants were rated as less happy than the controls during both mealtime and play ▪ No significant differences on either mental or motor scales
Waugh *et al.* *Case-control*	Total n = 20 Cases recruited from community (Any ED disorder)	▪ Two case mothers chose not to breastfeed prior to the birth of their child: one due to embarrassment and one due to being depressed ▪ Rate of 6 case and 10 control children being breastfed till weaning was statistically significant ▪ Case children consumed significantly more sodium and thiamin than the control children ▪ Mothers with ED made significantly less positive eating comments
Whelan *et al.*	Total n = 128 Clinical group (any ED disorder)	▪ Children in the feeding problem group were rated as significantly more disturbed than the disturbed comparison and control group (feeding disturbances such as refusal, faddiness and spitting) Child disturbance Severity of child disturbance was not related to the relationship between feeding problems and maternal ED
Blisset *et al*	Total n = 96 Clinical group (BN)	▪ Eating psychopathology did not explain mealtime negativity in boys ▪ Eating psychopathology failed to explain mealtime negativity in girls Food refusal ▪ Eating psychopathology failed to explain food refusal in boys Eating psychopathology added significantly to the variance explained in food refusal of girls*

Micali *et al*	Total n = 12,050 Cases recruited from community (Any ED disorder)	▪ Mothers with a history of ED were more likely to start breastfeeding than controls (83% with 76%) ▪ Also less likely to stop breastfeeding during the first year of infant life ▪ Those with BN were more likely to continue breastfeeding Infant feeding ▪ AN mothers reported more early onset persistent feeding difficulties in all domains except refusal to take solids ▪ Infants of mothers with BN differed in the rate of refusal to take solids from those AN mothers in the rates of being unsatisfied/hungry after feeding.
Reba-Harrelson *et al*	Total n = 13,006 Cases recruited from community (Any ED disorder)	▪ Mothers with BN and BED reported higher levels of disordered eating behaviours in their children than controls ▪ They also reported higher levels of anxiety symptoms in their children ▪ Mothers with BN reported higher levels of OCD symptoms in their children ▪ Maternally reported restrictive feeding was significantly associated with child disordered eating difficulties
Micali *et al*	Total n = 10,902 Cases recruited from community (Any ED disorder)	▪ Maternal lifetime ED predicted feeding difficulties at 1 month and 6 months ▪ ED symptoms in pregnancy found to predict feeding difficulties at 1 month ▪ Child 'difficult' temperament score was associated with late feeding difficulties
Easter *et al*	Total n= 9,423 Cases recruited from community (Any ED disorder)	▪ Children of mothers with all 3 ED groups (AN, BN and AN+BN) had higher scores on the health conscious/vegetarian dietary pattern across all 4 time points: 3, 4, 7 and 9 years* ▪ Differences persisted in maternal AN and BN groups after adjustments – children scored lower on the traditional dietary pattern across all 4 time points ▪ Trends showed higher energy intake of children with mothers with BN and AN+BN ▪ Children with mothers with BN had higher starch intake

de Barse *et al*	Total n = 4,851 Cases recruited from community (Any ED disorder)	▪ Mothers with a history of ED used less pressure to eat than mothers without ▪ Mothers with a history of AN were likely to use low levels of pressure to eat ▪ Children of mothers with an ED had higher levels of emotional overeating than controls – this was strongest with mothers with a history of AN
Torgersen *et al*	Total n = 46,628, mean age = 29.6 (4.6) Cases recruited from community (Any ED disorder)	▪ Percentages of mothers breastfeeding at 6 months: BN (79%), BED (76%), EDNOS-P (59%), AN (58%), no-ED (82%) ▪ Infants of mothers with BN had significantly lower odds of being in the *homemade traditional food* class than the *commercial jarred baby food* class ▪ Infants of mothers with BED were significantly less likely to be in the *homemade vegetarian food* class than the *commercial jarred baby food*
Evans and le Grange	Total n = 20 Cases (AN and BN)	▪ Positive correlation found in both case and control groups between mothers' satisfaction with their body size and their children's satisfaction with their own weight and shape ▪ Mothers in the clinical group complained of not feeling successful in breastfeeding ▪ Infants in the clinical group were schedule fed – this rigid adherence caused some confusion and anxiety for mothers when their infants displayed signs of hunger outside the recommended feeding times ▪ Half the children were described by their mothers as displaying behavioural difficulties such as hyperactivity, avoidant behaviour, enuresis, insecure attachment, depression, fears, personality problems, stuttering, violent temperament and oppositional defiant behaviour

Agras *et al*	Total n = 194 Cases (any ED disorder)	▪ Female infants of ED mothers sucked more rapidly than other infants ▪ ED mothers bottle fed their daughters for a mean time of 33.2 months compared with infants of NED mothers with 23.6 months ▪ Difficulties regarding weaning from the bottle of breast revealed a significant interaction between ED mothers and gender of children ▪ Significant effect of infants of ED mothers were reported to dawdle more while eating ▪ Significant interaction between ED group and gender for reported vomiting** ▪ From the ages of 2 to 5, there was a significant interaction between eating disorder status and gender for concerns over infant weight*** ▪ Significant main effect for the ED group for using food as non-nutritive purposes ▪ Significant effect of ED mothers reporting they fed their children on a less regular schedule than NED mothers ▪ Significant findings of children with mothers with ED presenting more negative affect than children of NED mothers
Stein *et al*	Total n = 58 Cases (any ED disorder)	▪ The most frequent antecedent to conflict was the mother's concern about the manner of eating; disagreement over who fed the infant and food refusal ▪ Mothers in the clinical group only acknowledged the infant's signals in a third of cases compared to over a half in the NED mothers group
Barbin *et al*	Total n= 251, mean age = 33.2 (3.1) Cases (any ED disorder)	Differences in global child psychopathology on internalising and externalising subscales of the CBCL amongst infants with mothers with and ED*

Zerwas *et al*	Total n = 48,964 Cases recruited from community (Any ED disorder)	Infant temperament ▪ Mothers with an ED had greater odds of reporting more difficult infant temperament ratings Women with AN and EDNOS-P had the highest odds of reporting the most difficult infant temperament*
Cimino *et al*	Total n = 64, mean age = 33.2 (3.1) Cases (AN and BN)	Children's longitudinal profiles ▪ Emotional-adaptive profiles were significantly higher in children in the exposed group on all CBCL dimensions across all three assessment time points Power of maternal EDs on the child's psychological profile ▪ Mother's psychoticism score was related to the child's anxiety/depression and T1 and T2
Kothari *et al* *cohort*	Cases recruited from community (Any ED disorder)	Intelligence and global cognition ▪ Children of AN mothers showed higher full-scale IQ and performance IQ than compared with NED mothers ▪ Children of AN mothers showed high picture arrangement scores on the WISC subtest whereas children of BN mothers showed low object assembly scores Working memory (WM) ▪ Children of AN mothers showed slightly better WM span scores after adjustments ▪ Children of AN+BN mothers showed better global WM scores

Kothari *et al* *cohort*	Cases recruited from community (Any ED disorder)	Griffiths development scales ▪ Children of women with a lifetime of AN showed lower scores on the development of locomotor and personal-social development than children of unexposed mothers Wechsler preschool and primary scale of intelligence-revised ▪ Children of women with a lifetime of AN showed lower scores on performance subtests of geometric design and block design ▪ They also showed lower scores on vernal tests of comprehension and similarities They also showed lower verbal IQ scores in comparison to the controls
Micali *et al* *cohort*	Total n = 9,443 Cases recruited from community (Any ED disorder)	Maternal ED and offspring psychopathology at age 7 ▪ Maternal ED predicted emotional disorders* ▪ Strong association present for maternal AN and AN+BN for offspring emotional disorders Maternal ED and offspring psychopathology at age 10 ▪ Maternal ED predicted offspring DSM-IV or ICD-10 disorder ▪ Maternal AN and AN+BN strong associated with emotional and anxiety disorders**
Micali *et al* *cohort*	Total n = 8,622 Cases recruited from community (Any ED disorder)	Childhood psychopathology ▪ Gender was predictive of having conduct problems, hyperactivity/inattention but not of emotional problems ▪ Children of women with AN (both genders) and boys of women with BN were more likely to have emotional problems ▪ Children of women with BN were more likely to present with conduct problems ▪ Girls of AN mothers were more likely to have emotional, conduct and hyperactivity problems ▪ Girls of BN mothers were more likely to have hyperactivity/inattention problems ▪ Boys of AN mothers were twice as likely to have emotional problems whereas boys of BN mothers were twice as likely to have emotional and conduct problems

Cimino *et al* *Case - control*	Total n = 251, mean age = 33.2 (3.1) Cases	Mothers' disorders and children's internalising and externalising problems ▪ Repeated analyses indicated that the internalising and externalising scores were not stable over time ▪ Externalising problems increased over time in the group of mothers with an ED
Kothari *et al* *cohort*	Total n = 1,128 Cases recruited from community (Any ED disorder)	Social cognition ▪ Children of women with a binging phenotype had higher odds of a poor social communication* ▪ Those with a binging and purging phenotype had children with higher odds of having poor social communication** DANVA ▪ Children of mothers with a binging and purging phenotype had lower odds of making errors when recognising emotional from high-intensity faces and lower odds of misattributing faces as sad*** Emotional triangle Binging and purging phenotype mothers' infants' showed poorer recognition of fear***

3.3. General aims

The general aim of the PhD is to investigate psychopathology, neurocognitive and neural profile of children at familial high risk of developing eating disorders. In the studies included in the PhD, high risk status was defined as being born to a mother with ED. The studies conducted include two different samples which will be described in detail below. This chapter aims to provide details on both samples, including recruitment, measures, MRI protocol and additional information from both study samples.

This will be achieved through the completion of the following objectives:

1.1.1. To study psychopathology in children at familial-high-risk for eating disorders in a large community sample (Study one).

1.1.2. To further our understanding of white matter microstructural differences in patients with ED to inform hypotheses of risk in children at high-risk (Study two).

1.1.3. To pilot the investigation of brain structural and diffusion parameter patterns as well as neurocognitive function in children at familial-high-risk for eating disorders (Study three). This study includes (a) study of neurocognitive function; (b) study of cerebellar and subcortical volumetric differences and (c) study of whole brain and region of interest white matter differences.

Study one:

Psychopathology and early cognitive development in children at high risk for developing an eating disorder (Chapter 5)

The aim of this study was to investigate the early cognitive development of children at high risk for eating disorders and whether they were at risk for developing other mental health disorders. For this study data was collected from a large cohort study (Danish National Birth Cohort) and the high risk status of children was defined via self-report of an ED diagnosis by the mother. The children of mothers who had self-reported ever having an ED were compared to the children of mothers who had not.

Study two:

Meta-analysis of white matter microstructure differences in patients with Anorexia Nervosa (Chapter 6)

The aim of this chapter was to systematically review and meta-analyse the literature on white matter microstructure in patients with ED.

Study three:

Neurocognitive differences in children at risk for ED: findings from BREDS (Chapter 7)

The aim of this study was to investigate whether children at high risk for developing ED showed differences in their neurocognitive profile when compared to children at low risk (of mothers without an ED). Data on maternal lifetime ED was collected using a structured interview and data on neurocognitive profile was collected using the following tasks: WASI (General intelligence), CANTAB (motor processing, inhibition control, set-shifting, risk taking behaviour, sustained attention and visual working memory), Reading the Mind in the Eyes (social cognition), Morphed Emotion Identification and BIS/BAS (reward).

Subcortical and cerebellar volumetric differences and amygdala connectivity in BREDS (Chapter 8)

The aim of this study was to investigate whether children at high risk for developing an ED showed differences in brain volume when compared to children at low risk (children of mothers without an ED). Data on maternal lifetime ED was collected using a structured interview and data on children's brain volume and regional cortical thickness was collected using a T1 weighted scan using a Magnetic Resonance Imaging (MRI) scanner.

White matter microstructure whole brain and ROI differences in children at familial high-risk for ED (Chapter 9)

The aim of this study was to investigate whether children at high risk for developing an ED showed differences in white matter microstructure when compared to children who are not at high risk. For this study high risk status in children was defined as the presence of a maternal ED over their lifetime. Data on maternal lifetime ED was collected using a structured interview and data on children's brain volume and regional cortical thickness

was collected using Diffusion Tensor Imaging (DTI) in an MRI scanner. Differences in whole brain white matter microstructure and region of interest tractography were studied.

Final conclusions (Chapter 10)

Final summaries of each result chapters, as well as conclusions, theoretical implications and future work are presented in this chapter. Overall limitations and considerations including thoughts on recruitment and controlling for multiple comparisons.

Chapter 4. Methodology

4.1. Sample 1: The Danish National Birth Cohort (DNBC)

Methodology for sample 1 will be described in detail within the results chapter (Chapter 5).

4.2. Sample 2: Brain in high-Risk for Eating Disorders Study (BREDS)

4.2.1. Study overview

The Brain in children at high-Risk for Eating Disorders Study (BREDS) in an observational prospective pilot study of daughters of women with a current or a history of an eating disorder.

Children at high-risk and controls were recruited from different ethical approval applications and NHS sites across London from 2015 until 2017. Figures 4.2 and 4.3 outlines participant recruitment.

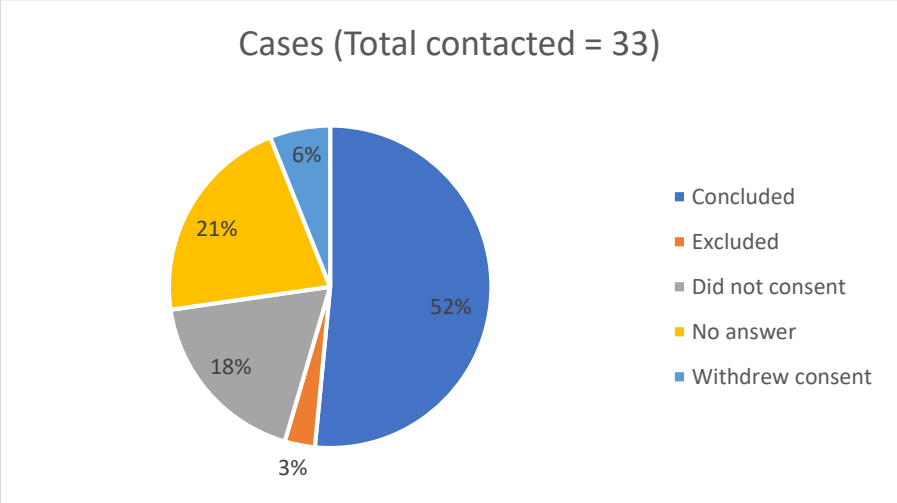


Figure 4.1. Cases contacted and percentage of recruitment outcome. Concluded: patients consented and who took part (N=17; 52%); excluded: met one of exclusion criteria (N=1; screened positive for ED) (3%); did not consent to take part (N=6; 18%); no answer after providing details (N=7; 21%); initial interest but did not sign consent form (N=2; 6%).

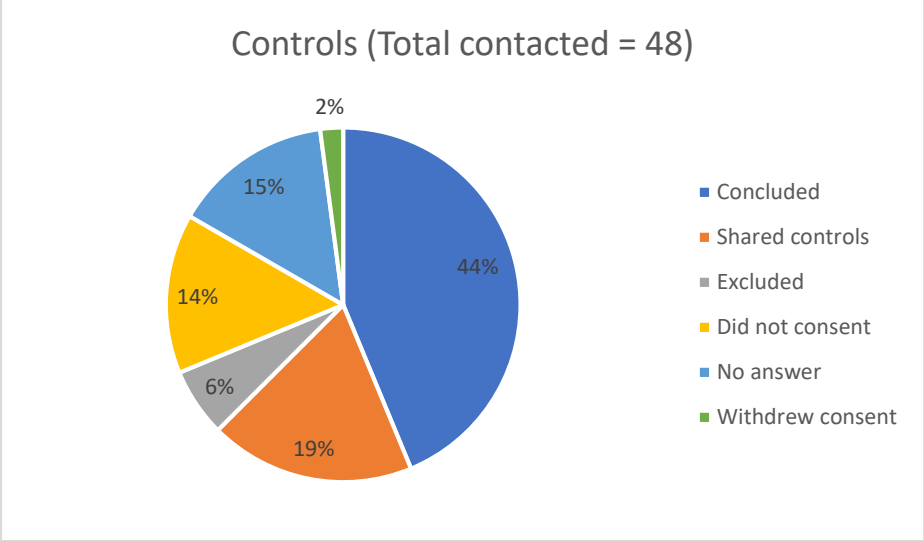


Figure 4.2. Controls contacted and percentage of recruited (N = 29; concluded and shared controls). Concluded: recruited and consented (44%); shared controls initiative at UCL (19%); excluded (N=1 screened positive for ED; N=1 had braces and N=1 mother with a lifetime diagnosis of depression/anxiety); did not consent after initial contact (n=7); no answer after initial interest (N=7); withdrew initial consent (N=1).

4.2.2. Aims and objectives

1. To pilot the first brain imaging study using quantitative brain structure and function MRI techniques including grey, white matter volume and white matter integrity, in female children (aged 8-15 years) at high-risk for anorexia nervosa (AN) and bulimia nervosa (BN) compared to children not-at-risk (children of mothers with no mental health disorders), matched for age and Tanner stage.

2. Investigate correlations between brain volume and white matter microstructure as well as neuropsychological functioning, social cognition, and reward processing in children at high-risk for developing an ED and comparing them to healthy controls and generate hypotheses to be tested in the full study.

4.2.3. Participants

Women and their daughters (between the ages of 8 and 15) were recruited. The sample included women with a history or a present ED and their healthy daughters. The healthy control sample was formed by mothers with no history of an ED or any other psychiatric disorder and their healthy daughters.

4.2.3.1. Recruitment

Several recruitment methods were used to recruit for the study: Initially, UCL ethics were obtained which allowed recruitment from the general population using posters, emails, social media, eating disorders societies (BEAT, Charlotte Helix) and a large university database that the PI had been involved in establishing. E-mails were sent to staff and students at UCL. A later amendment to initial ethics allowed for recruitment to be conducted through posters not only in the university but in different cafes and parks in London. Social media (Facebook, Twitter and eating disorders blogs) were also used to promote the study with the help of the eating disorder experts in the community. Webpages visited by mothers were also utilised to post questionnaires and assess possible participation in the study (such as netmums, mumsnet, Dulwich forum). A third amendment was requested to extend the UCL ethics and be able to recruit through schools. Personal contacts were used to contact several schools as well as the Schools in mind network. Information on the study was published in schools' newsletters with contact details.

After a year of slow recruitment, NHS ethics were drafted to be able to recruit from ED clinics. Once NHS ethics were granted, recruitment was attempted through the following NHS sites: SLAM, Vincent Square, St. Anns, Oxford health and Southern Health. In order to aid with recruitment, regular attendance of meetings in each of the sites was established. Only 2 mothers with a current ED were included through NHS sites. A further 5 showed initial interest but did not take part.

Finally, a webpage was developed in order to aid recruitment and Google Adwords were used to drive activity to the webpage. A total of 5 mothers with a history of ED were recruited through the webpage, with 3 contacted but no consent was granted.

Recruitment related documents, including ethics approvals and webpage screenshots can be found in the C and D. Overall, recruitment for the study was complex and this will be discussed in the final discussion chapter.

4.2.4. Demographics

Mothers were asked to report information on demographics during the day of testing. Data on maternal age, education, ethnicity and marital status was gathered.

Mothers were asked to report their acquired education using a likert scale with the following possible answers: (1) No formal qualifications; (2) no qualifications higher than CSE or GSCE level; (3) A-level or equivalent; (4) Higher qualifications. Due to the low number of mothers with low qualification level and for ease of use in statistical analysis, the variable was dichotomized into two categories: (0) qualifications up to A levels; (1) Higher qualifications. All mothers responded/ reported full information.

Mothers were asked to report their marital status using a likert scale with the following possible answers: (1) Single (no partner); (2) Single (with partner); (3) Cohabitating; (4) Married. Due to most mothers being in the last category and for ease of use in statistical analysis, the variable was dicothomized into two categories: (0) single (partner and no partner); (1) cohabitating or married.

Mothers were asked to report their ethnicity using a likert scale with the following possible answers: (1) White (British or other), (2) Black African, (3) Black Caribbean, (4) Asian (Indian, Pakistani, Bangladeshi), (5) Chinese, (6) Other. Due to most mothers being the first category and for ease of statistical analysis, the variable was dicothomized into two categories: (0) white; (1) other.

4.2.4.1. Tanner stages of development

Mothers were asked a set of questions with regards to their daughters development, including menarche. Pubertal development was assessed through a set of questions and pictures (Marshall & Tanner, 1969) (Appendix E). The questionnaire involves two pages

with a set of scaled drawings depicting external sex characteristics such as size of breasts and development of pubic hair. Participants can select between 6 options depending on the stage of development they are currently in. A series of questions were also developed regarding menstrual periods.

4.2.4.1. Analyses of demographics

The distribution of the demographics was studied according to the main predictor using chi-square (for categorical variables) and F-tests (for continuous variables) in order to provide a comparison between exposure groups. No differences were found between groups in maternal education, ethnicity and age. Both groups were matched in terms of child's age and development (Table 4.1.).

Table 4.1. Demographic group comparisons dependent on distribution of variable.

	ED (N = 17)	Unexposed (N = 20)	Statistic
Maternal education			
Up to A levels	3 (17.6%)	1 (5%)	$\chi^2 = 1.524$, p = 0.217
Higher education	14 (82.4%)	19 (95%)	
Maternal ethnicity			
White	14 (82.4%)	17 (85%)	$\chi^2 = 0.047$ p = 0.217
Other ethnicity	3 (17.6%)	2 (15%)	
Child development			
Menarche yes	9 (52.9%)	11 (55%)	$\chi^2 = 0.016$, p = 0.900
Menarche no	8 (47.1%)	9 (45%)	
Breast Development (6 stages)			$\chi^2 = 5.200$, p = 0.392
Pubic Hair Development (6 stages)			$\chi^2 = 6.448$, p = 0.265
Maternal age	44 (5.48)	45.55 (5.34)	t = 0.87, p = 0.39
Childs' age	11.94 (2.14)	12.25 (1.94)	t = 0.46, p = 0.65

4.2.3. Screening and psychopathology

4.2.3.1. Screener questionnaire

The screener questionnaire contained questions assessing both eating disorders and other psychiatric disorders (anxiety and depression) in mothers and children. The questions were extracted from the Structured Clinical Interview for Axis I DSM-IV Disorders (SCID-I) (First, Gibbon, Spitzer, Williams & Janet, 2002) screener questionnaire to assess the need to complete a more detailed assessment. If any of the girls screened positive for an ED they were excluded (2 participants were excluded after endorsing questions in the ED screener). Further investigations were done using the DAWBA in order to assess other mental health.

4.2.3.2. ED diagnosis

ED diagnosis was determined at baseline using the Structured Clinical Interview for Axis I DSM-IV Disorders (SCID-I) (First, Gibbon, Spitzer, Williams & Janet, 2002). The SCID-IV is a semi-structured diagnostic interview used to determine Axis I DSM-V disorders (American Psychiatric Association, 2000). The measure is formed by a series of open ended questions on the presence of ED symptoms (i.e. restricting, bingeing or purging) and cognitions (i.e. fear of fatness or denial of low weight). The measure also uses a series of skip rules in order to only ask for relevant questions. The SCID-IV has a high inter-rater reliability for the diagnosis of ED, with kappa being reported between 0.61 and 0.77 (Lobbestael, Leurgans & Agntz, 2010).

For the present study, we were interested in a lifetime history of ED symptoms, therefore relevant questions were repeated at appropriate times to determine the timeline of presence of symptoms.

Of the 17 recruited mothers, 14 had a history of AN while 3 had a history of BN.

4.2.3.3. Psychopathology

Child psychopathology was assessed using the DAWBA (Goodman et al., 2000) (Development And Well-Being Assessment), a computerised semi-structured interview. The DAWBA is commonly used as a reliable tool for the detection of mental health problems in children and young people. It's completed by both parent and child when the child is over 11 years old and by the parent only if the child is under 11.

Each child and parent were provided with a unique code and different passwords to access the questionnaire online. Using their unique ID and password each parent/child pair can log on and answer the questions individually and securely. The SDQ is a brief 25-item questionnaire, validated to assess psychopathology in children and adolescents (Goodman, 1997). The SDQ is scored into four categories: average, slightly raised, high and very high. Only scores of 2 (high) and 3 (very high) are relevant to the DAWBA skip rule. SDQ variables were made into a binary variable with average and slightly raised in one category and high and very high in another category.

Associations between maternal ED and SDQ scores were investigated using binary logistic regression analyses.

Table 4.2. Maternal ED and child SDQ scores: Odds ratios and (95%confidence intervals) from logistic regression.

	Adjusted OR (95% CI)	
	Cases (N = 17)	Unexposed (N = 20)
Total difficulties	1.392 (0.234 – 8.293)	Ref.
Emotional difficulties	2.112 (0.492 - 9.066)	Ref.
Conduct difficulties	1.127 (0.064 – 19.854)	Ref.
Hyperactivity difficulties	2.978 (2.33 – 8.026)	Ref.
Peer difficulties	1.734 (0.230 – 13.063)	Ref.

*p≤0.05, **p≤0.01, ***p≤0.001; [a] Adjusted for child's age at testing.

In order to minimise the time involvement of both parents and child, the DAWBA has skip rules which apply depending on the answers given to the questions.

- Initially the parent/child answers a set of questions which form the Strengths and Difficulties Questionnaire (SDQ) (Goodman, 1997). The SDQ is then scored by the program and was used to inform the use of the skip rules for each section (only positive scores of 2 or more in the SDQ sections are relevant to the DAWBA skip rule).

- The parent/child then goes on to complete each of the sections. In each section, they complete two or three screening questions at the beginning, if the screening questions are positive or they initially reported problems in the SDQ then the interview continues and the parent/child will see the rest of questions. However, if they respond negatively to the screener questions then the questions for that specific section end there and the entire section is skipped.

- If they go on to complete the entire section, they are then asked in detail about the presence or severity of symptoms in that specific domain. If the symptoms are present the rest of the interview continues, however, if they are not then the rest of the section might be omitted. Questions regarding how long the symptoms have been present and when they started are also asked. At the end of the section there is a question regarding the impact of symptoms on the child and family.

The following sections are included in the DAWBA:

- Separation anxiety
- Specific phobias
- Social phobias
- Panic attacks and agoraphobia
- Post Traumatic Stress Disorder
- Compulsions and obsessions
- Generalized anxiety
- Depression
- Attention and activity
- Awkward and troublesome behaviour
- Eating disorders

Once the questionnaire is completed, the program generates the probability of DSM5 psychiatric diagnosis. The full questionnaire is then reviewed by a clinician who rates the probability of disorder and provides a final DSM5 diagnosis.

For this study, the full DAWBA was rated blindly by a researcher (Psychiatrist) who is not involved in the recruitment or testing of participants.

While the questionnaire is confidential, it is explained to both child and mother that if there are any answers that depict risk to the young person or others, confidentiality would be broken an with the mother or appropriate services would be contacted. If necessary, referrals to appropriate services could be done. Only one participant endorsed questions with regards to a history self-harm which were discussed with the PI and with the mother.

4.2.4. Cognitive testing

4.2.4.1. Global intelligence

Intelligence and Global Cognition was assessed using the Wechsler Abbreviated Scale of Intelligence II (WASI-II)(Wechsler, 2011), a quick and reliable measure of intelligence and global cognition that has been well validated in a variety of populations (Irby & Floyd, 2013). The WASI comprises of four individual tests that together assess full-scale IQ (FSIQ). Two of the four tests go on to form the Verbal IQ (VIQ) score and the other two go on to form the Performance IQ (PIQ) score.

- VIQ is formed by Vocabulary and Similarities:
    - Vocabulary: participants are given several words and are asked to give a definition for them in their own words. This test measures semantic knowledge and verbal comprehension and expression.
    - Similarities: participants are given two words or concepts and are asked to describe in which way they are similar. This test measures abstract verbal reasoning and semantic knowledge.

- PIQ is formed by Block Design and Matrix Reasoning:
    - Block Design: This test measures visual spatial processing and problem solving as well as visual motor construction.
    - Matrix reasoning: this test measures nonverbal abstract problem solving and inductive reasoning.

4.2.4.1. Neurocognitive function

Neurocognitive Function was assessed using the CANTAB computerised tests of cognition (for test demonstrations see www.cambridgecognition.com). The tests were assessed using a tablet with the downloaded tests. Children were seated in front of the tablet and were instructed to carry out the test by touching the screen. Before each test the researcher explained the detailed instructions (following a script provided by the software developers) and asked if the child had understood, then proceeded to demonstrate the test allowing the child to do a practice test. All tests were run in the same mode for every child. Children were given short breaks whenever needed (between tests), and beverages were provided when requested.

One of the advantages of CANTAB is the number of outcome measures available through the software. Not all available outcome measures were used for analyses – only appropriate ones based on *a priori* hypotheses.

The following subtests were be used:

Motor Screening Test (MOT)

This task is a good introduction to the CANTAB touchscreen, and also assesses whether the participant has any sensorimotor and/or comprehension limitations that may limit collection of valid data. Participants are required to touch a flashing cross that appears on different locations on the screen.

Administration time: 2 minutes

Outcome measures: (1) Speed of response

(2) Accuracy of pointing

All participants completed this test.

Affective Go/No-go (AGN)

This task is an assessment of information processing biases for positive and negative stimuli. Over a series of blocks the participant is presented with a series of words from three categories: positive (i.e. joyful), negative (i.e. hopeless), or neutral (e.g. element). Words from two categories are presented during each block, and the participant is required to press the pad when they see a word matching a given target category.

Administration time: 10 minutes

Outcome measures: 12 outcome measures are provided which represent latency, and errors of commission and omission.

All participants completed this test.

Cambridge Gambling Task (CGT) – minimum age 11 years old

This task assesses decision-making and risk-taking behaviours, outside of a learning context. During the task the participant is shown a row of ten red and blue boxes, and has to guess whether a yellow token is hidden in a red or blue box. The participant is given a number of points, which are shown to be rising or falling in each trial, and they are able to gamble a chosen proportion of these points based upon their confidence in their guess (gambling stages). The goal is to accumulate as many points as possible. The task dissociates “risk-taking” from “impulsivity” due to a condition in which making a risky bet

requires the participant to wait patiently for it to appear (the ascending bet condition when the points are rising).

Administration time: up to 30 minutes

Outcome measures: (1) Risk taking

(2) Quality of decision making

(3) Deliberation time

(4) Risk adjustment

(5) Delay aversion

(6) Overall proportion bet

Only 10 girls at high genetic risk were old enough to complete the test and 17 girls of healthy control mothers were also old enough to complete the test.

Attention Switching Task (AST)

This task assesses the participant's ability to switch attention between an arrow's location and direction, and their ability to ignore task-irrelevant information in the face of interfering or distracting events. The arrow can be displayed either on the left or the right side of the screen, and can be pointing towards the left or the right. A cue at the beginning of each screen indicates whether a participant is required to press left or right according to the direction or the location of the arrow. In some trials information is congruent (arrow on right side of screen and pointing right), and in others information is incongruent, requiring higher cognitive demand. The task measures top-down cognitive control and is associated with the prefrontal cortex.

Administration time: 8 minutes

Outcome measures: Response latencies and error scores reflecting attention switching ability and interference of task irrelevant information (i.e. Stroop-like effect).

All participants completed this test.

Rapid Visual Information Processing (RVP)

This task is a sensitive measure of sustained attention in which digits from 2 to 9 appear in a pseudo-random order at the rate of 100 digits per minute. Participants are required to detect target sequences of digits (i.e. 2-4-6, 3-5-7, 4-6-8).

Administration Time: 10 minutes

Outcome measures: nine outcomes measures reflecting response accuracy, target sensitivity, and reaction times.

All participants completed this test.

Spatial Working Memory (SWM)

This task requires the participant to retain and manipulate visuospatial information, and measures strategy and memory errors. In each trial the participant is shown a number of boxes and through a process of elimination is required to find the one that contains a blue token. The number of boxes increases through trials.

Administration time: 8 minutes

Outcome measures: 24 outcome measures are provided reflecting errors, strategy, and latency.

4.2.4.2. Social cognition

Social cognition was assessed using the Reading the mind in the eyes (Baron Cohen) (Baron-Cohen et al., 2001) and Morphed Emotion Identification. Both tasks were completed in a computer using a web based experiment designed for research use (L. Germine et al., 2012) initially developed at Harvard University and which is currently managed by the non-profit Many Brains Project, the Laboratory for Brain and Cognitive Health Technology at McLean Hospital & Harvard Medical School, and the Human Variation Lab at Wellesley College .

Reading the mind in the eyes (RME)

RME measures social cognition by presenting the child with a series of 25 standardized photographs of the eye region of different male and female faces for a total of three seconds. Participants were presented with 36 pictures of the eye region and were given a forced choice question between four different mental states under each eye caption (Figure 4.3). The participant has to decide which mental state best describes what mental state the eyes depict. Test-retest reliability estimates are acceptable (Olderbak et al., 2015).

Figure 4.3. Reading the Mind in the Eyes web caption.

Morphed emotion identification

The task uses morphed face stimuli adapted from tests previously used in depression, schizophrenia and psychosis research (Bediou et al., 2007; Bediou et al., 2005; L. T.

Germine & Hooker, 2011). The presented stimuli were faces morphed between neutral and emotional expression (see Figure 4.4), with four different emotional expressions: happy, disgusted, angry and fearful. Faces were created using one male and two female faces. The faces contained 20, 30, 40, 50 and 60% of the emotional expression. Based on previous studies (L. T. Germine & Hooker, 2011) the range 20-60% was selected. Each trial began with a fixation cross for 250ms then the face was presented for 1000ms followed by the list of answer choices. The participant had to decide between angry, disgusted, fearful or happy. The answer choices remained in the screen for 7s or until the participant responded. Results were based on either correct or incorrect identification.

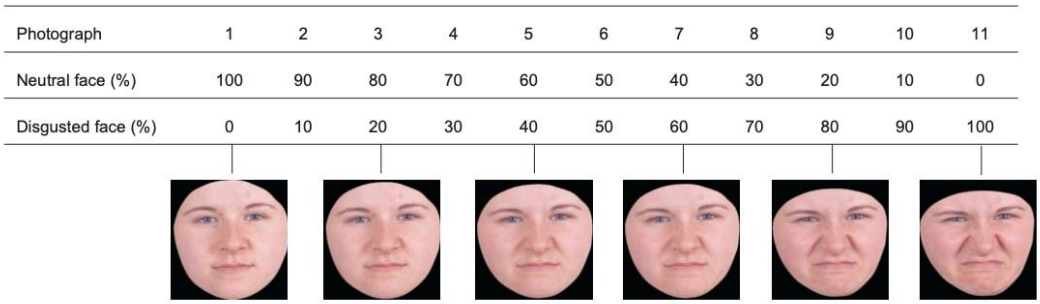


Figure 4.4. Example of morphed photographic image from neutral to disgusted expression. Figure taken from Bediou *et al*. Facial expression and sex recognition in Schizophrenia and depression (Bediou et al., 2005).

4.2.4.3. Reward processing

Reward Processing was assessed using the BIS/BAS scale (Carver & White, 1994): a self-report measure that assesses responsiveness to reward and punishment, and has been validated for use with adolescents as young as 11 years of age (A. Cooper, Gomez, & Aucote, 2007). The task was completed using pen and paper.

The self-report measure is completed using a 4-point scale (from 1 *strongly disagree,* to 4 *strongly agree*). Factor analysis (from initial tests with undergraduate students (Carver & White, 1994)) revealed a 7-time scale for BIS features, and three scales to assess BAS (Reward responsivity (5 items), Drive (4 items) and fun seeking (4 items). Crobach's α for

the BIS and BAS scales in the derivation sample were 0.74, 0.73, 0.76 and 0.66 respectively.

The scales measure the following constructs:

- Behavioural Inhibition System (BIS): sensitivity to punishment.
- Behavioural Action System (BAS):
    - Reward responsiveness: anticipation or occurrence of reward.
    - Drive: pursue of desired goals.
    - Fun seeking: desire of new rewards and impulsive approach to potential rewards.

4.2.5. MRI scanning

Patients and controls were scanned on a Siemens Avanto 3T clinical MRI system at Great Ormond Street Hospital (GOSH) using a 64-channel head coil by experienced research radiographers. An MRI safety questionnaire (Appendix F) was completed prior to scanning in order to screen for MRI-incompatible conditions, such as pregnancy or the presence of non-MR-safe medical implants, and to ensure the removal of any loose metallic objects. Recruited subjects were scanned with the following MRI protocol with a total duration of 45 minutes. The imaging protocol consists of three sequences; T1 MPRAGE, diffusion MRI and resting-state fMRI. A movie was playing during the acquisitions of all sequences except from when the resting-state images were being acquired. Analysis of the T_1-weighted and diffusion-weighted data were completed as part of this thesis. All T1-weighted and diffusion-weighted data were visually inspected for abnormalities, motion and other artefacts during scanning procedures. When possible, sequences were repeated in the scanner (with consent from the child) in order to adjust for movement. Abnormalities were discovered in one scan.

fMRI data was analysed separately by a post-doctoral researcher at Mount Sinai, NY, therefore the results from this data will not be included or discussed in this thesis.

An extra nine participants MRI data was include in the study from a shared control data initiative in the department. Information on exclusion/inclusion criteria was obtained by a researcher who contacted participants using our initial screener questionnaire.

All neuroimaging data was analysed with extensive help and supervision from members of the Neuroimaging department who played an instrumental role in designing pipelines for analyses and checking results.

All scans were converted from DICOM to NIfTI format using TractoR version 3.3.0 (Clayden et al., 2011) (http://www.tractor-mri.org.uk). The TractorR software includes R packages for reading, writing and visualising MRI images and it also includes functions designed for working with diffusion MRI and Tractography.

4.2.5.1. T_1-weighted scans

The T_1- weighted 3D MPRAGE (Magnetization Prepared Rapid Acquisition Gradient Echo) scan was acquired using the following parameters: flip angle=8°; TR=2300ms; TE=2.74ms; TI=909ms; voxel size=1x1x1mm^3; field of view=256mm; slice thickness=1mm.

Freesurfer

Following conversion to NIFTI, volumetric segmentation and cortical surface reconstruction was performed with the FreeSurfer image analysis suite (version 6.0. for Linux, documented at the website: https://surfer.nmr.mgh.harvard.edu/). FreeSurfer is a software package for the analysis and visualization of both structural and functional neuroimaging studies and has been described in technical detail previously (Fischl, Liu, & Dale, 2001; Fischl et al., 2002; Fischl, Salat, et al., 2004; Fischl, van der Kouwe, et al., 2004; Han et al., 2006; Jovicich et al., 2006; Segonne et al., 2004). FreeSurfer provides a full processing stream for structural MRI data.

For each individual the following steps were conducted using 'recon-all': FreeSurfer inputs whole head high resolution T1 weighted images after which the processing stream

includes motion correction (Reuter, Rosas, & Fischl, 2010), removal of non-brain tissue using a hybrid watershed/surface deformation procedure, automated Taliarach transformation, segmentation of subcortical white matter and deep grey matter structures, intensity normalisation and further steps to include grey and white matter surface reconstruction (Fig. 4.5.) (automatic subcortical segmentation processing stage (Fig. 4.6.): linear registration to the Gaussian Classifier Atlas, canonical normalisation and registration, registration and subcortical labelling for individual structures).

In order to minimize any methodological errors, all MRI volumes were visually inspected for data quality and any necessary edits (such as manual removal of non-brain tissue and modification of pial and white matter boundaries).

The recon-all command in FreeSurfer produces a number of files with statistics, including information on subcortical segmentation (*aseg*) and cortical parcellations (*aparc*). Both sets of files include information on volume for each participant which was imported into a data analysis table in SPSS.

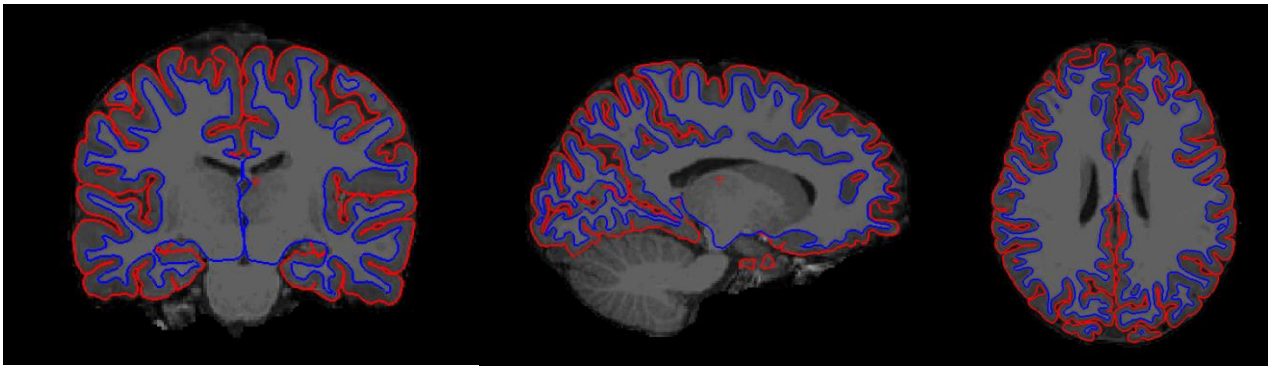


Figure. 4.5. Shows an example of a surface reconstruction with boundaries marked between pial and white matter surfaces overlaid on the participants T1 weighted-scan. Coronal, sagittal and axial views are showed (left to right respectively).

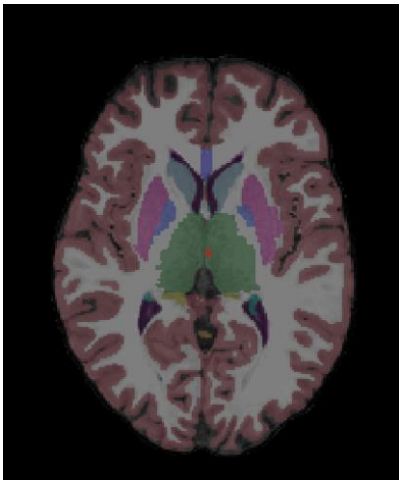


Figure 4.6. Shows a subcortical parcellation produced by freesurfer overlaid on the same participants T1 weighted-can.

4.2.5.1. Diffusion weighted scans

The diffusion-weighted scan protocol consisted of the following parameters: 60 unique gradient directions (b=1000s/mm^2) and 60 unique gradient direction (b=2200s/mm2) plus an additional b0 with negative phase encoding direction. TR=3050ms; TE=60ms; voxel size=2x2x2mm; 66 slices; slice thickness=2mm. Multiple fibers: beyond the diffusion tensor.

Pre-processing

The diffusion-weighted data were pre-processed using TractoR. The first step in pre-processing is the selection and brain extraction of the first reference b=0 scan volume for each participant (S. M. Smith, 2002). Top up was used to remove susceptibility distortions (J. L. Andersson, Skare, & Ashburner, 2003; S. M. Smith et al., 2004). Eddy was then used to correct for eddy-current distortions and to align the diffusion-weighted images (J. L. R. Andersson & Sotiropoulos, 2016). Finally, the data was reconstructed using diffusion tensor imaging and constrained spherical deconvolution (CSD), as implemented in MRtrix (J. Donald Tournier, Calamante, & Connelly, 2012). CSD was used as it provides better angular resolution than many other multiple-fibre reconstruction algorithms, while maintaining a modest computation time (Seunarine & Alexander, 2009; J. D. Tournier et al., 2007). The CSD reconstruction used a maximum spherical harmonic order of 6 for both the response and the fibre orientation distribution functions.

4.2.6. Inclusion/exclusion criteria

Participants were excluded if they exhibited any ED related behaviours or if their mothers had a primary lifetime diagnosis of a psychiatric disorder other than an ED. Participants were also excluded if they were left handed, or if their first language was not English. Exclusion criteria required for the use of an MRI machine (i.e. if the child has braces) was also applied (only one child was excluded based on MRI exclusion criteria).

4.2.7. Procedure

A visual representation of the protocol can be found in Appendix D.

Step One

Mothers initially recruited into the study were informed about the aims and details of the study. After consent was taken (initially on the phone) for both themselves and their child, they completed a screener questionnaire in order to assess inclusion and exclusion criteria.

Mothers who were eligible for the study were then called by a trained researcher who conducted a tailored version of the Structure Clinical Interview for DSM-V (SCID-I: 5), used to assess past or current mental health disorders in healthy control mothers, and to confirm lifetime diagnosis of ED in the experimental group. This tailored version of the SCID-I included the overview, ED modules, and questions concerning presence of anxiety, and mood disorders.

Step Two

If mother and child met inclusion criteria for the study, mothers were asked to talk through the study with their children and to confirm their willingness to participate in the study (separate information sheets tailored to the child's age (8 to 10 and 11 to 15 years old) were provided for this step).

All children and mothers that were willing to take part were then asked to complete an online DAWBA (Development and Wellbeing Assessment) to screen for the presence of ED and other psychiatric disorders in the child. For children under the age of 11 this was not done.

Step Three

The mothers of children who were eligible for inclusion (based on results from the DAWBA) were contacted to book in the testing session. A link was provided for the completion of online questionnaires prior to the assessment session (social cognition measures).

Step Four

Children attended one session at the Institute of Child Health, where they completed the selected tests from the Cambridge Neuropsychological Testing Assessment Battery (CANTAB: details below), the BIS/BAS measure, Tanner development stage and an MRI scan (details above).

Chapter 5. Cognitive development and psychopathology in children at high-risk for eating disorders: findings from the Danish National Birth Cohort

This chapter has been published in Acta Psychiatrica Scandinavia and findings were presented at the International Eating Disorders Conference 2015 and the Eating Disorders Research Society meeting in 2015[1].

5.1 Introduction

Research into recovered patients has been conducted in order to attempt to understand possible mechanisms for the development of the disorders, however, due to the nature of ED and the effect that malnutrition can have in cognitive processes and brain mechanisms, it is difficult to discern what are scars of the disorder and what are possible traits. Furthermore, recovery takes a long time and ED have high rates of relapses adding to the difficulty of discerning between state and trait difficulties. Another added difficulty is the low prevalence of the disorders and young age of onset which makes it harder to understand the effect that ED psychopathology can have on development and future scars. In an attempt to further our understanding of what can constitute as risk markers for the disorder and given the high genetic component of the disorders, studies have increasingly started investigating healthy first degree relatives. Both the study of recovered patients and healthy first degree relatives have helped to develop hypothesis of what could be risk mechanisms. Chapter 2 presented the evidence for possible neurocognitive markers for the disorder, such as weak central coherence and deficits in set-shifting, both of which have been proposed as endophenotypes for the disorder. Interestingly, both have been proposed to be altered across all ED subtypes and have been largely studied. Chapter 1 also presented evidence of comorbid psychopathology in ED and posted the question of the order of onset. There is evidence that anxiety disorders were present before the onset of ED and therefore could be considered as a risk factor for it's development. Risk models for the development of ED have been proposed, however, unless we are able to do prospective longitudinal studies of ED starting from a young age, we won't be able to elucidate what constitutes as a risk factor for the development of ED.

The prospective longitudinal study of children at high genetic risk for ED allows us to start to understand possible mechanisms for the development of the disorder as well as help distinguish what difficulties found in patients with ED could be present before the disorder and therefore possibly qualify as endophenotypes for the disorder. A place to start investigating has been guided by research into possible endophenotypes and risk mechanisms for the disorder, such as cognitive markers. This type of research has been conducted in other disorders such as ASD and ADHD and has led to findings into biological mechanisms for the development of these disorders.

It has been established that children of parents with psychiatric disorders are at a higher risk of developing a range of emotional, behavioural, cognitive and social difficulties (Leverton, 2003). However, this has not been as studied in children of parents with ED. Furthermore, it is not clear the role that early psychopathology can play in the development of an ED and if traits of other disorders such as anxiety, conduct or social difficulties could be considered as intermediate phenotypes for the disorder that play a role its development and maintenance or a general higher risk for any psychopatholoy.

The large majority of research on children of mothers with ED has focused on the early years of child development, when formal psychopathology is rare (Brinch, Isager, & Tolstrup, 1988). Case series have reported psychological disturbance in areas independent of eating in the offspring of mothers with ED, such as emotional, speech (Franzen & Gerlinghoff, 1997; Hodes, Timimi, & Robinson, 1997), behavioural problems (Timimi & Robinson, 1996) and general psychopathology(A. Stein et al., 2006). However, these findings rely on small clinical samples and therefore might be biased. Two recent studies(N. Micali et al., 2013; Nadia Micali, Stavola, Ploubidis, Simonoff, & Treasure, 2013) using data from a large population-based study, the Avon Longitudinal Study of Parents And Children (ALSPAC) investigated childhood psychopathology in children of mothers with ED at three and a half years (N. Micali et al., 2013) and in late childhood and early adolescence (Nadia Micali et al., 2013). Children of women with eating disorders, both anorexia and bulimia, had a higher risk of developing psychopathology at 3 and 1/2 years than unexposed children. These children also had higher prevalence of psychiatric disorders at age 10 years (N. Micali et al., 2013). Recent research has also found that children of mothers with ED show differences in cognitive performance compared to healthy children, such as difficulties with social understanding, visual-motor planning and abstract reasoning (R. Kothari, Solmi, Treasure, & Micali, 2013a). However, less is known

about mid childhood, when children start formal education, leading to new demands on cognitive and social functioning and potential difficulties becoming more prominent.

In order to help elucidate whether other psychopathology is present in children at high genetic risk of developing an ED and therefore could be a potential mechanism for the development of ED, we aimed to investigate children's psychopathology as measured by the Strengths and Difficulties Questionnaire at 7 years of age (which measures levels of conduct, emotional, hyperactivity, social problems and overall psychopathology). We also studied whether maternal eating disorders predicted early child development: motor, cognitive and language; as well as child difficult temperament at 18 months. Based on previous findings on gender differences in past studies in children of mothers with ED we focused on specific differences stratified by gender. We also aimed to explore whether child temperament might be a mediator in the associations between maternal ED and child psychopathology

5.2. Methods

5.2.1. Study overview

The Danish National Birth Cohort (DNBC)(Olsen et al., 2001) is a longitudinal and prospective population-based study of women (from pregnancy) and their children. The DNBC was developed in order to investigate exposure in the period from conception to early childhood that may have a long-lasting impact on health and disease susceptibility.

The initial recruitment aim was of 100,000 women in early pregnancy. Data collection started in 1996 and was extended to cover all regions in Denmark by 1999. The goal of 100,000 pregnant women had been reached by October 2002. Information has been collected through computer assisted telephone interviews with the women, twice during pregnancy, when their children were six and 18 months old, and seven and 11 years old.

5.2.2. Participants and procedure

Participants (pregnant women) were initially recruited through their General Practitioners (GPs) (approximately 3,500). When women visited their GP during their first ante-natal appointment (6 -12 weeks of pregnancy) the GP gave them an invitation to take part in the study. As almost all women have their GP as their first point of contact once they become pregnant this initial recruitment included a large number of women, however, a second recruitment procedure was also set in place through midwifes who look after pregnant women throughout pregnancy. Overall, more than 95% of the cohort where recruited through their GPs.

Initial inclusion criteria included wanting to carry the pregnancy to term and being able to speak Danish well enough to be able to take part on telephone interviews. No other exclusion criteria were used. Recruitment was undertaken in several Danish counties.

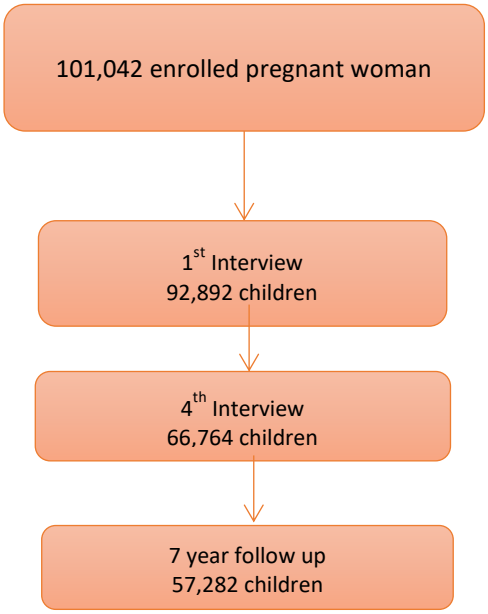
Information was collected on exposure and minor diseases (not registered in medical records) by using computerized telephone interviews in order to minimize the time involvement and increase response rate. The interviews were developed with external experts and steering groups. Information in a number of national registers were also used to access information on: maternal age, pregnancy-related smoking status, birth date, sex, birth weight and length, parity and multiple births (from the National Medical Birth Registry); information on hospital admissions, both outpatient and emergency room events, including diagnoses (from the National Hospital Discharge Register) as well as social conditions and occupational status of participants taking part in the study.

Initially, women enrolled agreed to complete 4 computerised assisted telephone interviews, four times at gestational weeks 12 and 30 and when the child was six and 18 months old. The telephone interviews lasted on average 18, 10, 16 and 10 minutes. As part of the study, participants were given a food frequency questionnaire at 25-26 weeks gestation as well. Participants also agreed to be invited to participate in subsequent data collection waves throughout childhood and the children who were born into the cohort will be given the opportunity to continue as part of the cohort after they turn 18 years old. Blood samples were also taken by GPs during routine visits in gestational weeks 6-12 and 24 months and an umbilical cord sample was also taken at time of birth by midwifes and nurses. After the initial four telephone interviews, further follow-ups were established, a 7

year follow up which was completed in August 2010, a 11 year follow up completed in August 2014 and a 14 year follow up (on dietary habits) which will be completed by 2017.

Initially the cohort enrolled 100,419 pregnancy (fewer than 100,000 women as some of them had more than one pregnancy in the cohort), however, the first interview was conducted on 92,889 (93% of the initial number). Information on number of people at each stage can be found in the flow chart (Figure 3.1.).

Figure 5.1: Flowchart of participation in the DNBC cohort.



5.2.3. Measures

Socio-demographic data

Information on socio-demographic data was obtained through the Danish National Patient Registry (DNPR), a population-based administrative registry that has been collecting data from all Danish hospitals since 1977. The data in the registry is structured with each variable having a finite number of possible values and includes information on administrative data (personal and admission data), diagnoses, treatments and examinations.

Information on maternal age, maternal social status (not in labour market, student, high and low grade professional and skilled or unskilled worker), parity (primiparae and multiparae), birthweight and gender of the child was extracted from the DNPR.

Maternal age: maternal age at the time of having the child included in the study. These data were used as a continuous variable.

Parity: mothers number of children was reported. The variable was dichotomized into primiparae or multiparae for ease of use.

Social status was based on parental current occupation. Maternal occupation was coded on a scale with four possible categories: (1) not in labour market, (2) student, (3) skilled or unskilled worker and (4) high/low grade professional. For this study the first two categories were combined into one.

Prematurity: data on weeks of gestation for the study child was extracted. The variable was dichotomized into less than 37 weeks gestation (premature) and more than 37 weeks gestation (term).

Gestational age (weeks): data on weeks of gestation for the study child was extracted. This data were used as a continuous variable.

Birthweight: information on birthweight of the study child was extracted and used as a continuous variable.

Child gender: information on the gender of the study child was extracted and was used as a dichotomous variable (male or female).

Maternal eating disorder

Information on maternal eating disorder was extracted from the first interview for the DNBC. During this interview women were asked whether they suffered from or had ever suffered from anorexia or bulimia nervosa (BN), and if they had BN at the time of the interview (N. Micali, Stemann Larsen, Strandberg-Larsen, & Nybo Andersen, 2015). Women were grouped into different exposure groups depending on having ever suffered from AN or from BN, and those who answered yes to having ever had both disorders were included in a separate group.

Women who reported ever having suffered from BN were also asked if they have suffered any episodes in the last six months. Women who reported having ever suffered from AN were also asked about the age at the onset of the disorder. These follow up information was then used to created further groups based on recency of the disorder.

Women were grouped into the following exposure groups: lifetime BN (N=1,751, 2.1%) and lifetime anorexia nervosa (AN) (N=1,673, 2.0%). Women who answered yes to having ever suffered from both disorders were included as a separate exposure group (N=658,0.8%), given previous evidence from Micali et al. 2007(Nadia Micali, Simonoff, & Treasure, 2007) that this group differs from the other two. Women with no history of ED were classified as unexposed (N=79,738, 95.1%).

A total of 83,820 women had complete data on maternal diagnoses.

Strengths and difficulties questionnaire (SDQ)

The SDQ is a brief 25-item questionnaire, validated to assess psychopathology in children and adolescents (Goodman, 1997); it has been validated in children across four large Danish cohorts, supporting its use in the Danish population [reliability (chronbach's alpha: 0.75-0.88)] (Janni Niclasen et al., 2012). The reliability and validity of the SDQ makes it a useful short measure of psychopathology in children and adolescents (Goodman, 2001) and all scales are associated with the relevant DSM-IV diagnoses (Goodman, 2001). Furthermore, the SDQ algorithm capitalises on the high level of detection of comorbidities; a well-recognised feature of child psychopathology (Angold, Costello, & Erkanli, 1999). The SDQ is completed by parents about their child's behaviour in the past six months and comprises five different subscales (emotional, conduct, hyperactivity/inattention, pro-

social and peer-relations) as well as a total scale. The responses for each item are coded in a 3-point Likert scale (not true, somewhat true, certainly true). We used recognised cut-offs for the SDQ (which roughly correspond to 80% of children: “normal” range, 10%: borderline range and 10%: clinical range), commonly used in clinical practice. The specific cut-off scores used were developed by Niclasen in 2012 based on a sample of almost 60,000 Danish children and are used to identify the 20% of the sample with the highest problem scores (http://www.sdqinfo.org/DanishNorms/DanishNorms.html) (J. Niclasen). The categories normal, borderline and clinical so derived were used as an ordered variable.

A total of 53,695 (63% of those originally enrolled) women completed the SDQ(Goodman, 1997) when the child was 7 years of age.

Developmental milestones

In the 18-month interview, mothers answered 11 questions pertaining to delays in their infant’s milestones. Five questions pertaining to motor development delays, (two assessed age for sitting and standing up, and three assessed whether the infant could climb stairs, remove his/her socks and shoes, and drink from a cup or glass by him-/herself). Six cognitive and language development questions were also asked. A developmental delay summary score for motor (0–5 points) and cognitive/language (0–6 points) development was calculated. Consistent with previous studies (J. L. Zhu, Basso, Obel, Hvidtjorn, & Olsen, 2009), to summarize motor, cognitive and language milestones, we defined as “delayed” those infants whose scores for motor or cognitive/language development corresponded to the highest 5% of all infants for each of the 2 summary measures (motor delay and cognitive/language delay). This cut-offs have been used previously in the DNBC cohort (J. L. Zhu et al., 2009).

A total of 58,637 children had complete data on developmental milestones.

Child temperament

Mothers were asked to indicate the frequency of a set of child characteristics. Whether the child was restless in his/her sleep, whether he/she was more or less active than other children, whether he/she was a happy child, and whether he/she was cautious and guarded. A summary score was obtained by adding the responses to three set of questions. Higher scores indicated a more difficult temperament. The scale was not normally distributed and could not be normalized therefore scores were dichotomised using the top 10% as indicating difficult temperament. The dichotomised variable was used in all analyses.

5.2.4. Scoring and data preparation

Women were eligible for the study if they had answered questions about their lifetime ED and had a live born child (N=83,820). We only included the first child amongst women who had more than one child in the cohort-to avoid non-independence of observations. Data on 48,403 children and mothers were available for the analyses investigating motor, cognitive and language development and temperament in children of mothers with ED and non-ED. Data on 46,156 children and mothers were available for the analyses investigating childhood psychopathology in children of mothers with ED and non-ED.

Patterns of missingness and attrition in the sample were investigated using logistic regression prior to any analyses, using missingness as the outcome variable and socio-demographic variables (maternal age, maternal social status and co-habituating status, and parity) and maternal ED as predictors. Missingness on the outcomes under study was associated with maternal ED; having missing data at 18 months. Attrition in the variables was associated with higher parity, higher maternal age and lower maternal social status.

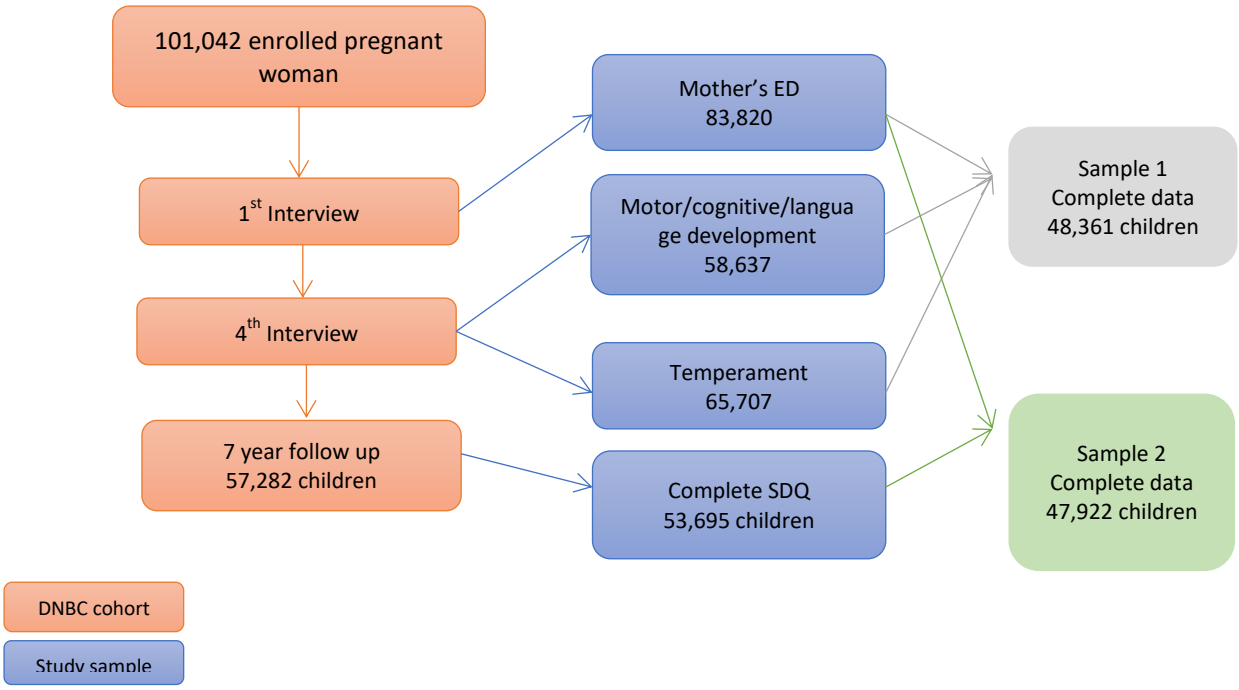


Figure 5.2.

5.2.4.1. Data analysis

All continuous variables were examined individually to check for inconsistencies/outliers and normality using tabulations and histograms. The primary outcome variables investigated in the paper were the following: child development, temperament and SDQ. Pearson/spearman (depending on the distribution of the variables) correlations were used to investigate correlations between outcome variables. The distribution of the covariates was studied according to the main predictor using chi-square (for categorical variables) and F-tests (for continuous variables) in order to provide a comparison between exposure groups.

Associations between maternal ED and SDQ scores were investigated using ordinal regression analyses, in which the OR represent the increase in the ordered odds of being in the abnormal (clinical), vs borderline, vs normal range for each subscale (and the total score). Associations between maternal ED and the outcomes at 18 months were investigated using logistic regression. All assumptions for ordinal regression analyses were met.

All analyses were stratified by gender based on previous results highlighting differences based on gender. Initially crude analyses were run. Additional *a priori* confounders (maternal age, maternal social status, birth-weight and parity) were included in adjusted models. All analyses were run using SPSS 21 (SPSS Inc., USA), and a two-tailed significance level of 5% was used.

Post-hoc mediation analyses

We investigated the role of temperament as a possible mediator of the associations between maternal ED and child psychopathology. Mediation was tested in SPSS following the methodology by MacKinnon and Dwyer for mediation with categorical variables (MacKinnon & Dwyer, 1993).

5.2.5. Ethical approval

The DNBC was approved by the Committee on Biomedical Research Ethics and all women who participated in the study gave informed written consent. The present study was approved by the DNBC steering committee (ref. 2010-33).

5.3. Results

5.3.1. Socio-demographic data

Results are shown in table 1. Socio demographic characteristics were studied across exposed and unexposed women. Co-habitation status (χ^2=51.26, p<0.01), parity (χ^2=31.74, p<0.01), social status (χ^2=106.61, p<0.01), birth weight (F=10.24, p<0.01) and maternal age (F=9.86, p<0.01) were found to be significantly different between groups.

Table 5.1. Socio demographics across sample studied: comparison using chi-square and F-tests depending on variable

	BN N=931	**AN** N=906	**AN+BN** N=360	**Unexposed** N= 46206	**Statistic**
Child gender, %					
Male	282 (50.54%)	280 (50.72%)	103 (53.37%)	14712 (50.37%)	χ^2=0.71, p=0.871
Female	276 (49.46%)	272 (49.28%)	90 (46.63%)	14487 (49.61%)	
Prematurity, %					
> 37 weeks	530 (95.84%)	516 (95.20%)	187 (97.40%)	27763 (9.56%)	χ^2=1.98, p=0.577
< 37 weeks	23 (4.16%)	26 (4.80%)	5 (2.60%)	1144 (3.96%)	
Parity, %					
Primiparae	348 (62.37%)	293 (53.08%)	102 (52.85%)	14761 (50.60%)	χ^2=31.74, p<0.0001
Multiparae	210 (37.63%)	259 (46.92%)	91 (47.15%)	14411 (49.40%)	
Education/employment status, %					
Skilled or unskilled worker	183 (33.27%)	196 (35.83%)	55 (29.25%)	12594 (43.50)	χ^2=150.49, p<0.0001
Professional occupation	234 (42.55%)	211 (38.57%)	71 (37.77%)	11545 (39.88%)	

Student/Unemployed	133 (24.18%)	140 (25.60%)	62 (32.98%)	4811 (16.62%)	
Gestational age (weeks), mean (SD)	40.14 (1.71)	39.87 (1.706)	39.97 (1.43)	40.02 (1.67)	F=2.50, p=0.057
Birthweight (g), mean (SD)	3599.03 (546.71)	3488.37 (528.41)	3505.90 (529.07)	3604.66 (544.13)	F=10.24, p<0.0001
Maternal age (years), mean (SD)	28.56 (4.30)	29.14 (4.81)	28.66 (4.84)	29.40 (4.16)	F=9.86, p<0.0001

5.3.2. Maternal ED and development, temperament, and mother child relation at 18 months of age

5.3.2.1. Cognitive and language development

Girls: girls of mothers with ED were comparable to girls of non-ED women (table 2).

Boys: Boys of women with AN were less likely to have delayed cognitive and language development in crude and adjusted analyses (OR=0.75, 95%CI: 0.62-0.91) compared to boys of unexposed women (Table 2).

5.3.2.2. Child temperament

Girls: Women with BN were more likely to perceive their daughter as having a difficult temperament in crude and adjusted analyses (OR=1.85, 95%CI: 1.30-2.65) compared to unexposed women (Table 2).

Boys: Women with AN and those with both AN and BN were more likely to perceive their son as having a difficult temperament in crude and adjusted analyses (OR=2.33, 95%CI: 1.34-4.07) compared to unexposed women. (Table 2).

5.3.3. Maternal ED and child SDQ at 7 years

Girls: Girls of women who reported BN had higher odds of conduct difficulties in crude and adjusted analyses (OR=1.41,95% CI: 1.09-1.83) compared to girls of unexposed women (Table 3). Girls of women who reported AN had higher odds of emotional difficulties in crude and adjusted analyses (OR=1.28,95% CI: 1.00-1.63) compared to girls of unexposed women (table 3).

Boys: Boys of women with BN had higher odds of having total, conduct, hyperactivity and peer difficulties in crude and adjusted analyses (respectively: OR = 1.51, 95%CI: 1.23-1.84; OR=1.32, 95% CI: 1.05-1.66; OR=1.33, 95%CI: 1.08-1.64; OR=1.37, 95%CI: 1.12-1.67) when compared to boys of unexposed women (Table 3). Boys of women with AN

had higher odds of total, emotional and conduct difficulties in crude and adjusted analyses (respectively: OR=1.37, 95% CI: 1.10-1.70; OR=1.56, 95%CI: 1.23-1.98; OR=1.32, 95% CI: 1.04-1.67) compared to boys of unexposed women (Table 3).

Boys of women with AN and BN had higher odds of total, emotional and peer difficulties in crude and adjusted analyses (respectively: OR=1.91, 95%CI: 1.41-2.59; OR = 1.92, 95% CI: 1.36-2.70; OR = 1.71, 95% CI: 1.26-2.32) compared to boys of unexposed women (table 3).

5.3.4. Post-hoc mediation analyses

There was a significant indirect effect of maternal BN on SDQ total, emotion, conduct, hyperactivity and peer scores mediated via child temperament (respectively: estimate=0.16, SE=0.01, p< 0.01; estimate=0.24, SE=0.01, p<0.01; estimate=0.08, SE=0.01, p< 0.01; estimate=0.07, SE=0.01 p< 0.01 and estimate=0.10, SE=0.01, p< 0.01). The direct effects from maternal BN to SDQ total, conduct and hyperactivity scores were also significant (respectively: estimate=0.05, SE=0.01, p<0.01; estimate=0.29 SE=0.10, p<0.01 and estimate=0.32, SE=0.09, p<0.01).

Child temperament also mediated the effect of maternal AN on SDQ total, emotion, conduct, hyperactivity and peer scores (indirect effects respectively: estimate=0.16, SE=0.01, p<0.01; estimate=0.24, SE=0.01 p<0.01; estimate=0.08, SE=0.01, p<0.01; estimate=0.07, SE=0.01, p<0.01 and estimate=0.10, SE=0.01, p<0.01). There were no significant direct effects.

Table 5.2. Maternal eating disorders and child's developmental, temperament and attachment variables at 18 months: odds ratios and (95%confidence intervals) from logistic regression

	Crude OR (95% CI)			Adjusted [a] OR (95% CI)			
	BN (N=446)	AN (N=460)	AN+BN (N=167)	BN (N=446)	AN (N=460)	AN+BN (N=167)	Unexposed (N=23458)
Female							
Delayed motor development	1.12 (0.63-2.00)	0.58 (0.26-1.31)	0.56 (0.14-2.26)	1.18 (0.66-2.12)	0.56 (0.25-1.27)	0.54 (0.13-2.20)	Ref.
Delayed cog/language development	0.87 (0.72-1.06)	0.86 (0.71-1.05)	0.81 (0.52-1.04)	0.91 (0.75-1.11)	0.88 (0.72-1.08)	0.76 (0.53-1.09)	Ref.
Child's difficult temperament	1.91*** (1.34-2.73)	1.58* (1.06-2.34)	1.15 (0.54-2.47)	1.85*** (1.30-2.65)	1.46 (0.98-2.19)	1.07 (0.50-2.29)	Ref.
	BN (N=491)	AN (N=449)	AN+BN (N=193)	BN (N=491)	AN (N=449)	AN+BN (N=193)	Unexposed (N=22258)
Male							
Delayed motor development	0.73 (0.41-1.30)	0.73 (0.41-1.30)	0.69 (0.26-1.88)	0.77 (0.43-1.38)	0.71 (0.40-1.27)	0.77 (0.23-1.72)	Ref.
Delayed cog/language development	0.91 (0.76-1.10)	0.73*** (0.60-0.88)	0.90 (0.66-1.23)	0.94 (0.78-1.13)	0.75** (0.62-0.91)	0.92 (0.67-1.26)	Ref.
Child's difficult temperament	1.47† (0.98-2.22)	1.60** (1.08-2.38)	2.44** (1.40-4.24)	1.36 (0.89-2.06)	1.56* (1.05-2.31)	2.33** (1.34-4.07)	Ref.

*p≤0.05, **p≤0.01, ***p≤0.001; [a] Adjusted for maternal age, maternal social status, parity and birth weight

Table 5.3 Maternal eating disorder and child psychopathology at 7 years of age (females): Odds ratios and (95%confidence intervals) from ordinal logistic regression.

	Crude OR (95% CI)			Adjusted [a] OR (95% CI)			
	BN (N=427)	AN (N=438)	AN+BN (N=161)	BN (N=427)	AN (N=438)	AN+BN (N=161)	Unexposed (N=21483)
Female							
Total difficulties	1.04 (0.81-1.32)	1.25 (0.99-1.56)	1.35 (0.94-1.94)	1.00 (0.78-1.27)	1.19 (0.95-1.50)	1.30 (0.90-1.87)	Ref.
Emotional difficulties	1.08 (0.83-1.40)	1.34** (1.05-1.71)	1.44 (0.98-2.12)	0.99 (0.76-1.29)	1.28*(1.00-1.63)	1.34 (0.91-1.99)	Ref.
Conduct difficulties	1.39** (1.07-1.80)	1.10 (0.84-1.45)	0.80 (0.48-1.34)	1.41**(1.09-1.83)	1.08 (0.82-1.43)	0.79 (0.48-1.32)	Ref.
Hyperactivity difficulties	1.23 (0.98-1.54)	1.04 (0.82-1.31)	1.23 (0.85-1.79)	1.20 (0.96-1.51)	1.00 (0.79-1.27)	1.20 (0.83-1.74)	Ref.
Peer difficulties	1.13 (0.89-1.44)	1.17 (0.93-1.48)	1.32 (0.91-1.91)	1.12 (0.88-1.43)	1.15 (0.91-1.46)	1.29 (0.88-1.87)	Ref.
Pro-social difficulties	1.02 (0.80-1.31)	1.08 (0.85-1.37)	1.03 (0.70-1.53)	1.05 (0.83-1.35)	1.11 (0.87-1.40)	1.07 (0.72-1.59)	Ref.

*p≤0.05, **p≤0.01, ***p≤0.001; [a] Adjusted for maternal age, maternal education/employment, parity and birth weight

Table 5.4 Maternal eating disorder and child psychopathology at 7 years of age (males): Odds ratios and (95%confidence intervals) from ordinal logistic regression.

	Crude OR (95% CI)			**Adjusted [a] OR (95% CI)**			
	BN (N=473)	AN (N=432)	AN+BN (N=185)	BN (N=473)	AN (N=432)	AN+BN (N=185)	Unexposed (N=22557)
Male							
Total difficulties	1.58***(1.29-1.93)	1.40**(1.13-1.74)	1.95***(1.44-2.64)	1.51***(1.23-1.84)	1.37**(1.10-1.70)	1.91***(1.41-2.59)	Ref.
Emotional difficulties	1.32*(1.04-1.68)	1.62***(1.28-2.05)	1.94***(1.39-2.72)	1.23 (0.97-1.57)	1.56***(1.23-1.98)	1.92***(1.36-2.70)	Ref.
Conduct difficulties	1.36**(1.08-1.70)	1.32*(1.04-1.68)	1.32 (0.92-1.90)	1.32*(1.05-1.66)	1.32*(1.04-1.67)	1.28 (0.89-1.83)	Ref.
Hyperactivity difficulties	1.37**(1.11-1.69)	1.01 (0.79-1.27)	1.18 (0.84-1.67)	1.33**(1.08-1.64)	1.00 (.078-1.26)	1.16 (0.82-1.64)	Ref.
Peer difficulties	1.40***(1.14-1.70)	1.21 (0.97-1.50)	1.74***(1.29-2.35)	1.37**(1.12-1.67)	1.19 (0.96-1.48)	1.71***(1.26-2.32)	Ref.
Pro-social difficulties	1.11 (0.88-1.40)	0.94 (0.73-1.22)	1.39 (0.99-1.96)	1.14 (0.91-1.44)	0.94 (0.73-1.22)	1.40 (0.99-1.97)	Ref.

*p≤0.05, **p≤0.01, ***p≤0.001; [a] Adjusted for maternal age, maternal education/employment, parity and birth weight

5.4. Discussion

A recent population-based prospective study showed that children at high-risk for ED (children of mothers with lifetime ED) had increased odds of specific psychopathology in adolescence. However attrition in early adolescence means small sample sizes were achieved by this stage (N. Micali, B. De Stavola, G. B. Ploubidis, E. Simonoff, & J. Treasure, 2014). The present study aimed to extend and replicate these findings by using data from a larger population-based study and investigate the development of psychopathology at seven years of age. Reproducibility is a defining feature of science and replication of results using different samples in different countries, with a different confounding structure can lead to increased confidence in the findings (Collaboration, 2015). Results show that children of women with eating disorders were at risk of developing psychopathology in mid-childhood across most domains (emotional, conduct, hyperactivity and peer difficulties); and were more likely to have a difficult temperament. Boys of mothers with AN were less likely to have a delayed language and cognitive development. Mediation analyses showed that there was a significant indirect effect of maternal AN and maternal BN on psychopathology mediated via child temperament at 18 months.

Boys of women with AN had lower odds of having delayed cognitive and language development. These results are consistent with previous findings from a study in children of mothers with AN (R. Kothari et al., 2013a). However, this study did not replicate the association between maternal eating disorder and motor development. This might be due to the latter study using an objective measure of development, rather than the maternally reported one used in the current study.

Mothers with ED tended to report their children as having a more difficult temperament. However, because the present study used maternal report rather than direct observational measures for child temperament, the nature of the associations remains unclear. Questions included in this section included ratings on the child's perceived restlessness, activity and happiness compared to other children their own age, all of which are subjective. One possible explanation is that children of mothers with ED are objectively more difficult than those of healthy control mothers, however, we cannot discard the possibility that mothers with ED may perceive their child as being more difficult. Mothers

with ED have been shown to have difficulties with greater anxiety, which in turn has been shown to predict a difficult infant temperament (Austin, Hadzi-Pavlovic, Leader, Saint, & Parker, 2005). It has also been suggested that social information processing bias (previously observed in ED (A. Harrison, Tchanturia, & Treasure, 2010)) may lead mothers to interpret their child's behaviour as more negative (H. J. Watson et al., 2014). However, even if these difficulties are in 'the eye of the beholder' mothers perception is likely to impact on their own sense of being a mother (Crockenberg & Acredolo, 1983) and are likely to negatively impact on the child's later development.

Boys of women with BN had higher odds of developing psychopathology across most domains. In particular, findings that hyperactivity in boys was associated with maternal BN are consistent with a previous study by Micali *et al* (N. Micali et al., 2013). This might be due to the fact that BN and attention deficit and hyperactivity (ADHD) disorder share several key features. Studies have shown that individuals with a diagnosis of ADHD are at greater risk of developing an ED (Biederman et al., 2010; Sonneville et al., 2015; Yoshimasu et al., 2012) and this association is particularly true for BN/binge eating (Nazar et al., 2008). However, in this study, this was only significant for boys. One possible explanation for this finding is that it might be due to the gender distribution of hyperactivity and attention difficulties, which are more common in boys. An alternative explanation is that mothers might be more likely to report these difficulties selectively by gender. Despite this, the findings partly underscore the strong association between hyperactivity/inattention and BN. Whether the nature of this association is via a shared genetic link or whether hyperactivity/inattention is an intermediate phenotype for BN remains to be determined.

Both girls and boys of women with AN had higher odds of having emotional problems (internalising problems such as anxiety). These results are consistent with previous research which highlighted the association between AN and emotional/anxiety disorders (Pamela K Keel, Klump, Miller, McGue, & Iacono, 2005; Tracey D Wade, Bulik, Neale, & Kendler, 2000), and are consistent with results from studies by Micali *et al* (N. Micali, B. De Stavola, et al., 2014; N. Micali et al., 2013) using a large community cohort (ALSPAC). Results are also consistent with the hypothesis that anxiety may represent an intermediate phenotype for ED (Goddard & Treasure, 2013) across diagnoses. The study by Goddard and Treasure recruited family trios (an offspring and both her parents) in which the offspring either had an eating disorders or was a healthy control and examined trait

anxiety, social anxiety and emotional difficulties. They found that trait anxiety was elevated in ED offspring and in parents of ED offspring who had never had an ED. These results support a predispositional model of trait anxiety in the development of EDs (L. R. R. Lilenfeld, Wonderlich, Riso, Crosby, & Mitchell, 2006; J. Swinbourne et al., 2012; J. M. Swinbourne & Touyz, 2007) and are consistent with previous studies that have suggested anxiety is a familial risk factor for EDs with genetic underpinnings (L. R. Lilenfeld et al., 1998; Strober, Freeman, Lampert, & Diamond, 2007). Kaye *et al* (W. H. Kaye et al., 2004) proposed that premorbid high anxiety in individuals with ED can make them more vulnerable to perceive feedback from their environment in a negative way. Furthermore, testimonies from ED patients, also show calorie restriction as a way of gaining control and reducing anxiety levels (becoming intrinsically rewarding) (Dignon, Beardsmore, Spain, & Kuan, 2006).

Another interesting finding relates to peer difficulties in this sample. Results showed that boys of women with a lifetime history of BN (both those who reported BN only and AN+BN) have more peer problems when compared to children of healthy women. The questions in the SDQ that load to the peer problems reflect problems engaging and communicating with other children, which could be a reflection of social communication problems. We have previously shown that children at risk for BN had difficulties in recognising emotions and had worse social communication (R. Kothari, Barona, Treasure, & Micali, Under review) compared to controls. These results might point to an overlap between BN and social communication difficulties. Interestingly, in the literature it is patients with AN who have been suggested to have social communication difficulties. Gillberg and colleagues (C. Gillberg & Råstam, 1992) proposed that some cases of AN reflected underlying social communication difficulties. Since then, more research has investigated traits present in both disorders and common possible intermediate phenotypes have been suggested for both disorders. Furthermore, the interpersonal model for AN (Chapter 2) proposes that anxiety and social communication difficulties can be a vulnerability for the development of the disorder (J. Treasure & U. Schmidt, 2013). Zucker and colleagues (Zucker et al., 2007) identified premorbid traits of anxiety as well as comorbidities with ASD (as well as social phobia and OCD) as aspects of central importance in the development of a risk model for AN.

It is important to take into account the possibility that children of parents with a psychopathology have a general higher risk of having any psychopathology, and that

findings of higher internalizing or externalising disorders are not intermediate phenotypes but a general non-specific risk for psychopathology. However, it has been found that specific anxious/depressed behaviours, somatic problems or conduct behaviours in children were best predicted by the same problem behaviour in the parent (van Meurs, Reef, Verhulst, & van der Ende, 2009). Similar results have been found for the relationship between depression in children of mothers with depression (Beck, 1999), although they were also found to be at risk of developing conduct behaviour (Beck, 1999). Importantly, research has consistently found a higher prevalence of ED in first degree relatives of those with an ED (Bulik, 2005; Bulik et al., 2006; L. R. Lilenfeld et al., 1998; Mazzeo & Bulik, 2009), and numerous twin studies suggest that liability to AN and BN is significantly influences by additive genetic factors (Klump, Miller, Keel, McGue, & Iacono, 2001; T. Wade, Martin, & Tiggemann, 1998; Tracey D Wade et al., 2000; Tracey D Wade et al., 2011). It is also important to consider the complex interplay of genetic and environmental factors in the development of psychopathology. Many associates have been found between parental practices as childhood anxiety, such as parental control (McLeod, Wood, & Weisz, 2007; Weijers, van Steensel, & Bögels, 2018). Of course, children also learn from their parents (e.g. vie conditioning or operant learning procedures, or via imitation and listening), and through their own personalities and ways of copying, in this sense parental psychopathology can plan a role as part of a learned behaviour. For example, parental stress has been found to predict child behaviour problems (Ashford, Smit, van Lier, Cuijpers, & Koot, 2008) (e.g., Ashfort et al., 2008). Thus, parents may play a role in the development of their child's psychopathology in multiple ways, both through genetic charge and environmentally. In ED, one way of trying to discern between genetic and environmental parental load is by studying children or mothers with a current ED and those with a past ED. The hypothesis being that in both cases children will have a higher-genetic risk, but for current cases their environment might be another predictor. In the current study, mediation analysis were used to understand the direct or indirect nature of maternal ED diagnosis. We used the data collected with regards to child temperament, where mothers reported their child as having a difficult temperament which we hypothesise will have an impact on their behaviour and parenting. Our mediation analyses suggest some differences in mediation pathways across maternal diagnosis; for example, whilst child temperament partially mediated the effect of BN on child psychopathology (pointing to a potential genetic and environmental effect), for maternal AN, there was no direct effect of the ED on child psychopathology.

Finally, there were some significant differences in maternal age and parity between groups which may shed some light into differences between maternal diagnosis. There was a higher proportion of mothers with only one child in mother with BN compared to mothers with AN, AN+BN and unexposed. It is possible to hypothesise that mother with BN find motherhood a ore overwhelming experience, with difficult conflicts between the demands of bringing up a child and the bulimic disorder. This in turn could create tension in the mother-child relationship. Mothers with BN and those with AN+BN were also younger than those with AN and unexposed. BN has been linked to a more impulsive personality profile (Seitz et al., 2013) which could partly explain a younger age in mothers with BN. These significant differences between maternal ED groups provide some more insight into differences between diagnoses and could possibly be linked with neuropsychological profiles identified in AN and BN. Further investigation is warranted.

5.4.1. Strengths and limitations

The main strengths of this study are the large sample size, the prospective data collection, and the availability of register-based information on socio-demographic data. This is the largest study investigating maternal ED and the effect of childhood psychopathology to date.

Limitations have to be taken into account: firstly the information on maternal ED was obtained from self-report during interviews. However, a recent study validated self-reported ED in a similar population based sample of pregnant women with very good sensitivity and specificity of self-reported AN and BN (N Micali et al., 2012). Furthermore, there is evidence from past research that self-report measures might be as reliable, as other, more frequently, used measures (Anna Keski-Rahkonen et al., 2006). Another important limitation is that data on the children were obtained from mothers (shared method variance). The variables on child temperament and difficulties looking after the child were based on questionnaires designed for DNBC and have therefore not been validated. While measures obtained from mothers can be subjective, it is an important way of acquiring information in large samples and at a young age, when the child cannot self-report and an informant (e.g. teacher) is not available. Taking this into consideration, it is important that future studies should explore the psychometric properties of these measures in order to validate them. One possible future study, could consider if early temperament can predict later childhood psychopathology as a way of helping clarify the

validity of the measure. We found evidence of selective attrition amongst women with ED, therefore generalizability of this study might be affected. It is difficult to know whether the selective attrition might be reflective of more severely ill mothers being lost to follow up or whether children with lower levels of psychopathology might be lost to follow up, therefore whether results are biased towards the null or not. However in a previous similar study (Nadia Micali et al., 2013), children with higher psychopathology were more likely to be lost to follow-up therefore suggesting that OR might be biased towards the null. This means that we may be underestimating the effect found in our study if we expect more severely ill mothers to be lost to the study.

5.5. Conclusion

This study has important research implications for our understanding of shared diathesis for psychopathology and ED. At risk studies have the potential to provide important information about intermediate phenotypes. Further investigation of specific risk pathways and mechanisms in the intergenerational transmission of psychopathology in children of parents with ED is needed. Studies focusing on children at risk and on understanding risk mechanisms will be indispensable for developing early intervention programs as well as prevention strategies, and to improve our understanding of ED and their pathophysiology.

Chapter 6. White matter alterations in anorexia nervosa: evidence from a voxel-based meta-analysis

This chapter has been published in Neuroscience and Biobehavioural reviews (Barona et al., 2019) and findings were presented at International Conference on Eating Disorders in 2017.

6.1. Introduction

As established in Chapter 1, in the last decades there have been a number of advances in our understanding of volumetric and functional brain differences in ED's. The heterogeneity of findings and large number of areas involved in the pathophysiology of ED's suggests a critical role of interconnections of cortical and subcortical regions, warranting therefore further exploration of the structural connectivity in ED. More recently thanks to the development of neuroimaging techniques that allow us to study white matter structures through their water diffusion properties we have been able to investigate these connections and microstructure of white matter bundles.

As established in Chapter 2, diffusion tensor imaging (DTI), a non-invasive magnetic resonance method based on the diffusion characteristics of water, has been used to study WM microstructure (Beaulieu, 2002; Le Bihan & Johansen-Berg, 2012). One of the most commonly used parameters for measuring WM microstructure is fractional anisotropy (FA), a scalar value that measures the directionality of water diffusion. To date, the large majority of DTI studies in AN have been based on small samples ($n < 25$) and have mostly used voxel-based approaches to study water diffusion in the brain, with only three studies to date utilising tractography, however, results have been mixed (for a review see Monzon et. al. 2016 (Martin Monzon, Hay, Foroughi, & Touyz, 2016)). WM microstructure alterations have been found in acute patients with AN, both adults (J. Cha et al., 2016a; Frieling et al., 2012b; Hayes et al., 2015; Kazlouski et al., 2011a; Nagahara et al., 2014; Shott, Pryor, Yang, & Frank, 2016; E. Via et al., 2014; Yau et al., 2013) and adolescents (J. Cha et al., 2016a; G. K. Frank, M. E. Shott, J. O. Hagman, & T. T. Yang, 2013; S. Gaudio et al., 2017; Hu et al., 2017; Travis et al., 2015; Vogel et al., 2016), in multiple WM

tracts, including the fornix, cingulum, posterior thalamic-radiations, fronto-occipital fasciculus, superior longitudinal fasciculus and cerebellum. Studies have also found differences in recovered patients (J. Cha et al., 2016a; Frieling et al., 2012b; Shott et al., 2016; Yau et al., 2013). However, other studies have also yielded non-significant findings in both acute and recovered patients. Only three studies have used tractography to provide further detail on WM microstructure. Travis and colleagues in 2015 (Travis et al., 2015) studied adolescents with acute AN and found both increased FA (in thalamic radiation and left anterior longitudinal fasciculus) and decreased FA (in fornix and superior longitudinal fasciculus); whilst Hayes and colleagues in 2015(Hayes et al., 2015) studied adults with acute AN who were treatment-resistant and found decreased FA in several areas including the fornix and cingulum. More recently Pfuhl and colleagues (Pfuhl et al., 2016) found no differences in WM microstructure in acute or recovered patients with AN. Although most studies have found FA to be reduced, two studies, one by Frank and colleagues in 2013 (G. K. Frank, M. E. Shott, J. O. Hagman, & T. T. Yang, 2013) and a second by Vogel and colleagues in 2016 (Vogel et al., 2016) reported an increase in FA (Vogel only reported increase while Frank and colleagues reported both increased and decreased FA), both studies only included adolescents. Available evidence therefore suggests that FA alterations are present in AN, however there is significant between-study variability as to the spatial distribution of these abnormalities making any conclusions on WM microstructure in patients with AN complex.

To date only two studies have investigated WM microstructural differences in BN (He, Stefan, Terranova, Steinglass, & Marsh, 2016; Mettler, Shott, Pryor, Yang, & Frank, 2013). Both studies found alteration in WM microstructure in patients with BN. The studies showed alterations in projection, association and commissural tracts. Specifically both studies found alterations in the inferior fronto-occipital and uncinate fasciculi. Other areas showed to be altered were the corona radiata, corpus callosum, forceps minor and major, SLF and cingulum were also found. Although both studies showed lowered FA in patients with BN compared to healthy controls, these alterations in BN are poorly explored and therefore understood.

The current study aims to identify whether consistent changes in regional FA could be identified in AN patients compared to controls in order to inform further research in children at-risk. To this purpose, we conducted a search of the available DTI literature in AN followed by a meta-analysis of eligible studies using anisotropic effect size-signed

differential mapping (AES-SDM). As the most common method used in the area of study are voxel-based methods, with only three tractography studies published to date, the meta-analysis will focus only on voxel-based methodology. We also performed a meta-regression to examine the mediating effect of Body Mass Index (BMI). Two exploratory subgroup analyses were also undertaken to determine the effect of age (looking only at adult studies) and stage of the disorder (looking only at acute studies).

6.2. Methods and materials

6.2.1. Data source

We adhered to the guidelines detailed in the PRISMA Statement (Moher, Liberati, Tetzlaff, & Altman, 2009) and registered this study in PROSPERO (registration number CRD42016042525). A systematic and comprehensive search of databases, including Embase, PubMed and Psychinfo was carried out for studies published between January 1990 and May 2018. The search was performed using the following MESH terms and keywords: ('eating disorders' or 'anorexia nervosa' or 'AN' or 'anorexia') and ('dti' or 'diffusion tensor imaging' or 'diffusion weighted imaging' or 'tractography'). The search was finalized on the 1st May 2018.

6.2.2. Study selection

Eligible DTI studies fulfilled the following inclusion criteria: (1) conducted voxel-based analyses; (2) compared FA values between AN and healthy controls; (3) reported whole-brain results in 3-dimensional coordinates (x, y, z); (4) used a threshold for significance and (5) were published in English.

Studies were excluded if: (1) it was a tractography based study; (2) studies of eating disorders other than AN.

6.2.3. Quality assessment and data extraction

Two authors independently screened, extracted and cross-checked the data from retrieved articles based on the eligibility criteria outlined above. The quality of the final studies was

also independently checked by both authors using the Newcastle-Ottawa Scale (Wells et al.) for assessing the quality of non-randomized studies in meta-analyses. For each study the following data were extracted: demographic information (age), sample size (sample size), age (mean age), BMI (mean BMI), AN type and AN status (acute vs recovered) and the three-dimensional peak coordinates of case-control differences in each study.

6.2.4. Voxel-based meta-analysis

A voxel-based meta-analysis of regional case-control differences in FA values of WM was performed using SDM software v4.31 (http://www.sdmproject.com) (Radua & Mataix-Cols, 2009). The above method improves upon existing methods and has been used in several meta-analyses of voxel-based studies (Emre Bora, Fornito, Yücel, & Pantelis, 2010; Radua, Via, Catani, & Mataix-Cols, 2011). The software's main advantage is that it uses restricted maximum likelihood estimation of the variance with the reported peak coordinates to recreate maps of the positive and negative FA differences between patients and controls, rather than just assessing the probability or likelihood of a peak.

The method has three steps: (1) coordinates and magnitude of cluster peaks of case-control differences (e.g. the voxels where the differences between patients and health controls were highest in each study) are selected according to inclusion criteria, (2) the coordinates are used to recreate statistical maps, effect-size maps and their variances derived from t-statistics (Z- or p- values for significant clusters which were then converted to t-statistics using the SDM online conversion utilities), and (3) individual study maps are entered into the meta-analysis and the outcome is further tested in terms of sensitivity and heterogeneity.

In more detail, after the conversion of the coordinates to MNI space, an SDM map is created for each study using a specific mask (e.g. TBSS). The coordinates are then used to recreate statistical maps and effect sized maps. Pre-processing of reported peak coordinates (from each study) are done by recreating the clusters of difference by means on an anisotropic un-normalized Gaussian Kernel, so that the voxels more correlated with the peak coordinate have effect-sizes similar to those of the peak. Finally, the resulting individual maps are meta-analyzed to complement the main outcome with sensitivity and

heterogeneity analyses (The meta-analytic value of each voxel in the m-a map is defined as the proportion of studies reporting a coordinate around the voxel).

We first calculated the mean of the FA voxel peaks in the different studies, accounting for the variance and inter-study heterogeneity. A systematic whole-brain voxel-based jack-knife sensitivity analysis was performed using the leave-one out method. Exploratory subgroup analyses were also conducted to check for main differences based on age (adults vs. adolescents (under 18 years old) and illness status (acute vs. recovered).

The analytical parameters of SDM were those suggested by the software developers (Emre Bora et al., 2010; Radua & Mataix-Cols, 2009; Radua et al., 2012; Radua et al., 2011) and as follows: anisotropy = 1.0; isotropic full-width at half-maximum (FWHM) = 20 mm; voxel p = 0.005; cluster = 10 voxels with 500 repetitions of standard randomization tests. A specific mask and correlation template for white matter was used. We used FSL visualization software (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/) to visualize SDM maps that were overlaid onto a high resolution brain template (White matter atlas; http://www.dtiatlas.org/). An uncorrected $p < 0.001$ was used, which has been described by the authors to be empirically equivalent to a corrected $p < 0.05$. All parameters where used as suggested by the authors of the meta-analytic method (28, 29, 31) and as previously used in other studies.

The coordinates for one of the main significant area were extracted and separately mapped into a standard diffusion image to provide a three dimensional image of the most likely tracts traversing a bounding 5mm box centred on the coordinates using MRtrix software (http://www.mrtrix.org/).

6.2.5. Meta-regression

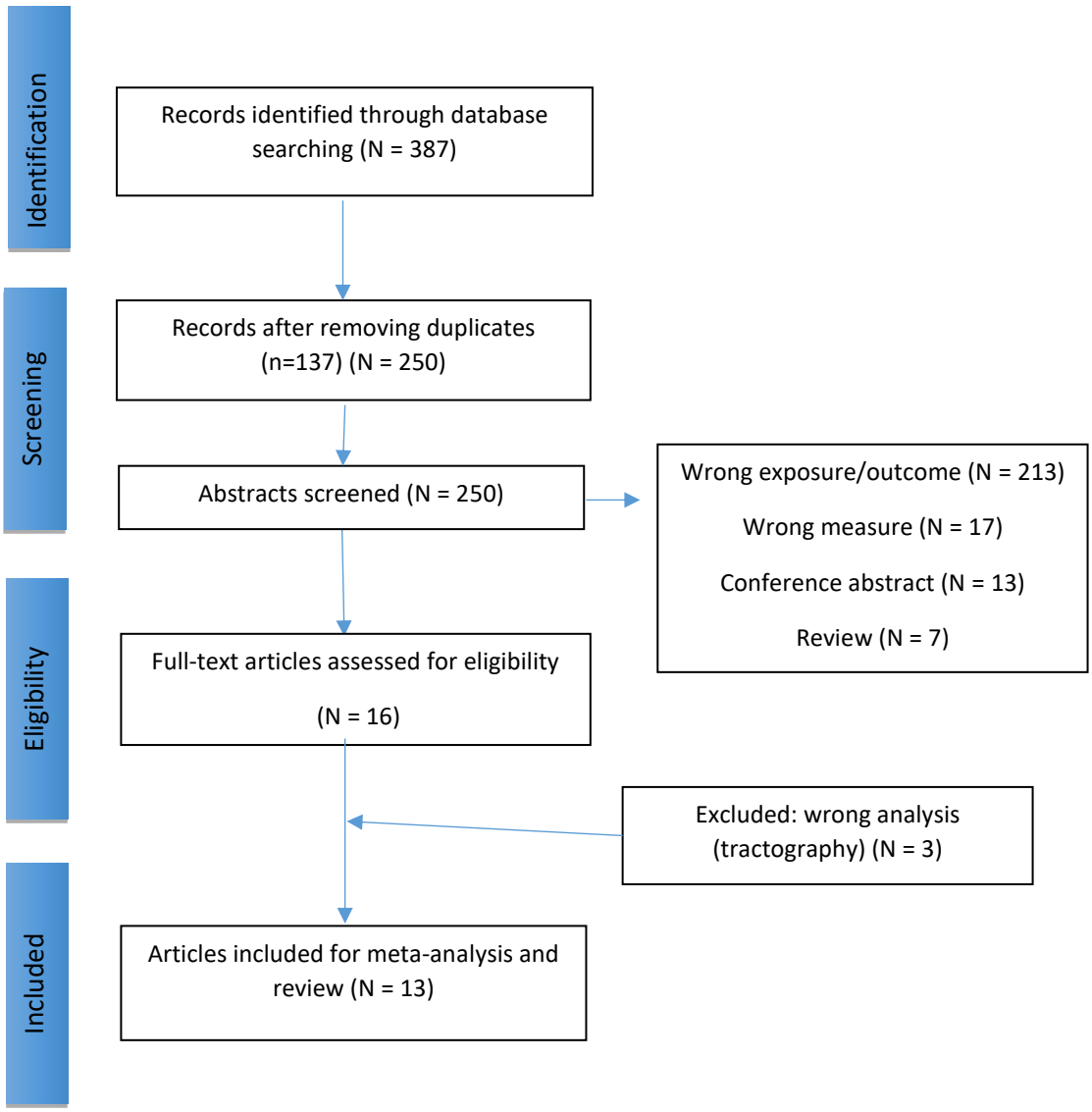
The effect of BMI was examined by means of simple linear regression, weighted by the square root of the sample size and restricted to only predict possible SDM values (i.e. from -1 to 1) in the observed range of values of the variable. The main output for each variable is a map of the regression slope. In order to minimize the detection of spurious relationships we decreased the probability threshold to 0.0005. Required abnormalities

need to be detected both in the slope and in one of the extremes of the regressor. Findings in regions other than those detected in the main analyses were discarded. Further regression analyses were done using only adult and acute subgroups. Since the BMI used for adolescent sample was not standardized and a low BMI for adult could a near normal for adolescents we run the regression analyses using the adult sample only. The acute group has a lower BMI and therefore we could expect the correlation to exist only in this group. Meta-regression results should be taken with some caution because of the limited variability in the data and the low sample size.

6.3. Results

The search strategy yielded a total of 387 studies, of which 13 met criteria for inclusion. The PRISMA diagram in Fig. 6.1 shows selection and exclusion of studies.

Figure 6.1. Flow diagram for the identification and exclusion of studies.



6.3.1. Meta-analysis: Included studies and sample characteristics

Table 6.1 summarizes the characteristics of the 13 studies included in the meta-analysis. Table 6.2 summarizes the technical characteristics of studies included in the meta-analysis.

The included studies reported FA alterations of WM in individuals with AN (N = 227, mean age 22.5) relative to healthy controls (N = 243, mean age 21.9).

Table 6.1. Summary of demographics of studies included in meta-analysis conducted with patients with AN using whole-brain voxel-based analysis.

	ED status	AN subtype	Age group	Cases	Controls	Mean age_cases (sd)	Mean age_controls (sd)	BMI_cases (sd)
Kazlouski, 2011	Acute	R and B/P	Adult	16	17	23.9 (7)	25.1 (4)	16.5 (1)
Frieling, 2012	Both	R and B/P	Adult	21	20	26.8 (6.9)	24.8 (2.6)	15.2 (1.4)
Frank, 2013	Acute	R and B/P	Adolescents	19	22	15.4 (1.4)	14.8 (1.8)	16.2 (1.1)
Yau, 2013	Recovered	R	Adult	12	10	28.7 (7.9)	26.7 (5.4)	21.2 (1.5)
Via, 2014	Acute	R	Adult	19	19	28.3 (9.5)	28.6 (8.6)	17.0 (1.1)
Nagahara, 2014	Acute	AN	Adult	17	18	23.8 (6.6)	26.2 (5.6)	13.6 (1.3)
Shott, 2015	Recovered	R	Adult	24	24	30.2 (8.1)	27.42 (6.3)	20.8 (2.4)
Vogel, 2016	Acute	R and B/P	Adolescents	22	21	15.0 (1.6)	15.2 (1.3)	15.4 (1.1)
Cha, 2016	Acute	AN	Both	22	18	19.5 (2.4)	20.5 (2.9)	17.3 (1.2)
Bang, 2017	Recovered	AN	Adult	21	21	27.6 (5.0)	26.1 (4.8)	20.5 (1.7)
Gaudio, 2017	Acute	R	Adolescents	14	15	15.7 (1.6)	16.3 (1.5)	16.2 (1.2)
Hu, 2017	Acute	R	Both	8	14	17.6 (2.2)	19.1 (3.1)	14.3
Olivo, 2017	Acute	EDNOS	Adolescents	12	24	15.3	14.1	18.7
Total				227	243	22.5 (5.0)	21.9 (3.9)	17.4 (1.4)

Table 6.2. Summary of technical imaging data and patient status at scanning time of studies included in meta-analysis conducted with patients with AN using whole-brain voxel-based analysis.

	MRI	Software	p value	Patient status at scanning
Kazkiysju, 2011	3T	SPM5[a]	P < 0.05, corrected (FDR[c])	within one or two weeks of admission, no electrolyte abnormalities
Frieling, 2012	3T	SPM2	P < 0.05, corrected (FDR)	recovered: BMI of 18.5 and no ED behaviours one year; acute: AN diagnosis
Frank, 2013	3T	SPM8	p < 0.005 uncorrected	within two weeks of start of treatment no rehydration
Yau, 2013	-	FSL	-	Recovered: 12 months
Via, 2014	1.5T	FSL[b]	p < 0.05, corrected (FWE[d])	at least one week of rehydration
Nagahara, 2014	3T	FSL	p < 0.05, corrected	no signs of rehydration
Shott, 2015	-	FSL	p < 0.05, corrected (FWE)	Recovered: 12 months, no ED behaviours
Vogel, 2016	3T	FSL/SPM	P < 0.05, corrected	Weight at admission
Cha, 2016	1.5T	FSL	-	at least 24 hours of rehydration
Bang, 2016	3T	FSL	p < 0.05, corrected (FWE)	Recovered: 12 month

Gaudio, 2017	1.5T	FSL	p < 0.05, corrected (FWE)	duration of less than 6 months and scanned within a week of diagnosis
Hu, 2017	3T	SPM	P < 0.05, corrected (FDR)	at least one week of rehydration
Olivo, 2017	3T	FSL	P < 0.05, corrected (FDR)	upon diagnosis

[a]SPM: statistical parametric mapping; [b]FSL: FMRIB software library; [c]FDR: false discovery rate; [d]FWE: family-wise error rate

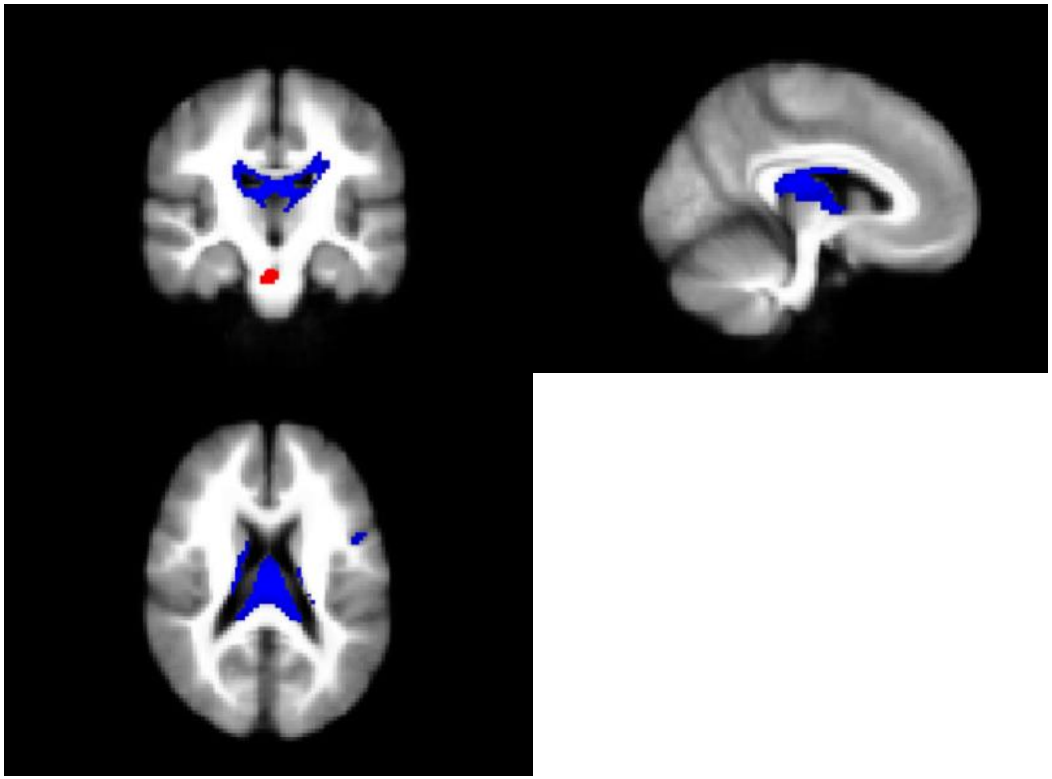
6.3.2. Regional differences in FA

MNI coordinates for the SDM meta-analysis were obtained from all 13 studies. As shown in table 6.3 and figure 6.2 and 6.3, patients with AN had significantly lower FA values in five clusters compared to healthy controls. Three of the five clusters exhibited a peak in the corpus callosum (CC) (MNI: -2,-18,18; 18,-58,30 and -22,24,30). The first peak (MNI = 2,-18,18) included voxels in the corpus callosum, thalamus, anterior thalamic projections, caudate nucleus, cortico-spinal projections and pons; the second peak (MNI = 18,-58,30) included voxels located in the corpus callosum, right precuneus and right cuneus and the third peak and smallest (MNI = -22,24,30) included voxels only in the corpus callosum. The two other peaks were found in the left superior longitudinal fasciculus II (MNI = -31,-34,36) and the left precentral gyrus, BA44 (MNI = -48,6,20). In addition, patients with AN had significantly higher FA values in the right cortico-spinal projections (MNI: 6,-16,-24), and right and left lingual gyrus BA18 (respectively: MNI = 10,-86,-12 and MNI = -28,-90,-14).

Table 6.3. Significant regional differences in FA values in patients with AN compared to healthy controls and results from Jacknife sensitivity analysis. less than 20 voxels are not represented in the breakdown of voxels.

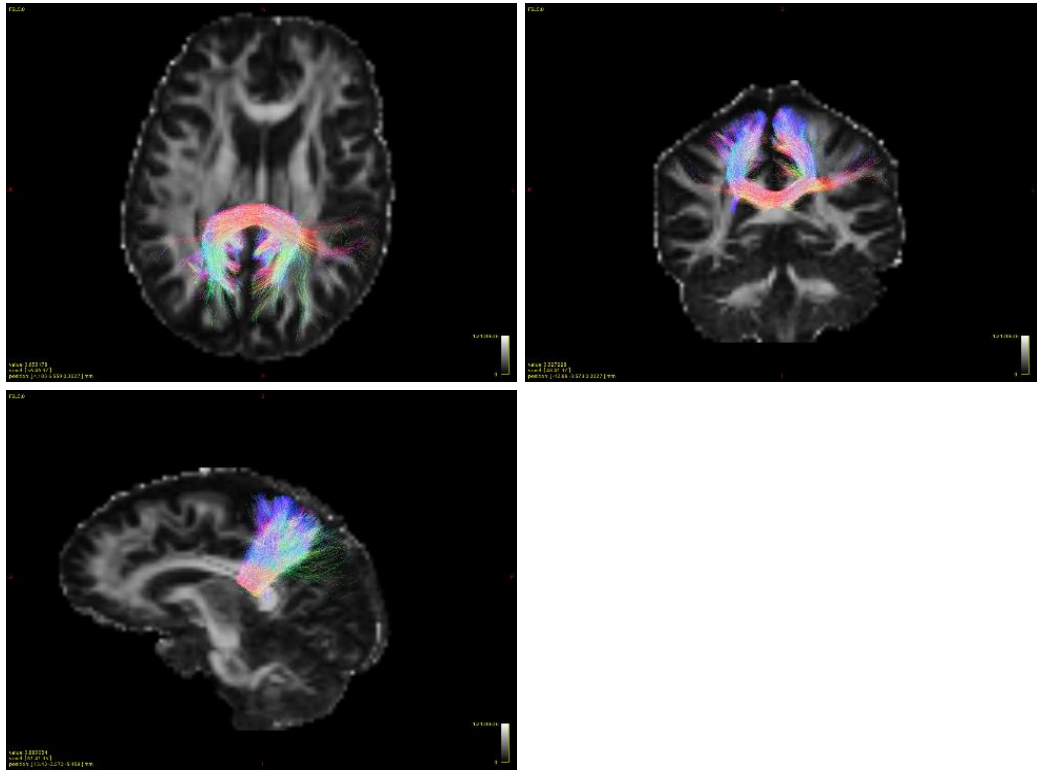
Region	Maximum			Cluster		
	MNI coordinates x, y, z	SDM value	Uncorrected p	Number of voxels	Breakdown (number of voxels)	Jacknife sensitivity analysis
Clusters of decreased FA						
Corpus callosum	-2,-18, 18	-1.954	0.000012696	2070	Corpus callosum (945), thalamus (395), anterior thalamic projections (382), caudate nucleus (56), cortico spinal projections (77), pons (57)	8/13
Corpus callosum	18,-58,30	-1.770	0.000065684	262	Corpus callosum (211), right precuneus (47), right cuenus (35)	10/13
Corpus callosum	-22,24,30	-1.335	0.001392961	91	Corpus callosum (91)	10/13
Left superior longitudinal fasciculus II	-31,-34,36	-1.387	0.001008451	66	Left superior longitudinal fasciculus II (66)	7/13
Left precentral gyrus, BA44	-48,6,20	-1.304	0.001658022	31	Left precentral gyrus BA44	9/13
Clusters of increased FA						
Right cortico-spinal projections	6,-16,-24	1.008	0.000757337	69	Right cortico spinal projections (61), right pons (29)	2/13
Right lingual gyrus, BA18	10,-86,-12	1.006	0.000788093	31	Right lingual gyrus	8/13
Left lingual gyrus, BA18	-28,-90,-14	1.001	0.000844777	20	Left lingual gyrus	9/13

Figure 6.2. Signed differential mapping-generated FSL map of areas showing altered FA in patients with AN identified in main meta-analysis.



Significant regional differences in FA values in patients with AN compared to healthy controls. Clusters of decreased FA are marked in blue and increased FA are marked as red

Figure 6.3. Tractography images of main replicable cluster in the CC (MNI coordinates = 18,-56,28)

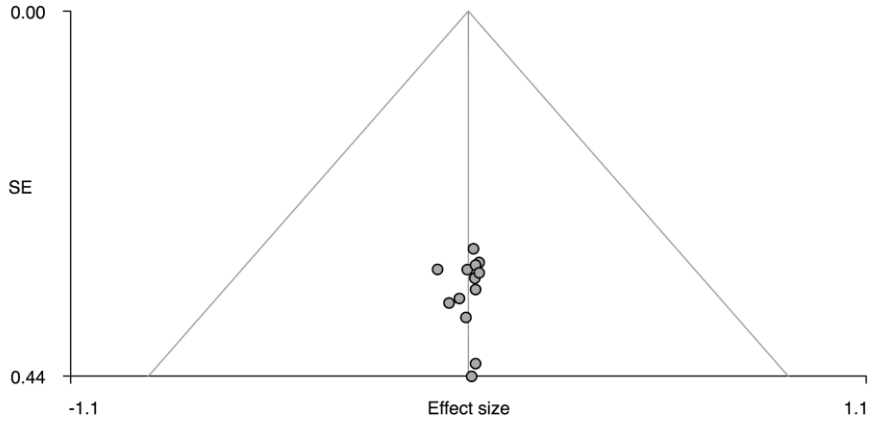


Three dimensional images showing WM tracts traversing a bounding box centred at x = 18, y = -58, z = 30 (the most replicable coordinate from main analyses) was mapped with MRtrix in a single typical individual. Axial view (A), coronal view (B) and sagittal view (C).

6.3.3. Sensitivity analysis

As shown in table 6.3, whole-brain jack-knife sensitivity analyses of the pooled meta-analysis indicated that FA reductions of AN patients in the second and third cluster in the CC (respectively: MNI: 18,-58,30; MNI: -31,-34,36) were highly replicable; these findings were detected in ten out of the thirteen studies. Increased FA in the right cortico-spinal projections was present in only two of the thirteen studies. As shown in figure 6.4, a funnel plot showed that all studies contributed to findings in the CC. Egger's test suggested no publication bias for these areas (Egger's p = 0.864).

Figure 6.4: Funnel plot demonstrating contribution of each study to the corpus callosum clusters.



6.3.4. Meta-regression

BMI was not associated with AN-related FA reductions in any of the clusters for the combined or adult sample.

BMI was associated with acute AN-related FA reductions in the cluster found in the Left precentral gyrus, BA 44 (MNI: (-48,6,20)).

6.3.5. Findings in the acute stage and in adults

As shown in table 6.4 and figures 6.5 and 6.6, the subgroup analyses revealed the following results.

Acute stage (Table 6.4 and Figure 6.5): patients with active AN had both significantly lower and higher FA values in different clusters when compared to healthy controls. There were five significant clusters of decreased FA: the first peak came up as undefined by the software (MNI = 2,-14,14) with voxels located in the corpus callosum, thalamus, anterior thalamic projections, pons, cortico spinal projections, right caudate nucleus and left superior longitudinal fasciculus. The second cluster was located in the corpus callosum (MNI = -22,24,30), the third cluster was located in the left precentral gyrus BA44 (MNI= -48,6,20), the fourth cluster was located in the left pons (MNI = -22,-14,-8) and the fifth cluster in the cingulum (MNI = 16,-56,28). In addition, acute patients also showed a number of small clusters of increased FA when compared to healthy controls (Table 4 and Figure 3).

Adults (Table 6.44 and figure 6.6): adult patients with AN had significantly lower FA values in two clusters when compared to adult healthy controls. The first cluster exhibited a peak in the right caudate nucleus (MNI: 16,2,24) and the second cluster exhibited a peak in the corpus callosum (18,-50,26).

Table 6.4. Significant regional differences in FA values in subgroups of patients with AN (adult subgroup; acute subgroup)

Adult subgroup

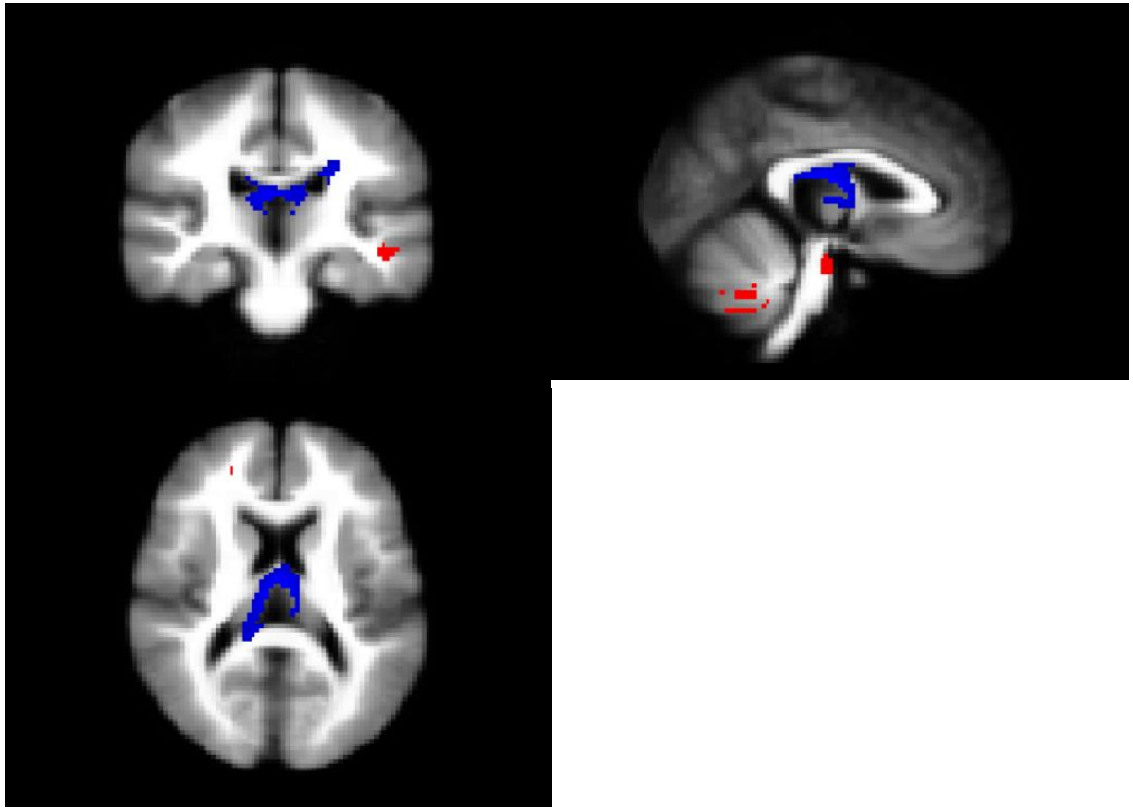
Region	Maximum			Cluster		
	MNI coordinates x, y, z	SDM value	Uncorrected p	Number of voxels	Breakdown (number of voxels)	Jacknife sensitivity analysis
Right caudate nucleus	16,2,24	-1.733	0.000043690	1387	right caudate nucleus, corpus callosum	4/7
Corpus callosum	18,-50,26	-1.483	0.000351667	196	corpus callosum, cingulum, precuneus	6/7

Acute subgroup

Region	Maximum			Cluster		Jacknife sensitivity analysis
	MNI coordinates x, y, z	SDM value	Uncorrected p	Number of voxels	Breakdown (number of voxels)	
Clusters of decreased FA						
Undefined	2,-14,14	-1.935	0.000019729	1055	corpus callosum, thalamus, anterior thalamic projections, left pons, left cortico-spinal projections, right caudate nucleus, left SLF	6/9
Corpus callosum	-22,24,30	-1.447	0.000822186	203	Corpus callosum	7/9
Left precentral gyrus, BA44	-48,6,20	-1.401	0.001111686	77	Left inferior frontal gyrus (opercular part), left precentral gyrus	7/9
Left pons	-22,-14,-8	-1.285	0.002091289	43	Left pons, left optic radiations	7/9
Right median network, cingulum	16,-56,28	-1.285	0.002850354	20	Corpus callosum, cingulum	7/9
Clusters of increased FA						
Undefined	-2,-52,-42	1.027	0.002499163	216	Cerebellum	7/9
Right cortico-spinal projections	8,-18,-28	1.065	0.001279950	85	Cortico spinal projections, pons	4/9
Corpus callosum	-46,-22,-8	1.030	0.002317071	57		

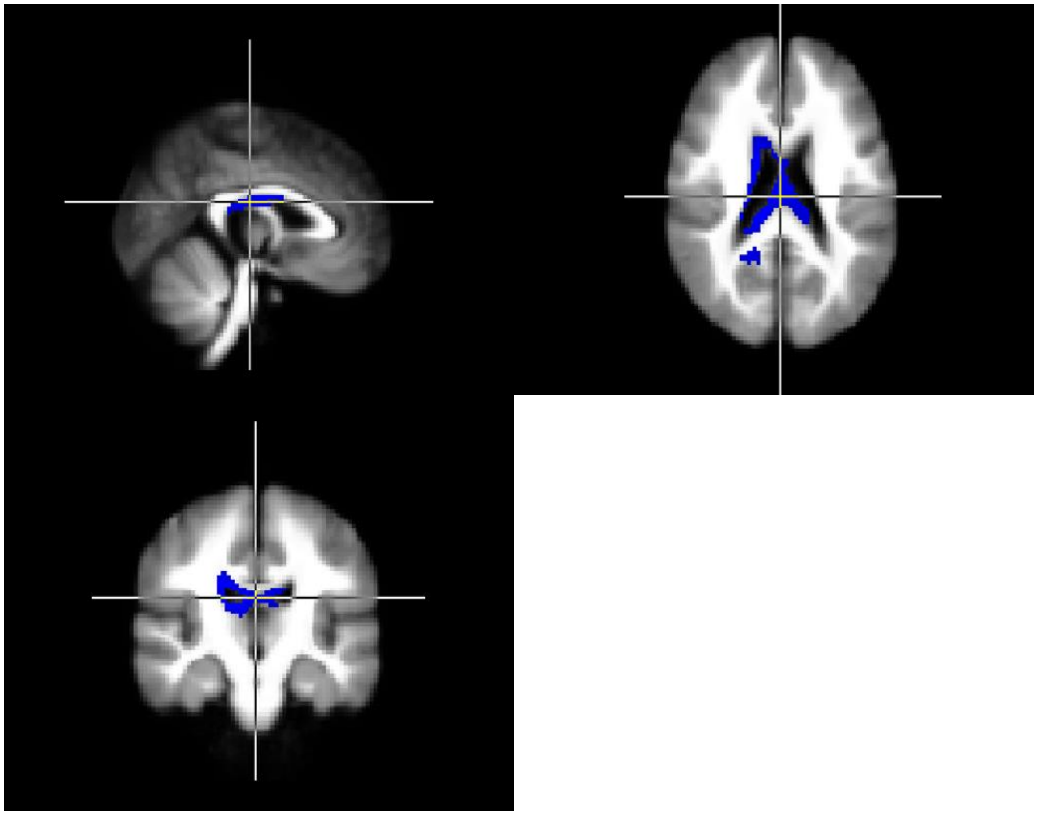
Corpus callosum	-20,44,18	1.030	0.002317071	50	7/9
Corpus callosum	16,52,-8	1.030	0.002317071	46	5/9
BA 18	8,-86,-14	1.062	0.001305223	39	
Left lenticular nucleus, putamen, BA48	-28,10,0	1.065	0.001258790	30	8/9
Left lingual gyrus, BA18	-28,-88,-16	1.061	0.001336217	26	6/9
Right arcuate network, post. Segment	46,-36,10	1.062	0.001305223	25	8/9
Left superior temporal gyrys, BA48	-54,-16,4	1.027	0.002499163	25	7/9

Figure 6.5. Signed differential mapping-generated FSL map of areas showing altered FA in patients with acute AN.



Significant regional differences in FA values in patients with acute AN compared to healthy controls. Clusters of decreased FA are marked in blue and increased FA are marked as red.

Figure 6.6. Signed differential mapping-generated FSL map of areas showing altered FA in adult patients with AN.



Significant regional differences in FA values in adult patients with AN compared to healthy controls. Clusters of decreased FA are marked in blue.

6.4. Discussion

This is the first meta-analysis comparing white matter microstructure between patients with AN and healthy controls. We found that patients with AN had reduced FA in the CC, left SLF and precentral gyrus; and increased FA in the right-corticospinal projections and lingual gyrus. The areas in the peri-splenial CC were robust and survived sensitivity analyses. FA reduction in the most posterior part of the CC survived sensitivity analyses in adult patients; this was also the case for the area in the precentral gyrus in the acute subgroup.

The CC is the largest WM tract containing more than 300 million axons connecting regions in both hemispheres and has been found to be critically involved in the integration of emotional, attentional, perceptual, and cognitive functions. The rostrum, genu, and rostral body of the CC contain fibres connecting homologous regions in the left and right prefrontal cortex; the midbody is formed by fibres connecting premotor, motor, and posterior parietal regions between the two hemispheres; and the splenium comprises connections between the superior and inferior temporal and occipital cortex (Hofer & Frahm, 2006). Based on our results, AN was associated with changes in the perisplenial CC consistent with a tractography study by Travis et al. (Travis et al., 2015) which found FA reduction in patients with AN in subdivisions of the CC, including parietal, temporal and occipital projections. Although the most robust peaks of case-control differences in the main analysis were located in the perisplenial CC (MNI = 18,-58,30; MNI = -22,24,30), the largest cluster (MNI = 18,-58,30), extended to the right precuneus and right cuneus. We used MRtrix in order to identify the major tracts which traverse this cluster (Figure 6.3). The reduction in FA in these tracts would most likely impact the transfer of information between parietal and occipital regions and within the limbic system. Both structural and functional neuroimaging studies have also consistently found the parietal cortex to be involved in AN (e.g. reduced GM and differential activation during tasks). This brain region is involved in the integration of proprioceptive and visual information of the body (Shimada, Hiraki, & Oda, 2005), (Zhang et al., 2013). Furthermore, atypical patterns of activation in task fMRI studies have been found in areas of the occipitotemporal region (including the extrastriate body areas) and parietal cortex in patients with AN when observing digitally distorted images of their bodies (Grönemeyer & Herpertz, 2010; Uher et al., 2005). Abnormal structural connectivity in these regions involved in body-image information processing might explain distorted body perception, a key feature in AN (APA, 2013).

The largest cluster in the main analysis (MNI = -2,-18,18) was found in the CC as well, with most voxels located within the CC. However, there was involvement of other areas, including the anterior thalamic projections (ATR) and thalamus. Almost the same cluster, with same involvement of areas (CC, thalamus and ATR) was found (although classified as undefined) in the acute subgroup. This is of special importance as it could point to some stability of brain alterations and could point to more stable markers of the disorder and neuropsychological differences associated with the brain alterations. However, it is also true that due to the the nature of these analyses (with an heterogeneous sample) and our small sample, we are only able to propose this as a hypothesis. Due to the large number of acute studies included in the main sample, it is also possible that these studies are driving the effect found in the main analysis. More studies are therefore needed in order to help elucidate this hypothesis. The ATR are fiber pathways that connect the anterior part of the thalamus with the frontal lobe, while the thalamus is part of a network that projects relevant sensory information to numerous brain areas (Fan, McCandliss, Fossella, Flombaum, & Posner, 2005) and also plays a role in higher-order cognition through relevant cortico-thalamic-cortical projections (Klostermann et al., 2006; Sherman, 2007). Cognitive behavioural inflexibility is an AN trait and has be conceptualized as impaired cognitive set shifting (rigid approaches to changing rules) and impaired behavioural response shifting (perseverative behaviours). Numerous studies in the literature have found this trait in patients with AN, both ill and recovered as well is healthy sisters (Roberts et al., 2007). These findings suggest that impaired set shifting could be an endophenotype in AN (Holliday et al., 2005). Our results of decreased FA in areas of the thalamus and anterior thalamic projections suggests a possible dysregulation in the pathways that project information relevant for set shifting and behavioural response. In fact, areas of the fronto-striato-thalamic pathway have been found to be altered in patients with AN (Shafritz, Kartheiser, & Belger, 2005; Zastrow et al., 2009).

A main limitation of the findings in these areas, is that they could be influenced by partial volume effects (PVE) given their proximity to the ventricles, and therefore results should be considered carefully. In 2017 Kaufmann (Kaufmann et al., 2017) questioned previous results of altered fornix FA in AN after using a new method by which he corrected DTI images for increased CSF. Given the proximity of the fornix to ventricles it is logical to think that as CSF increases (and thus the ventricles), this could lead to increased partial volume effects and falsely reduced FA. This was reiterated in a commentary by Seitz (Seitz, 2017)

who suggested that not only new studies should correct for PVE, but that some of the old studies with findings of altered FA in the fornix should be re-assessed. Given that the studies which found differences in FA in the fornix are included in this meta-analysis, and are likely largely contributing to the two main clusters around the peri-splenial CC, it is important to take these results with some caution.

A main finding is the cluster of reduced FA in the left precentral gyrus in an area also known as the pars opercularis of the inferior frontal gyrus, in Broadman area 44. Although this cluster was the smallest in the main combined analyses, it was also found in the acute sample and was one of the most robust findings for this group. Furthermore, this cluster was found to be associated with BMI in the acute sample as well. This cluster is located in a motor area which lies in front of the central sulcus in the frontal lobe. Specifically the cluster was found in the Broadman area number 44 which has be recently found to be involved in suppression of response tendencies (Forstmann, van den Wildenberg, & Ridderinkhof, 2008), such as during go no go tasks. Interestingly, the fact that this area was found to be associated with BMI indicates that it's associated with weight and therefore could be more an effect of the reduced weight during the acute stage of the disorder rather than a more permanent marker of the disorder. However, this area was also found in the combined analysis. One possible explanation is that this area is more affected than others by the decreased in weight and the physiological aspects of the disorder and can be a permanent scar of the disorders. However, we can't disregard the possibility that differences in this area are present before the onset of the disorder and are further affected by it. Kothari and colleagues in 2013 (R. Kothari, Solmi, Treasure, & Micali, 2013b) studied a large community sample of children at high risk for ED's and found poorer behavioural inhibition in children of mothers with Bulimia Nervosa (BN) as well as a trend towards significance in children of mothers with AN. Therefore it is possible that alterations in the area already exist and become exacerbated during the acute stage of the disorder. Interestingly, Broadman area 44 is an area of GM and unlike WM, GM is a heterogeneous mixture of neurons, axons and neuroglia. This makes it harder to conclude the meaning for changes in FA, as anisotropic diffusion is much lower than in WM. In fact, interpretation of DTI diffusivity parameters in GM is not greatly reported and still a matter of debate. An important limitation is that it could be biased by PVE, given the convoluted structured of GM and proximity to CSF spaces. One possibility, would be to study ADC in the cortex, being careful to segment out the effects of CFS.

We did not find an association between BMI and FA in combined or adult samples. This is contrary to previous findings of a positive correlation between FA and current BMI in adult AN patients (Kazlouski et al., 2011a; Nagahara et al., 2014; Yau et al., 2013). However, in order to control for spurious results, the meta regression is limited to results found in the main areas reported in the meta-analysis, therefore, we can only conclude that there is no association in these areas. Studies have indeed reported associations with BMI involving regions which were not identified in this meta-analysis. Therefore, one possible explanation for this discrepancy is that FA decreases found are independent of low BMI and malnutrition and a manifestation of trait abnormalities in AN. However, another possible explanation is that FA decreases in these areas are secondary to malnutrition and do not recover with weight restoration. It is important to note that due to the small sample size of this meta-analysis, we cannot discard that the lack of association could be due to methodological issues and not a real lack of association. Therefore, this requires further investigating in future meta-analyses.

The last major cluster of reduced FA in the main analysis was found in the SLF, a major intra-hemispheric fiber tract composed of four separate components. Our results show involvement of the left parietal part of SLF II which occupies the central core of the WM above the insula, lateral to the corona radiata and CC (Makris et al., 2005). The SLF II fibers connect posterior parts of the superior temporal cortex with dorsolateral and ventrolateral prefrontal areas (Makris et al., 2005) and plays a major role in visual and oculomotor aspects of spatial function (Makris et al., 2005). Similar to the area in the CC extending towards the precuneus, the SLF is functionally relevant to body image distortion in AN. The SLF II is the major WM tract connecting areas of important for body self-image (parietal) and body image perception (prefrontal and parietal network). The inferior parietal areas involved in proprioception, spatial judgment and the integration of visual information, forming the neural basis for representation of body self-image (S. Gaudio & Quattrocchi, 2012). Several studies have found differences in parietal areas in patients with AN as well as in prefrontal areas during visualization of own body image during fMRI studies (S. Gaudio & Quattrocchi, 2012).

As well as areas identified in the main meta-analysis, a cluster of decreased FA was found in the pons only in the acute group. The pons connects the limbic forebrain and brainstem and is vital to autonomic functions necessary for life, including food pleasure (Kent C. Berridge, 2009). Areas in the pons and thalamus receive information from the lower

brainstem in relation to the properties of food and project to the amygdala and frontal cortex, which perform higher order functions pertaining to the rewarding and aversive aspects of food. Thus, alterations in pontine white matter could disrupt the food reward system, which has been shown to be altered in eating disorders (Kent C. Berridge, 2009; Lassek, 1942). Malnutrition during the acute phases of the illness may contribute to persistent reduction in FA in the pons. This possibility is supported by reports of central pontine myelinolysis (CPM) in AN (Amann, Schäfer, Sterr, Arnold, & Grunze, 2001; Keswani, 2004; Ramírez, Arranz, Martín, & San, 2007); this is a demyelinative lesion in the pons that was first reported in cases of malnutrition and alcoholism. Since decreased FA only appeared in subgroup acute analysis, it is possible that alterations found in the pons are due to malnutrition.

Analyses in adult patients only implicated the right caudate nucleus and the most robust area in the CC. The caudate nucleus is one of the structures that makes up the dorsal striatum, a component of the basal ganglia. This area has shown to be involved in several roles, including executive functions (Grahn, Parkinson, & Owen, 2008; Villablanca, 2010) (especially inhibitory control and planning) and it has been shown to also play a role in the reward system as part of the cortico-basal ganglia-thalamic loop. The basal ganglia has also been found to be of importance in disorders such as obsessive compulsive disorder (OCD) and attention-deficit/hyperactivity disorder (ADHD), which have been found to co-occur with ED's (Murphy, Timpano, Wheaton, Greenberg, & Miguel, 2010).

Finally, one small cluster of decreased FA in the cingulum was found in the acute subgroup and showed involvement of the CC as well. The cingulum is a projection of white matter fibers that allow communication between components of the limbic system and that is located adjacent to the CC. The area of decreased FA was found in the middle towards the anterior part of the cingulum. The anterior part of the cingulum has been linked with emotion, motivation and executive functions (Bubb, Metzler-Baddeley, & Aggleton, 2018). Alterations in this structure have been found to be associated with altered emotion identification in AN, as well as difficulties with cognitive control.

Both the main analyses and acute ones found areas of increased FA, however, these results should be taken with some caution as they were driven by results in adolescent studies and therefore disappeared when analyzing adult groups only. Three out of the four

studies on adolescents found increased FA and were therefore driving the findings of increased FA. Furthermore, results only show small sized clusters (less than 100 voxels) compared to those found for decreased FA. In the combined analyses, only three clusters of increased FA were found, however, the acute subgroup had a larger and more widespread number of increased FA clusters. Only nine studies were included in the acute sample and three of those were the studies on adolescents with increased FA, therefore it is likely that these studies had more weight in this analyses than they did in the combined ones were 13 studies were included.

Given that increased FA was a finding mainly reported in adolescent samples, it is possible to hypothesise reasons for the differences from a developmental perspective. Both GM and WM follow a trajectory of development from birth until early adulthood. The onset of puberty initiates a number of changes, not only physical but psychological and social. It is during this time that there are substantial increases in hormonal levels as well as changes in brain structure and function. Alongside these changes, there is a sharp increase in difficulties associated with the regulation of emotion and behaviour, with more mental health difficulties (Dahl, 2004). The normal trajectory of GM and WM development is opposite, with GM volume decreasing with age and WM volume increasing (Lenroot et al., 2007). In general, maturation of WM in adolescence is associated with increase in both myelination and fibre density (Paus et al., 1999). It is possible that different mechanisms could play a role in the still-developing brain of adolescents and they may be differentially affected by starvation, with a restructuring of WM. It is however possible that increased FA found in studies in adolescent patients could be explained by preexisting WM differences in AN and be considered a vulnerability. Localisation of the most robust FA findings were found in areas that have been shown to play a role in supporting rewarding behaviours (O'Doherty et al., 2004) which have been hypothesized to be altered in AN and could play a role in development (A. Harrison, O'Brien, Lopez, & Treasure, 2010) (Harrison 2010). It is clear that further studies in at risk populations are necessary in order to clarify these differences.

The first cluster found in both the combined and acute sample analyses is located in the corticospinal projections. Interestingly, to date, studies on white matter microstructure in adolescents with AN have found mixed results, with some brain regions showing increased FA and others showing decreased FA compared to controls. The differences in the direction (increased rather than decreased FA) of FA might be due to an unmasking effect.

The corticospinal tract has significant crossing fibres, therefore, if one of these pathways has low FA this might result in a higher FA overall. Interestingly, in the cluster breakdown of the largest peak of decreased FA, some voxels were located in the cortico spinal projections. However, it is important to note that there are only four studies investigating adolescent samples have been published to date and therefore more studies are needed in order to understand the differences between adolescent and adult samples.

The two most robust findings of increased FA in the acute sample were found in the putamen and the arcuate network. The putamen is a structure located at the base of the forebrain and together with the caudate nucleus it forms the dorsal striatum which is one of the structures that composes the basal ganglia. Alterations in the dorsal striatum are of interest for ED as they have been shown to play a role in supporting rewarding behaviors based on previous experience (O'Doherty et al., 2004). Dorsal striatum responds to reward and punishment and contributes to reward based decision making (Balleine, Delgado, & Hikosaka, 2007). Although the results show increased FA, it could still reflect damage as increased tissue anisotropy of the basal ganglia is thought to reflect microstructural GM damage (Cavallari et al., 2014; Hannoun et al., 2012). This results would therefore support those found by Frank and colleagues (G. K. Frank, M. E. Shott, J. O. Hagman, & V. A. Mittal, 2013) who found alterations in GM in the putamen which predicted sensitivity to reward. Therefore, alterations in the putamen could therefore play a role in differences in sensitivity to punishment and reward found in patients with AN (A. Harrison, O'Brien, et al., 2010).

An undefined area with voxels mostly in the cerebellum was also a replicable finding of increased FA. Findings in the cerebellum are of interest for the pathophysiology of AN due to the role it plays in feeding behavior. The cerebellum connects to the hypothalamus through cerebello-hypothalamic circuits which are thought to be involved in the regulation of food intake and feelings of satiety (J. N. Zhu & Wang, 2008). Studies have also focused on the role that the cerebellum plays in cognition and emotional experience (Stoodley & Schmahmann, 2009) which again is of interest for the development and maintenance of AN. Volumetric reductions in cerebellar GM have been found in patients with AN (Boghi et al., 2011) as well as deficits in functions associated to the cerebellum. This meta-analysis has found increased FA in this area, however, because FA is sensitive to several tissue characteristics it needs to be interpreted carefully, a range of tissue characteristics might change in such a way that the resulting FA obscures subtle changes. More research is

needed to further understand alterations in the cerebellum and the direction as well as associations with deficits in AN.

The cross-sectional design of the available studies does not address the issue of causality and specifically the contribution of nutritional status. GM and WM volumes can show significant reductions in otherwise psychiatrically healthy individuals after only 2-3 days of dehydration (Streitburger et al., 2012) and WM volumes can be restored following re-feeding. Furthermore, a commentary by Frank in 2015 (G. K. Frank, 2015) discussed the variance in time between start of re-alimentation and scanning as another point of variability that might account for differences between structural MRI studies in AN. In fact, the studies included in the meta-analysis show a degree of variance with regards to this issue, with six of the ten studying acute patients (12,14,15,20) controlling for the effect of re-feeding in some respect, most of them scanned patients after one to two weeks of rehydration. However, it is not known whether more subtle WM differences persist over time following recovery. Axons in the corticospinal tracts and the corpus callosum are some of the largest axons in the brain (Lassek, 1942) and therefore have thicker myelin and larger concentrations of lipids (Paus, 2010). As such, these areas could be predisposed to greater degree of myelin loss secondary to malnutrition or chronicity.

Whilst the meta-analysis sheds some light into what could be more stable WM microstructure alterations in AN, it does not help elucidate if these alterations are a cause or a consequence of the disorder. Although subgroup analyses are exploratory because of the sample size, there are differences in the number of areas found between the main analysis (which include both acute and recovered) and only the acute group, with the second subgroup analysis finding a larger number of areas relevant to AN. One possible explanation for this is that in the acute stage, patients have a more widespread number of white matter micro-structural abnormalities, and that one year after recovery (criteria for inclusion in both studies with recovered patients) only some differences persist. These differences could either be more permanent scars of the disorder or could possibly be present before the onset and therefore contribute to the development of the disorder. To date, four studies have investigated recovered patients, however, only three met criteria for inclusion in this meta-analysis and therefore we were not able to run subgroup analyses. Taken together, these four studies (J. Cha et al., 2016a; Frieling et al., 2012b; Shott et al., 2016; Yau et al., 2013) showed inconsistencies in WM integrity abnormalities identified, with two studies finding decreased FA, one finding increased FA and one only

finding decreased MD. It is important to note that not all studies use the same criteria for recovery making comparability of the results complex. However, the studies included in the meta-analysis used a 1-year criterion of weight restoration and no engagement in ED behaviours for their recovered patients. Only one of the studies had a short recovery criteria, only weight restoration for two weeks (19). Although this study was included in the meta analysis, whole brain white matter differences were only reported during the acute period and not in the second stage at weight restoration. Of the studies with more stringent recovery criteria (one year recovery and not ED behaviours), two did not find any differences in FA, however, these studies only included nine and twelve recovered patients, while the study by Shott and colleagues (16) included 24 patients providing them with increased power for their analyses. Importantly, more studies with recovered patients are needed to ascertain if differences do persist after recovery or if WM alterations are a temporary scar of the disorder. Furthermore, although, methodological differences in the studies could account for some variability in results, they are unlikely to account for differences in the direction of DTI parameter changes. Taken together, these studies suggest that some FA alterations may be present in weight recovered patients but also that WM integrity and myelin may be improved with weight restoration in AN in some brain areas. It is unclear whether the differences found in recovered patients are an antecedent or a scar of the disorder (related to severity of illness or specific symptoms), and/or an artefact of heterogeneous methodology employed to measure WM integrity. Importantly, there is a need for longitudinal prospective studies investigating an at-risk population that will allow us to investigate differences before the onset of the disorder.

Limitations

Overall, results from DTI studies should be taken with caution as there are some limitations to the technique that should be acknowledged. One of the most common issues with DTI is the data interpretation, usually higher MD and lower FA indicate damaged or impaired fiber integrity due to increased diffusion and loss of coherence in movement direction. However, this is not always true, and it can be dependent on brain region, cellular basis or the sample studied. Studies in schizophrenic patients have shown an overall reduction of FA, however, increased FA (Kubicki et al., 2007) has been found in interhemispheric auditory fibers in those patients suffering from hallucinations. Therefore, it cannot be said that increased FA is always better. Another potential limitation involves the questions of how much FA abnormalities truly reflect altered WM integrity. Usually, FA is described as a marker for WM integrity, however it can be altered due to a variety of reasons (Jones et

al., 2013; Jones & Leemans, 2011) such as larger axon diameter and lower packing density of fibers (both contributing to fewer barriers to diffusion leading to lower FA), or increased membrane permeability and reduced myelination.(Jones et al., 2013) Crossing fibers (previously discussed in this section) are also problematic in DTI result interpretation. A single voxel can be composed by fiber populations with different spatial orientation which can result in an average increase in FA, however, in this case, this is not due to changes in axonal or myelin structure.

The present meta-analysis has several limitations. First, the number of studies utilizing DTI in AN is rather small, and not all studies of AN could be included due to methodological differences, therefore the results are preliminary and further meta-analyses are necessary as studies continue to be published. Second, although the meta-analytic methods used provide good control of false positive results, it does not so for false negative results (Radua et al., 2012). Third, the studies included in this meta-analysis used different statistical thresholds. However, although thresholds involving correction for multiple corrections are desired, it is still statistically correct to include those studies using more liberal thresholds (Radua et al., 2012). Fourth, the heterogeneity of the MRI acquisition, including differences in acquisition parameters and the use of two different methods (TBSS and VBA) to undertake whole brain analysis may lead to inconsistencies between studies. However, these limitations are inherent to all meta-analyses and in spite of them, some findings were quite robust. Meta-analysis combining TBSS and VBA analysis have been published including a study by the developers of the software. As published in the methods, to allow combinations of VBA and TBSS studies the TBSS template included in AES-SDM was adopted. Fifth, the studies varied in terms of patient characteristics, not only in terms of illness status (acute and recovered) and duration of illness, but also for the inclusion of both binge/purge and restricting types of AN. As further studies are published, future meta-analytic studies should subcategorize between types of AN in order to ascertain neural differences between both binge/purge and restricting AN as well as different stages of illness duration. Sixth, the main finding in this meta-analysis involves a cluster of voxels in the CC, however, Figure 2 shows how these areas surround ventricular areas. Although the image is just a rough illustration of the location, it is worth noting that since ventricles are enlarged in AN, a biased estimation of FA in these areas can be due to partial volumes effects (Jones & Cercignani, 2010). Finally, this meta-analysis only focused on FA as it is the most commonly reported variable to show differences in WM

microstructure (both in the wider literature and in the studies included in this meta-analysis).

Conclusions

In conclusion, by summarizing WM microarchitecture studies to date, we have demonstrated significant FA alterations in patients with AN, affecting white matter tracts in the CC and in subcortical regions. This evidence implicates regions likely to be involved in body image processing and cognitive behavioural inflexibility. Further studies are needed to examine the direction of causality of these findings. These results integrate previous findings from DTI studies in AN and provide a more coherent picture of the most prominent and replicable abnormalities in WM integrity in patients with AN.

Chapter 7. Neurocognitive differences in children at high-familial risk for ED: findings from BREDS

7.1. Introduction

There is a large body of evidence indicating neurocognitive deficits in patients with ED (see Chapter 1 for summary). Earlier neurocognitive studies in ED focused on general cognitive deficits, however, subsequent research has examined specific constructs that have been hypothesised to play a role in ED psychopathology. Areas of executive functioning (multidimensional construct which refers to “top-down” processes that allow an individual to adapt information processing and behaviour to their goals (Diamond, 2013)) have been studied in ED, including working memory, attention, cognitive flexibility and cognitive and behavioural inhibitory control.

Inhibitory control refers to a set of processes that allow the individual to suppress or interrupt responses. There is some evidence of ineffective inhibitory control in ED, with binge-eating type ED falling within deficient inhibitory control and restricting type ED characterized by excessive inhibitory control. This construct has been generally assessed using go/no-go and stop signal tasks (requiring participants to inhibit responses to a specific target which is less frequently presented). The majority of research has been conducted with adults with binge-type ED, with nine systematic reviews conducted to date (K. E. Smith et al., 2018). Evidence suggests that inhibitory control deficits are present in patients with binge-purge type disorders while the evidence is not as clear for restricting subtypes.

In the last decades, set-shifting (or cognitive flexibility, which refers to the ability to shift thoughts or actions according to situations) has obtained evidence as a transdiagnostic mechanisms contributing to ED psychopathology (although most of this literature focuses on AN patients) (Lang, Treasure, et al., 2016; Naor-Ziv & Glicksohn, 2016; Talbot et al., 2015). It has been found to be altered in both ill patients, recovered and more recently in first degree relatives of patients. To date there have been 12 systematic reviews, including meta-analyses focused on set-shifting difficulties (K. E. Smith et al., 2018). Interestingly,

one meta-analytic review failed to find significant differences in set-shifting in children and adolescents with AN compared to HC (Lang, Stahl, et al., 2014), suggesting that deficits may be an effect of longer term duration of the disorder. However, this review included fewer studies than a later one by Wu and colleagues (Wu et al., 2014) which did not find differences between adolescents and adult samples. With regards to central coherence (focus in details rather than global integration when processing information), there is less evidence to support its possible *trait* status (Garrett et al., 2014; N. Kanakam et al., 2013; Lang, Roberts, et al., 2016; Lang, Treasure, et al., 2016; C. Lopez et al., 2009; Talbot et al., 2015; Tenconi et al., 2010).

Other executive functions such as working memory (ability to hold and work with information in mind in order to guide behaviour) have also been investigated in EDs suggesting some dysfunction in these measures of executive function (K. E. Smith et al., 2018). Lastly, decision making (construct which involves multiple processes of executive function) has also been examined in ED, using tasks such as Iowa Gabling Task (IGT) (Bechara, Damasio, Damasio, & Anderson, 1994) and Game of Dice Task (GDT) (Brand, Franke-Sievert, Jacoby, Markowitsch, & Tuschen-Caffier, 2007). It has been proposed that decision making may be altered across different ED subtypes given inconsistencies in goals and actions (related to binge-purging subtypes) and persistence in actions despite the need to modify behaviour (restriction given seriously low weight). Overall findings vary depending on tasks, with results from the two main tasks (IGT and GDT) showing impairments in both AN and BN with medium to small effect sizes (K. E. Smith et al., 2018).

Approach and avoidance processes and their motivation (linked to reward) have been proposed to play a role in the development and maintenance of ED (Ehrlich et al., 2015; A. Harrison, O'Brien, et al., 2010; Amy Harrison, Treasure, & Smillie, 2011; Wu et al., 2016). The BIS/BAS scale has been used to measure sensitivity to reward and punishment in ED populations and was developed based on Gray's Reinforcement Sensitivity Theory, who proposed two broad motivational systems that regulate approach and withdrawal behaviour: the activation system (BAS, sensitive to reward) and the inhibition system (BIS, sensitive to punishment) (A. Cooper et al., 2007; Jeffrey Alan Gray, 1987). Higher sensitivity to reward and punishment have been proposed to be contributing factors to ED psychopathology (Loxton & Dawe, 2001). As seen in Chapter 2, there has been extensive research into neural correlates of alterations in the reward system and areas in the frontostriatal regions have been shown to be altered and correlated to higher sensitivity

for reward and punishment in patients with ED (Jiook Cha et al., 2016; Olivo et al., 2017; Shott et al., 2016; Steward et al., 2018).

To date very few studies have been published studying the neurocognitive profile of children at risk. A large community based study by Kothari and colleagues found that children of women with AN showed higher full scale IQ, increased WM capacity, better visuo-spatial function and decreased attentional control, while children of women with BN showed poor visuo-spatial functioning (R. Kothari et al., 2013b). A later study found that younger children at high risk for ED had difficulties in social understanding, visual-motor function, planning and abstract reasoning (R. Kothari, Rosinska, Treasure, & Micali, 2014). More recently, Kothari and colleagues found differences in social cognition in children at high risk for ED (R. Kothari et al., 2015).

Overall there are limitations to neurocognitive research that need to be taken into account. As described in Chapter 1, a large number of tasks have been used to assess these constructs with conflicting results at times, most likely because these tasks can tap into different executive functions and therefore may not be necessarily measuring the same thing. Importantly there are possible confounds that may influence neurocognitive functioning which need to be taken into account as well as duration of illness and differences between adults and adolescents. It is important to consider neurodevelopment, particularly since ED generally have an onset in adolescence. Frontal regions, which continue to develop into late adolescence, are key areas in executive functions. Furthermore, frontolimbic circuits underlie evaluation and response to reward, risk and emotion regulation (Steinberg, 2005) all of which play an important role in maintenance and/or development of ED.

As established in Chapter 1 and 3, available literature makes it difficult to ascertain if these neurocognitive deficits in patients are an effect of the acute illness (*state*) or are present prior to the onset and therefore play a role in the development of the disorder (*trait)*. Different approaches have been taken to ascertain what is *state* and what is *trait*, including studying recovered patients, first-degree relatives and more recently, children at high-risk for developing the disorder.

The aim of this chapter is to expand on previous findings on neurocognitive dysfunction in children at risk for ED by studying specific executive function constructs: attention, working memory, inhibition, set-shifting, decision making and measures of reward. The chapter will also explore measures of emotion identification and theory of mind in children at risk for ED. Further in Chapters 8 and 9, neurocognitive measures will be investigated within neural correlates.

7.2. Methods

Data from both cases and controls were collected as part of the Brain in high-Risk for Eating Disorders Study. A total of 21 healthy controls and 17 at-risk cases were recruited as part of the study. For inclusion in this study, demographics, data on maternal lifetime ED and childrens' neurocognitive measures were included.

Full details on the study's protocol, inclusion and exclusion criteria can be found in chapter 4 (Methodology). Assessments were conducted when the children were between the ages of 8 and 15 years old, with mean age: children of mothers with ED 11.94 and children of healthy control mothers 12.25.

7.2.1. Demographics

Information on maternal demographic details (age, marital status, ethnicity and education) was collected at the time of testing.

7.2.2. Exposure

Data on maternal eating disorders was gathered via an in depth interview using the Structured Clinical Interview for DSM-IV-TR (Research version)(First, Spitzer, Gibbon, & Janet, 2002) which was adapted for the new DSM-5 diagnoses. A full explanation of both the measure and the interview protocol can be found in chapter 4 (Aims and methodology). Mothers received a diagnosis of AN or BN, current or past with a detailed description of lifetime ED.

7.2.3. Neurocognitive measures

Data was collected on General Intelligence (WASI) (Wechsler, 2011), social cognition (Reading the Mind in the eyes (Baron-Cohen et al., 2001) and Morphed Emotion Recognition (Bediou et al., 2005)), reward (BIS/BAS (A. Cooper et al., 2007)) and a range of neuropsychological constructs using subtests of the *Cambridge Neuropsychological Test Automated Battery* (CANTAB) (J. Fray, W. Robbins, & J. Sahakian, 1996; Luciana, 2003). The following CANTAB tests were used:

- Affective go/no-go: affective shifting task that requires inhibitory control over different components of cognitive and emotional processing. Measures of affective response bias (difference between correct latency for positive and negative blocks; a higher score means a bias towards negative words) and correct latency (time to respond correctly to each word in all blocks).

- Attention switching task: set-shifting. Measures of correct trials, reaction time, switching cost (difference in response between blocks with switching rule vs rule that remains constant, a positive score means quicker response in non-switching blocks and closer to 0 means less variation between rules) and congruency cost (differences in response time between congruent vs incongruent, positive scores indicate faster response on congruent trials).

- Spatial working memory: measures of errors (number of times a subject touches a box that doesn't contain a token) and strategy (use of heuristic search sequence to complete the task in the most efficient way, lower scores mean more effective use).

- Rapid visual information processing: measure of sustained attention capacity with a working memory component. Higher scores in mean latency indicate longer time taken to respond correctly and higher scores in probability of hits corresponds with a higher probability of responding correctly.

- Cambridge gambling task: measures of delay aversion (if a participant is unable to make a decision, higher scores indicate the size of bet is determined by presentation in sequence rather than deliberation), risk adjustment (extent to which betting behaviour is moderated by the ratio boxes, higher scores means higher bet when majority of boxes are congruent with chosen colour).

More detailed information on each CANTAB test can be found in Chapter 4 (Section 4.2.4.1.).

Table 7.1. CANTAB Task, construct it evaluates and measurements exported.

CANTAB Task	Evaluation	Measurements
Motor screening	Simple motor processing speed	Reaction time (ms) and accuracy
Affective Go/No-go	Inhibition control	Accuracy and affective response
Attention switching	Set-shifting	Accuracy, switching and congruency cost
Cambridge Gambling	Risk-taking behaviour	Delay aversion, risk taking and adjustment
Rapid Visual Processing	Sustained attention	
Spatial working memory	Visual working memory	

Further detailed information on other neurocognitive measures can be found in the following sections in Chapter 4:

- Global intelligence as measured by the WASI can be found in section 4.2.4.1.
- Social cognition as measure by Morphed Emotion Identification and Reading the mind in the eyes can be found in section 4.2.4.2.
- Reward processing as measured by the BIS/BAS scale can be found in section 4.2.4.3.

7.2.4. Missing data

All participants completed the WASI for measurement of general intelligence. All participants completed five of the six CANTAB test. The Cambridge Gambling Task can only be used in children over the age of 11, therefore, complete data is only available on 10 at-risk girls and 17 healthy controls.

The social cognition measures which were completed remotely (separately from other measures) have missing data: complete data is missing from 3 of the 17 at-risk girls and 1 of the healthy controls.

7.2.5. Statistical analyses

All variables were examined individually to check for inconsistencies/outliers and normality using tabulations and histograms. The primary outcome variables investigated in this chapter were: general intelligence, CANTAB battery of tests, social cognition and reward measures. The distribution of demographic variables/covariates was studied according to the main predictor using chi-square (for categorical variables) and F-tests (for continuous variables) in order to provide a comparison between exposure groups (table 7.2).

Means and standard deviations of the neurocognitive measures were initially investigated in order to visually explore the data (see figures 7.1 to 7.3). Associations between neurocognitive measures and at risk status were investigated using linear regression analyses. All assumptions for linear regression were met. Additional *a priori* confounder (child age at testing and IQ) was included in adjusted models. All analyses were run using SPSS version 25 (SPSS Inc., USA).

Effect sizes for statistically significant results were also included as they provide a good measure of a studies practical significance. In a pilot study such as this, effect sizes can help gauge the research significance when a small sample is utilised.

Supplementary analyses for significant results were run based on maternal diagnosis. Associations between neurocognitive measures and at risk status stratified by maternal diagnosis were investigated using independent t-test.

Sample size for a larger scale study was calculated using pilot study results. Analysis was run using G*Power version 3 (Faul, Erdfelder, Lang, & Buchner, 2007).

7.3. Results

7.3.1. Demographics

Results are shown in table 7.2. There were no significant differences between the groups in any of the demographic variables studied, both maternal and child based.

Table 7.2. *Demographic group comparisons dependent on distribution of variable.*

	ED (N = 17)	Unexposed (N = 20)	Statistic
Maternal education			
Up to A levels	3 (17.6%)	1 (5%)	$\chi^2 = 1.524$, p = 0.217
Higher education	14 (82.4%)	19 (95%)	
Maternal ethnicity			
White	14 (82.4%)	17 (85%)	$\chi^2 = 0.047$ p = 0.217
Other ethnicity	3 (17.6%)	2 (15%)	
Child development			
Menstrual period yes	9 (52.9%)	11 (55%)	$\chi^2 = 0.016$, p = 0.900
Menstrual period no	8 (47.1%)	9 (45%)	
Breast Development (6 stages)			$\chi^2 = 5.200$, p = 0.392

Pubic Hair Development (6 stages)			$\chi^2 = 6.448$, $p = 0.265$
Maternal age	44 (5.48)	45.55 (5.34)	$t = 0.87$, $p = 0.39$
Child age	11.94 (2.14)	12.25 (1.94)	$t = 0.46$, $p = 0.65$

7.3.1. General Intelligence

Girls at high- and low-risk were comparable in General Intelligence.

Table 7.3. *Linear regression of children's IQ: comparisons of girls at high-risk and girls of healthy control mothers (B coefficients and 95% confidence intervals)*

	Crude *B* (95% CI)		Adjusted [a] *B* (95% CI)	
	Girls at risk	Unexposed	Girls at risk	Unexposed
Full-scale IQ	2.32 (-9.01, 13.64) p=0.68	Ref.	1.94 (-8.11, 12.00) p=0.69	Ref.
Verbal IQ	3.67 (-8.41, 15.74) p=0.54	Ref.	3.25 (-7.30, 13.81) p=0.53	Ref.
Performance IQ	-2.46 (-12.08, 7.16) p=0.60	Ref.	-2.80 (-11.50, 5.91) p=0.51	Ref.

*p≤0.05, **p≤0.01, ***p≤0.001; [a] Adjusted for child age at testing

7.3.2. Neurocognitive

7.3.2.1. CANTAB

See figures 7.1 to 7.3 for distribution of mean scores between groups and Table 7.4 for linear regression results.

Girls at-risk for ED showed higher switching costs scores in the attention switching task in comparison to girls at low-risk (table 7.4) (effect size: Cohen d = 0.742). Girls at-risk had more variability in scores between switching a non-switching blocks (responses were quicker in non-switching). Girls at-risk for ED showed better working memory (table 7.4.) (Effect size: Cohen d = 0.603).

Girls at risk and girls of healthy control mothers were comparable in all other CANTAB suite tasks.

Table 7.4. *Linear regression of children's CANTAB scores: comparisons of girls at high-risk and girls of healthy control mothers (B coefficients and 95% confidence intervals)*

	Crude B (95% CI)		Adjusted [a] B (95% CI)	
	Girls at risk (N = 17)	*Unexposed (N = 20)*	*Girls at risk (N = 17)*	*Unexposed (N = 20)*
MOT				
Mean latency	-27.82 (-158.173 to 102.60) p=0.67	Ref	-36.71 (-163.35 to 89.91) p=0.56	Ref
AST				
Correct trials	2.37 (-2.54 to 6.27) p=0.23	Ref	2.62 (-1.18 to 6.42) p=0.17	Ref
Mean latency	65.29 (-39.67 to 170.24) p=0.22	Ref	56.52 (-42.89 to 155.92) p=0.26	Ref
Switching cost	**76.01(7.83 to 144.19) p=0.03***	Ref	**71.18 (5.21 to 137.15)* p=0.035**	Ref.
Congruency cost	25.91 (-8.18 to 59.99) p=0.13	Ref	23.10 (-9.26 to 55.46) p=0.16	Ref
AGN				
Mean correct	-0.17 (-52.40 to 53.05) p=0.99	Ref	-5.21 (-53.36 to 42.93) p=0.83	Ref
Total omissions	-0.05 (-4.07 to 3.40) p=0.97	Ref	-0.48 (-4.09 to 3.12) p=0.79	Ref
Total commissions	-2.14 (-11.22 to 6.94) p=0.64	Ref	-3.02 (-11.38 to 5.34) p=0.47	Ref
Affective response	12.23 (-11.56 to 36.01) p=0.30	Ref	11.83 (-12.34 to 36.01) p=0.327	Ref
RVP				
Mean latency	3.15 (-58.07 to 64.37) p=0.92	Ref	-2.55 (-59.42 to 54.31) p=0.93	Ref

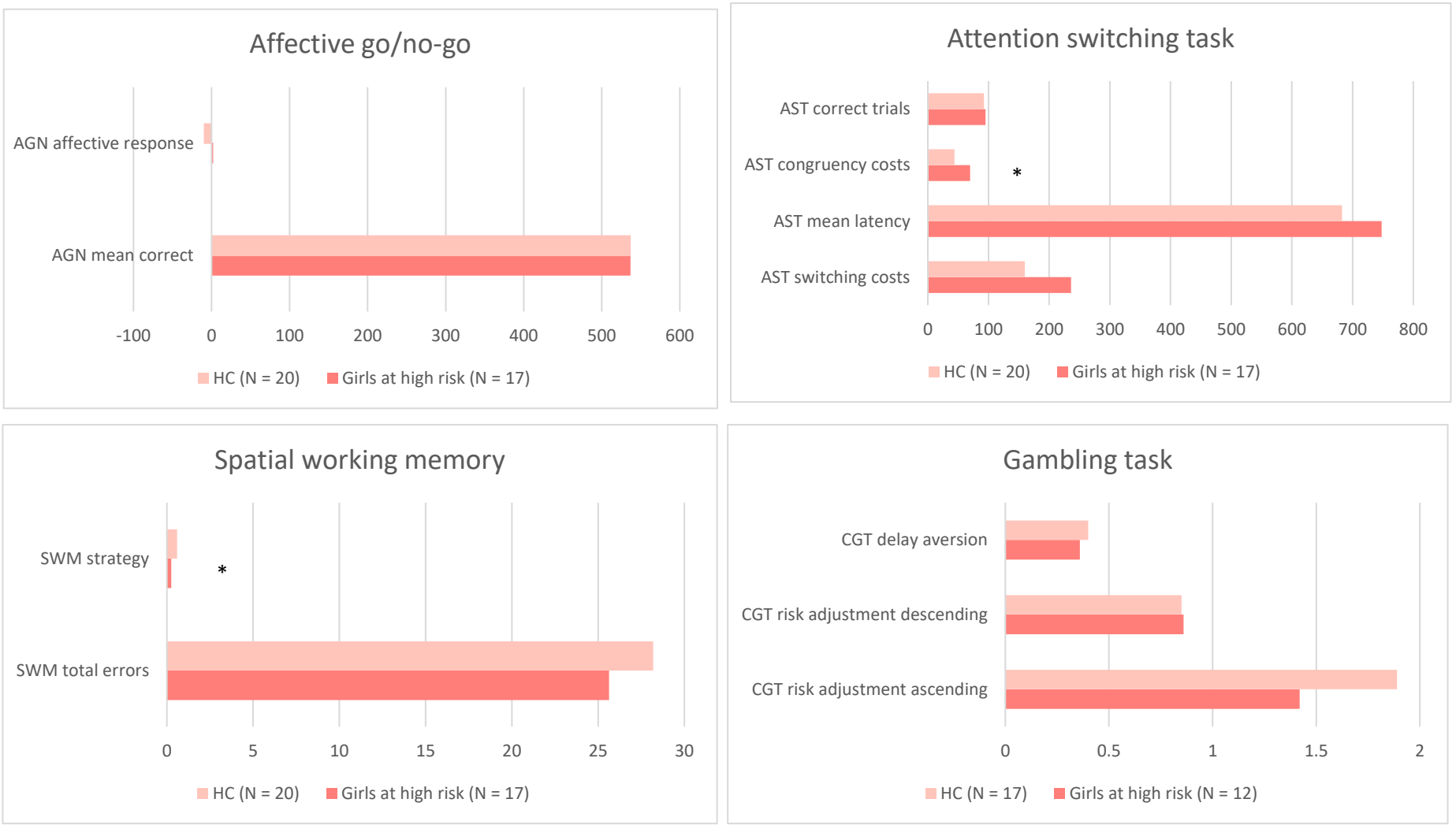
SWM				
Total errors	-2.55 (-17.62 to 12.52) p=0.73	Ref	-3.92 (-17.99 to 10.16) p=0.58	Ref
Strategy	**0.39 (1.61 to 16.92) p=0.02***	Ref	**3.90 (1.34 to 17.02) p=0.02***	Ref

*p≤0.05, **p≤0.01, ***p≤0.001; [a] Adjusted for child age at testing and child IQ

	Crude B (95% CI)		**Adjusted [a] B (95% CI)**	
	Girls at risk (N = 10)	*Unexposed (N = 17)*	*Girls at risk (N = 10)*	*Unexposed (N = 17)*
CGT				
Risk adjustment ascending	-0.48 (-1.48 to 0.53) p=0.34	Ref	-0.53 (-1.58 to 0.52) p=0.31	Ref
Risk adjustment descending	0.01(-0.80 to 0.82) p=0.97	Ref	-0.12 (-0.92 to 0.67) p=0.75	Ref

*p≤0.05, **p≤0.01, ***p≤0.001; [a] Adjusted for child age at testing and child IQ

Figure 7.1. CANTAB battery: means plotted by task



7.3.2.2. Social cognition

Girls at high- and low-risk were comparable in social cognition tasks (Table 7.5 and figure 7.2).

Table 7.5. *Linear regression of children's social cognition scores: comparisons of girls at high-risk and girls of healthy control mothers (B coefficients and 95% confidence intervals)*

	Crude *B* (95% CI)		**Adjusted [a] *B* (95% CI)**	
	Girls at risk	Unexposed	Girls at risk	Unexposed
RME	-1.65 (-5.46, 2.16) p=0.38	Ref.	-1.56 (-5.36, 2.25) p=0.41	Ref.
Morphed emotion recognition	0.95 (-4.78, 6.69) p=0.73	Ref.	1.35 (-3.04, 5.75) p=0.53	Ref.

*p≤0.05, **p≤0.01, ***p≤0.001; [a] Adjusted for child age at testing and child IQ

7.3.2.3. Reward

Girls at risk showed higher scores in BAS drive than girls of healthy control mothers and higher scores in fun seeking showed a trend towards significance (Table 7.6. and Figure 7.3).

Table 7.6. *Linear regression of children's reward scores: comparisons of girls at high-risk and girls of healthy control mothers (B coefficients and 95% confidence intervals)*

	Crude B (95% CI)		**Adjusted [a] B (95% CI)**	
	Girls at risk (N = 17)	Unexposed (N = 20)	Girls at risk (N = 17)	Unexposed (N = 20)
BIS	0.136 (-0.41 to 3.13) p=0.14	Ref	1.29 (-0.46 to 3.05) p=0.15	Ref
BAS drive	2.09 (0.75 to 3.42) p=0.001**	Ref	2.04 (0.70 to 3.38) p=0.000**	Ref
BAS fun seeking	1.36 (-0.17 to 2.89) p=0.09[b]	Ref	1.26 (-0.19 to 2.7) p=0.09[b]	Ref
BAS reward	1.31 (-0.27 to 2.89) p=0.14	Ref	1.28 (-0.32 to 2.87)p=0.14	Ref

*p≤0.05, **p≤0.01, ***p≤0.001; [a] Adjusted for child age at testing and child IQ; [b] trend towards significance p≤0.1

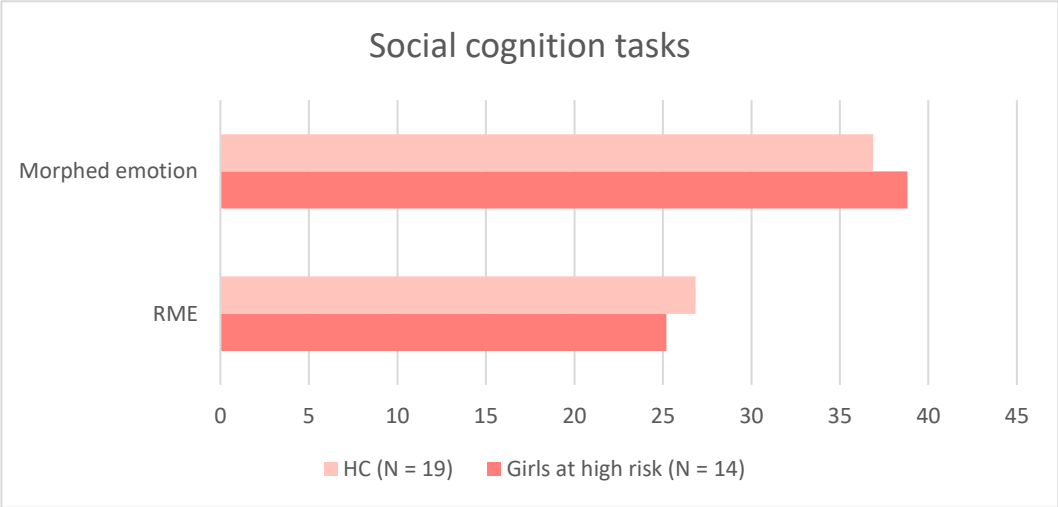


Figure 7.2. *Social cognition: task means plotted into graphs. Reading the mind in the eyes and Morphed emotion identification. Average scores for RME are between 22-30 with higher scores depicting better identification of emotions. Morphed emotion identification, higher scores means better recognition of emotions.*

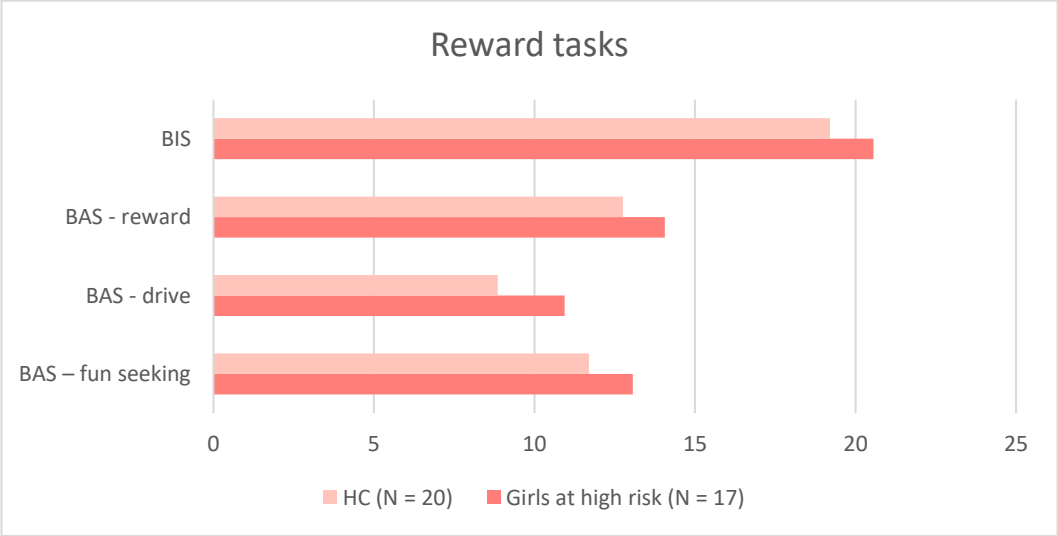


Figure 7.3. *Reward task BIS/BAS: task means plotted into graphs for all BIS/BAS scales: BIS, and BAS reward, BAS drive and BAS fun seeking. Higher scores in BIS depict punishment-sensitivity. Lower scores in BAS has been associated with lower reward sensitivity.*

7.3.2.4. Exploratory analyses

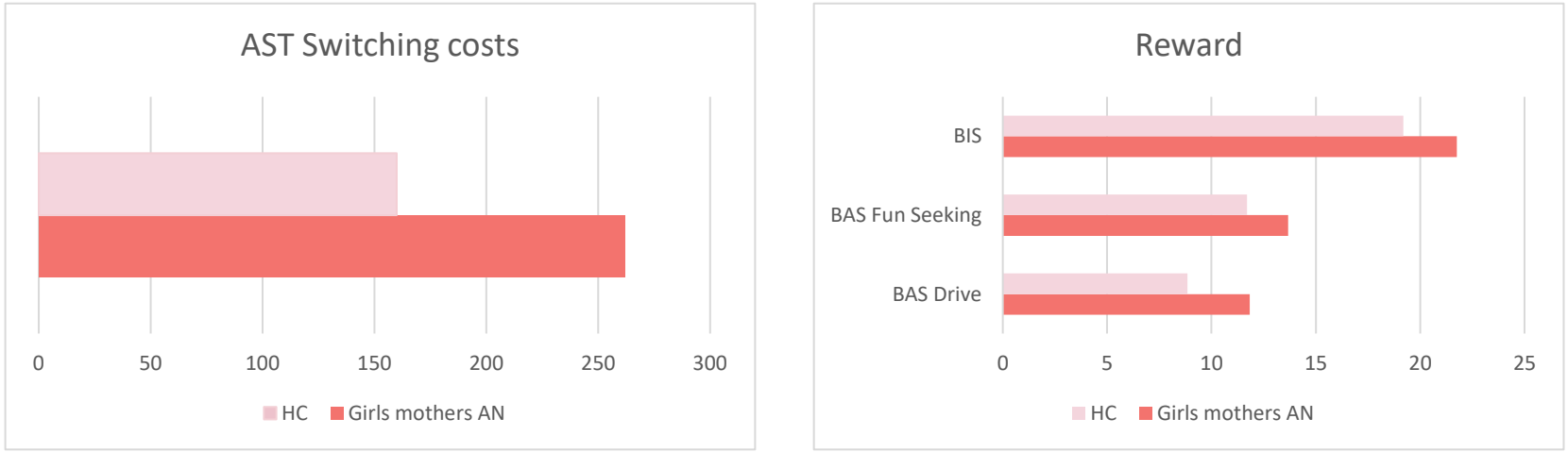
Results from girls of mothers with AN are merely exploratory and should be considered with caution.

Girls of mothers with AN showed higher switching costs scores in the attention switching task in comparison to girls at low-risk ($t = -2.80$; $p = 0.01$). Girls of mothers with AN had more variability in scores between switching a non-switching blocks (responses were quicker in non-switching).

Girls of mothers with AN showed higher scores in BAS drive ($t = -3.47$; $p = 0.002$), BAS fun seeking ($t = -2.42$; $p = 0.022$) and BIS ($t = -3.01$; $p = 0.005$) than girls of healthy control mothers. Scores depict higher reward and punishment sensitivity.

No significant differences were found in SWM between girls of mothers with AN and girls of healthy control mothers.

Figure 7.4. AST switching costs and Reward task BIS/BAS: task means plotted into graphs.



7.3.2.5. Sample size

One of the aims of this pilot study was to be able to examine the sample size needed in a larger scale study, and the feasibility of such a study (given that this was the first ever study of children at risk for ED). Specifically in this study we wanted to examine the feasibility of recruitment and find the appropriate sample size for a larger scale study.

G*Power (Faul et al., 2007) was used to calculate sample size based on neurocognitive results. Calculations for a later larger study showed that the sample size in each group should be 49 at an alpha of 0.05 and a power of 0.80.

7.4. Discussion

The main findings from this chapter are difficulties in attention switching, more effective working memory strategy and higher drive towards pursuing goals (measures by BAS drive) in girls at risk for ED compared to girls of healthy control mothers. No other measures showed significant differences, however, the main aim of this study is to generate hypothesis for a larger study and therefore limitations in power should be considered.

One of the main findings form this study is difficulties in set-shifting in children at risk (taking significantly longer to shift rule than children at low risk). Cognitive flexibility or set-shifting is an aspect of executive function that refers to the brain's ability to transition from thinking in one dimension or concept to another. The quicker you are able to shift your thinking from one rule or concept to another, the greater your cognitive flexibility is. Cognitive flexibility can therefore affect how quickly we are able to adapt to changes in our environment and has been related to difficulties found in individuals with psychiatric disorders such as ASD and OCD (characterized by maladaptive patterns of repetitive, inflexible cognition and behaviour) (Gruner & Pittenger, 2017). Patients with OCD have difficulties shifting between mental processes that influence changes in their behaviour (especially in the context of their symptoms). Individuals with ASD have trouble adapting

to variable demands in their environment and can show rigid behaviours and difficulties changing plans or altering routines and overall show deficits in tasks that require cognitive flexibility. It has also been suggested that ineffective set-shifting could underlie AN phenotypic presentation of inflexible thinking (rigidity in restricting calories and inability to change behaviour in the presence of extreme low weight). While the literature is clearer for adults, showing greater and more consistent deficits in set-shifting, results are not as clear in adolescents (Shott et al., 2012; K. E. Smith et al., 2018). This may suggest that difficulties in set-shifting are a consequence of malnutrition and become worse the longer the patient is ill, which may indicate that these findings in recovered patients are a permanent scar of the disorder. On the other hand, it is possible that some deficits are present even before the onset and play a role in the aetiology of the disorder, but are also worsened by the effects of malnutrition which may explain why differences are more evident in adults with ED. It is of course important to take into consideration the effect of neurodevelopment during adolescence and how this may affect differences in set-shifting. It has been suggested that tasks that require cognitive flexibility also require inhibitory control (Koch, Gade, Schuch, & Philipp, 2010) (as the individual has to inhibit a prepotent response (previous rule) in order to shift to another one) and therefore better inhibitory control could be masking difficulties in set-shifting (Weinbach, Bohon, & Lock, 2019). Results from our study suggest that difficulties in set-shifting might be present in children at risk and therefore could play a role in the pathophysiology of ED. These findings build upon findings from Holliday *et al.* study in 2005 (Holliday et al., 2005) and Tenconi *et al* in 2010 (Tenconi et al., 2010), both of which found that set-shifting difficulties were not only evident in patients with AN but also shared by their healthy sisters. Interestingly, while findings of cognitive inflexibility are regularly shown in patients with AN, the same cannot be said for patients with BN, where findings are less clear, with some studies finding altered set-shifting in BN (Roberts et al., 2010) and others not (Darcy et al., 2012; F. Van den Eynde et al., 2011). Results from exploratory analyses showed that when stratified by maternal diagnoses, girls of mothers with AN had significant difficulties in set-shifting compared to controls. The results from this study were in agreement with the hypothesis that specific set-shifting difficulties may constitute a biological marker or heritable endophenotype for AN (as they are present in first degree relatives who do not have the disorder).

Results also show better working memory (as shown by better use of strategy in a working memory visuospatial test) in girls at high-risk compared to girls of healthy control mothers.

A systematic review of studies published between 2010 and 2017 which examined working memory in patients with ED, showed that 45% of studies found that patients with AN had better performance in working memory tests than healthy controls or those who binge; 18% showed worse performance and 37% reported no differences (S. J. Brooks, 2016; S. J. Brooks, Funk, Young, & Schiöth, 2017). As with previous systematic reviews of neurocognitive measures, the heterogeneity in findings is possibly due to a number of factors such as mixing ED subtypes, different duration of illness and the use of different experiments (such as testing verbal versus visuospatial working memory). These results confirm previous results from a large community study by Kothari and colleagues in children at high-risk for ED (R. Kothari et al., 2013a). It has been suggested that working memory may underpin the ability to maintain attention on longer term goals (Hofmann, Gschwendner, Friese, Wiers, & Schmitt, 2008) which in turn may facilitate engaging in actions towards them (Hofmann, Schmeichel, & Baddeley, 2012). It is therefore possible that better working memory may facilitate sticking to diet goals. Interestingly, when stratifying by maternal diagnosis, results showing better use of strategy in SWM stopped being significant. This could mean that full sample results could be driven by girls of mothers with BN. Given the small sample, it is also possible that we do not have the power to stratify as differences are not as strong. Upon visual inspection of the data, two of the three participants with mothers with BN had significant higher scores compared to the average for the maternal AN group and could therefore be driving the differences. Because of the small BN sample, its not possible to further investigate if these differences were due to outliers or there is a significant difference between maternal diagnosis. A larger sample will allow to further investigate. Working memory has also been implicated in behavioural control, linked to excessive appetite control in AN (S. J. Brooks, 2016). It is therefore possible that better working memory could be considered an intermediate phenotype of ED.

The current study found differences in the approach system (Behavioural Activation System BAS) (A. Cooper et al., 2007) in children at risk, reflecting higher reward sensitivity. The BAS system has been conceptualised as a feedback loop where we are constantly monitoring the environment for signals of reward. When a cue is associated with reward, the BAS system is activated. As such, individuals with high BAS are thought to be more sensitive to reward cues and be more prone to engage in approach behaviour (Carver & White, 1994). The BAS section of the BIS/BAS questionnaire contains a further three subscales: BAS *drive* (which reflects persistence in pursuing goals even in the event of

delayed reward or just a potential for reward); BAS Reward responsiveness (measures seeking new incentives) and BAS Fun seeking (associated with positive feelings when incentives are attained). On the other hand, the Behavioural Inhibition System (BIS) underlies the inhibition of behaviour (associated with an increase in attention to conditioned cues of punishment). The Reinforcement Sensitivity Theory (RST) (J. A. Gray, 1970) provides a framework that explores the different brain systems that are responsible of responding to punishment and reward and how these are reflected in individual personality. This theory suggests that personality dimensions such as anxiety and impulsivity are controlled by the behavioural activation system and that individuals with altered sensitivity in these dimensions may then be at increased risk for psychopathology (Bijttebier, Beck, Claes, & Vandereycken, 2009). A meta-analysis by Harrison and colleagues (A. Harrison, O'Brien, et al., 2010) showed that patients with AN-restricting had a tendency to be lower in novelty seeking than HC, while patients with AN-binge/purge and patients with BN did not differ in novelty seeking compared to controls. Overall, the meta-analysis found that patients with AN-restrictive and binge/purge as well as patients with BN demonstrated higher harm avoidance than HC. These findings suggest that there is a difference in performance depending on phenotypic presentation of the disorder (restrictive vs. binge/purge). Patients with BN and AN-binge/purge showed higher reward sensitivity; while a high level of harm avoidance may be a more transdiagnostic feature across phenotypic presentations (Dawe & Loxton, 2004). Interestingly, more recent studies have shown that patients with both restricting and binge/purge presentations of AN reported higher sensitivity to reward (Glashouwer, Bloot, Veenstra, Franken, & de Jong, 2014; Jappe et al., 2011). Inconsistencies in the literature could be due to differences in measures used, although it has been suggested that individuals with AN-binge/purge may not differ from AN-restrictive in their sensitivity to reward but perhaps in their impulsivity (Glashouwer et al., 2014).

It has long been shown that individuals with ED show high co-morbid anxiety disorders, that often predate the emergence of the ED (W. H. Kaye et al., 2004). Patients with AN have also been shown to be perfectionistic and controlling as well as showing temperament traits such as shy, fearful, worrying behaviours and avoidance of perceived punishment. These characteristics have led to the hypothesis of the role that heightened sensitivity to experiences that are associated with reward or punishment may play in the development of ED behaviours, as a way of mitigating the emotional and physiological reactions.

This study only found higher sensitivity in girls at risk compared to girls at low-risk as measured by BAS drive. Our results of higher sensitivity to reward are in line with findings in patients with ED (Glashouwer et al., 2014; A. Harrison, O'Brien, et al., 2010; Jappe et al., 2011) although as discussed earlier, differences between phenotypic presentations are still not clear. Larger studies may be able to explore further differences in reward sensitivity based on maternal ED phenotypic expression (AN-binge/purge vs AN-restrictive) to further clarify differences. Interestingly, BAS drive has also been shown to be correlated with measures of self-directed perfectionism (beliefs that striving for perfection in oneself is important) (Stoeber & Corr, 2015) which has also been shown to be a possible maintaining or risk factor in AN (Bardone-Cone et al., 2007; Fairburn et al., 2003). It is possible that results of high reward sensitivity in this sample could also be tapping into this construct.

This study did not find higher sensitivity to punishment in girls at ED risk compared to girls at low risk although supplementary analysis stratified by maternal diagnosis showed a difference between girls of mothers with AN and healthy girls. The BIS system has been implicated in the expression of negative affective states such as fear and anxiety in response to punishment cues. It has been suggested that elevated anxiety might have a basis in dispositional punishment sensitivity (Amy Harrison et al., 2011; Jappe et al., 2011). In fact, a factor analysis of the STAI (measure of state and trait anxiety) and BIS suggested that they are strongly correlated constructs (A. J. Cooper, Perkins, & Corr, 2007). Girls at risk did have higher scores in the BIS scale, which became significant once stratifying by maternal diagnosis. It is therefore possible, that higher sensitivity to punishment is linked to AN rather than BN. Given the directionality of results and differences when stratifying by maternal diagnosis, it is worth further investigating this in a larger sample.

Overall, as shown in Chapter 2, fMRI studies have also shown altered reward processing in patients with both AN and BN using both taste and monetary paradigms (J. Cha et al., 2016a; Olivo et al., 2017; Steward et al., 2018; Wu et al., 2016) suggesting alterations in the ventral striatal region. Voxel-based morphometry methods have also shown positive associations between sensitivity to punishment and reward and gray matter volume in both the amygdala and the hippocampal area (Barros-Loscertales et al., 2006). Gray (J. A. Gray, 1970) proposed that elevated scores on trait anxiety measures (reflecting overactive

BIS – higher sensitivity to punishment) would represent a specific risk factor for generalise anxiety disorders. It is possible to hypothesise that alterations in the ventral striatal region and increased gray matter in amygdala and hippocampal area could represent an endophenotype related to reward sensitivity.

Interestingly, this study did not confirm past findings of alterations in social cognition measures in children at risk (R. Kothari et al., 2015). This could be due to a number of factors. First of which is the sample studied. While Kothari and colleagues studied a large sample, we were only able to study less than 40 girls in total, therefore, lack of findings could be due to a lack of power. Secondly, differences could be due to the use of tasks and the way these were administered. While Kothari and colleagues used three measures to asses social cognition (Social Communication Disorders Checklist, Faces subtest of the Diagnostic Analysis of Non-verbal accuracy and Emotional Triangles), we used two measures focused on assessing emotions based on faces and eyes (the second measure was design to explore more complex theory of mind traits). There were also limitations in the way our tasks were administered, as they were done remotely online, hence testing environment was not controlled for, other variables unknown to the researchers could have influenced performance. Interestingly, Kothari and colleagues did not find differences overall in emotion recognition, but found that children of mothers with binge-purge type disorders had differential facial emotional processing and poorer recognition from fear and social motion cues. It is possible that by exploring an overall measure of emotion recognition we were not able to differentiate between recognition of different types of emotions or measures of misinterpretation rather than recognition. While research has suggested that patients with BN may have difficulties categorising emotional faces (Kucharska-Pietura et al., 2004; Kuhnpast et al., 2012; Legenbauer et al., 2008; Pollatos et al., 2008), Kothari and colleagues only found misinterpretation of emotions in children at risk. It has been suggested that deficits in the ability to recognise emotional information in faces could contribute to interpersonal stress by misunderstandings in interpersonal relationships and poor social communication (Kuhnpast et al., 2012). It is possible that given that a part of the research in poor emotional identification focuses on patients with BN, these difficulties are more associated with risk for binge-purge subtype ED. While we did not have the power to run analyses based on maternal ED subtype, in our sample we only had a total of 3 mothers with a lifetime diagnosis of maternal BN (two of which had had both AN and BN and only one of which was purely BN) and 14 with a lifetime diagnosis of AN. This differences between maternal ED subtype could offer an explanation for why

we did not find similar results to those found by Kothari and colleagues. It is of course not clear what the relation between emotion recognition and ED is and it warrants further study.

It is worth noting the differences between what processes our two measures (Morphed emotion identification and Reading the mind in the eyes) are tapping into. Reading the mind in the eyes, requires higher order mentalization of emotional states and therefore is used as a measure of theory of mind (Baron-Cohen et al., 2001; Olderbak et al., 2015), while morphed emotion identification is a clearer emotion recognition task. While differences did not reach significance, differences in performance based on different social cognition tasks warrant some thoughts. Girls at risk performed better than controls in morphed emotion identification but worse than controls in Reading the mind in the eyes. As suggested in the previous paragraph, it is possible that difficulties in emotion recognition are linked to binge-purge type ED while difficulties in theory of mind which require higher order processing and understanding of others emotional state might be linked to restricting type ED. This could possibly explain why children in our sample, the majority of which were at high-risk for AN performed worse in these tasks, while they performed better in the morphed emotion recognition one. Interestingly, deficits in theory of mind are one of the main findings in ASD (U. Frith & Happe, 1994). In recent years, the has been an increase in the exploration of the overlap in deficits in ASD and those in AN after finding an overrepresentation of ASD and/or ASD traits in samples of girls with AN (Heather Westwood & Tchanturia, 2017; Zhou et al., 2018). Interestingly, deficits in set shifting abilities are also commonly found in people with ASD and are one of the significant deficits shown in this chapter between children at risk and children of HC mothers (H. Westwood et al., 2016).

Limitations

There are limitations that need to be considered. Although this is a pilot study, it is important to consider the small sample a limitation. Another limitation is the fact that some of the tasks (social cognition) were completed at home, which can limit the comparability between subjects. Some of the measures were also for 11 years old and over, which meant that not all of our subjects could complete them, reducing further our current sample. As previously stated, this is a pilot study and therefore we did not correct for multiple

comparisons. Given that the aim of the study is to develop hypotheses that may guide a full study in children at risk, it is important to include possible false positives.

Interim conclusion

In conclusion, this chapter shows that girls at high-risk show alterations in set-shifting and reward processing. While difficulties in cognitive flexibility refer to the ability to shift from thinking in one dimension to a different one. This influences how quickly we adapt to our environment and could therefore represent an aspect of rigidity which is shown in patients with ED. Difficulties in reward processing have long been proposed to play a role in psychopathology. The role that they play in ED has only recently been investigated and theories of ED development based on reward processing have been developed. Given the role that reward plays in food consumption, alterations in reward systems have been suggested to play an important role in the pathophysiology of ED.

Chapter 8. Subcortical and cerebellar volumetric differences in children at familial high-risk for ED: findings from BREDS

8.1. Introduction

As outlined in Chapter 2, neuroimaging studies in patients with ED have exponentially increased over the last decades, although they continue to lag behind those of other psychiatric disorders. As demonstrated, volumetric differences in ED have been found in patients with AN during acute stages of the disorder (Phillipou et al., 2014; Seitz et al., 2016). However, it has been proposed that these more obvious differences are most likely due to the pathophysiology of the disorder characterized by extreme weight loss and self-starvation. Further research is needed to clarify this as results are not conclusive. As demonstrated by studies with recovered patients, some of these alterations may normalize following successful treatment of the disorder (Wagner et al., 2006). However, recent well controlled studies have also demonstrated some subcortical and cerebellar volumetric alterations in patients who have been long-term recovered (Roberto et al., 2011; Seitz, Buhren, Von Polier, et al., 2014). Therefore, further research into subcortical and cerebellar volumetric differences is important to shed light into possible neural risk markers for these disorders.

The phenotypic presentation of ED has been widely studied and has informed our current treatments as well as helped develop hypotheses for possible risk mechanisms that can help guide neuroimaging research. In 2015, Culbert and colleagues (Culbert, Racine, & Klump, 2015) wrote a review in which they outlined possible risk factors based on results from studies using integrative methodologies (e.g. twin studies, gene-environment interactions) and/or data at the biological and behavioural level (e.g. neuroimaging). This review identified a variety of risk factors that could predispose an individual to develop an ED: these included factors such as negative emotionality, inhibitory control, and cognitive inflexibility amongst others. In 2016 McAdams and Smith wrote a targeted review focused on identifying cognitive neuroimaging data with translational relevance for the treatment of ED (McAdams & Smith, 2015). The authors reviewed 32 papers and identified three major neural systems which are disrupted in ED: reward, decision making, and social processing. In order to provide a neuroanatomical context to the review, the authors also performed a Neurosynth meta-analysis that highlights the neural regions most commonly identified in

studies of reward (functional MRI of reward studies using food-based and monetary paradigms, N=560 studies), as well as studies involving decision making (N=569 studies) and those using social cognition paradigms (N=960 studies). Results from the nerosynth meta-analysis highlighted the nucleus accumbens (NA), putamen, caudate, anterior insula as well as the midbrain, the anterior cingulate and ventromedial prefrontal cortex (vmPFC) as the neural regions most commonly identified in studies of reward. Many areas highlighted in studies in patients with ED involved in decision making overlapped with those in reward, such as the caudate and insula with the addition of part of the frontal cortex. Lastly, there was some overlap between areas highlighted in studies of social cognition and those in reward and decision making, such as areas in the medial prefrontal cortex and insula. However, more areas were highlighted such as the bilateral temporoparietal junctions, bilateral amygdala, and smaller clusters in the right temporal pole, right fusiform and superior frontal gyrus. Findings from this review conclude patients with ED have altered neural systems that mediate reward response, decision-making and social behaviours, and highlighted subcortical areas such as NA, caudate, cingulate, insula, amygdala and areas from the prefrontal cortex (vmPFC) as being altered in this group of patients.

As established in earlier chapters research suggests that alteration in brain circuits related to cognitive control and emotion are of importance to the maintenance (Walter H Kaye, Wierenga, Bailer, Simmons, & Bischoff-Grethe, 2013) and possibly development of AN. Neuroimaging studies have found alterations in prefrontal and anterior cingulate cortices in relation to cognitive control in AN (Ehrlich et al., 2015; Sato et al., 2013), while altered areas related to reward sensitivity have also been identified in both BN and AN (Jiook Cha et al., 2016; G. K. Frank, Shott, & Mittal, 2013; Garcia-Garcia et al., 2013; A. Harrison, O'Brien, et al., 2010; W. H. Kaye et al., 2011; Titova et al., 2013; Wu et al., 2016). Ample evidence exists with regards to the association between AN and alterations in emotion processing. In fact, the comorbidity between AN and Autism Spectrum Disorder (ASD) has been largely studied (Coombs, Brosnan, Bryant-Waugh, & Skevington, 2011). Most evidence in AN suggests an alteration of circuits related to the perception and processing of emotional stimuli, and studies investigating emotional processing in depressed and anxious adolescents have identified the amygdala as playing a central role in the mediation and processing of emotional stimuli (Fusar-Poli et al., 2009). In fact, research in patients with AN found that when exposed to emotionally salient stimuli that are specific for ED pathophysiology (such as images of food and bodies) both patients with AN and those

recovered (relative to HC) show greater activation in cortical and subcortical brain circuits, including the anterior cingulate, prefrontal cortex and amygdala (Bang, Ro, & Endestad, 2016; Seeger, Braus, Ruf, Goldberger, & Schmidt, 2002; Vocks et al., 2010).

Although as established, research into children at familial-high risk for developing ED is of great importance, little has been published to date. As highlighted in Chapter 3, early research on the neuropsychological and cognitive profile of children at high-familial risk has already identified that children of mothers with ED show poorer performance on social cognition tasks, have higher odds of having emotional difficulties and have decreased attentional control (Barona, Nybo Andersen, & Micali, 2016; Barona et al., 2017; R. Kothari et al., 2015; R. Kothari et al., 2014; R. Kothari et al., 2013b).

Alterations in reward, decision making, and social cognition processes as well as other interpersonal processes (such as negative emotionality) and their neural correlates have been proposed as possible risk mechanisms for ED and have been found to be altered not only in recovered patients but in healthy first degree relatives (e.g. siblings or parents) of patients with ED. The aims of this chapter are: first, to explore whole brain volume and subcortical brain volumetric differences in children at risk, in order to help elucidate if areas correlated to these functions (reward, decision and social cognition) are found to be altered in children at high-risk of developing ED. Second, given the suggested role that specific regions such as anterior cingulate, orbitofrontal cortex, and insula play in the development of ED, this study focused on these specific regions to explore hypothesis-driven analyses.

8.2. Methods

8.2.1. Participants

Data from both cases and controls were collected as part of the Brain in high-Risk for Eating Disorders Study. For further details on methodology see Chapter 4.

Information on maternal and child demographics, maternal ED diagnosis and children intelligence and neurocognitive measures can be found in Chapter 4.

As a summary, this study explored measures of general intelligence (WASI) , social cognition (Reading the Mind in the Eyes and Morphed Emotion Recognition) (Baron-

Cohen et al., 2001), CANTAB neurocognitive measures: motor screening, inhibitory control, set-shifting of cognitive flexibility, risk-taking behaviour, sustained attention and visual working memory (J. Fray et al., 1996) and reward measures (BIS/BAS) (A. Cooper et al., 2007). Significant differences between groups were found in set-shifting (girls at risk took a significantly longer time to switch when compared to controls) and BAS drive (girls at risk had higher drive towards pursuing goals when compared to controls) (see Chapter 7 for detailed information).

8.2.3. MRI Acquisition

Data acquisition and pre-processing proceeded as described in Chapter 4.

8.2.4. Pre-processing

The raw MRI data (DICOM file) was converted into NIFTI format using Tractor software (Clayden et al., 2011) (http://www.tractor-mri.org.uk). The TractorR software includes R packages for reading, writing and visualising MRI images and it also includes functions designed for working with diffusion MRI data and Tractography.

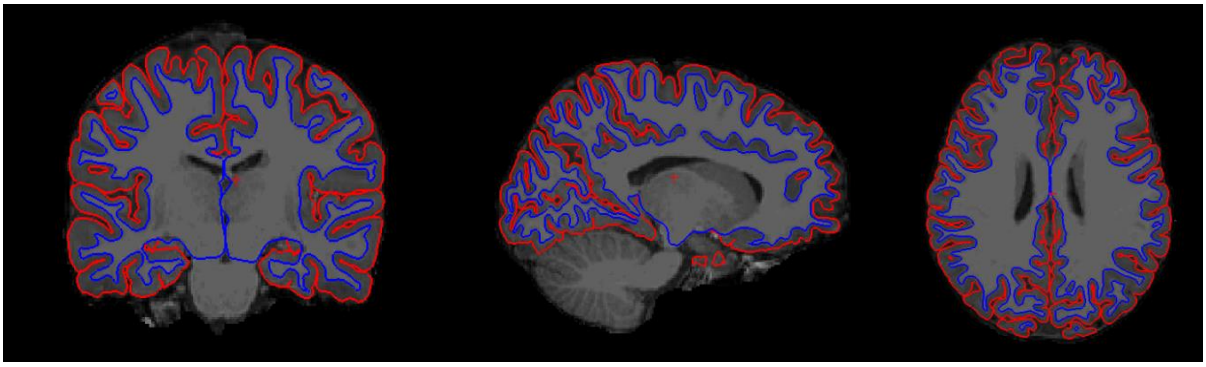
8.2.4.1. Freesurfer

Freesurfer was used to acquire full surface reconstructions (Fig. 7.1) and subcortical reconstructions (Fig. 7.2) (full details in Chapter 4). FreeSurfer image analysis suite (http://surfer.nmr.mgh.harvard.edu/), provides a set of tools to reconstruct topologically correct surface models of the inner and outer cortical boundaries. FreeSurfer produces a number of files with volume information, including data on subcortical segmentation (*aseg*) and cortical parcellations (*aparc*) volumes.

Following automated processing steps outlined in Chapter 4, each individual cortical reconstruction was visually inspected to ensure optimal grey/white matter classification with the support of quality assurance tools implemented in FreeSurfer (https://surfer.nmr.mgh.harvard.edu/fswiki/QATools). Manual edits were used where necessary. Following this process, measurements were directly obtained from the

generated files, including information on 34 cortical structures per hemisphere, as labelled by the Desikan-Killiany atlas (Desikan et al., 2006) (Fig. 8.2).

Figure 8.1. shows an example of a surface reconstruction with boundaries marked between pial and white matter surfaces overlaid on the participants T1 weighted-scan. Coronal, sagittal and axial views are shown (left to right respectively).



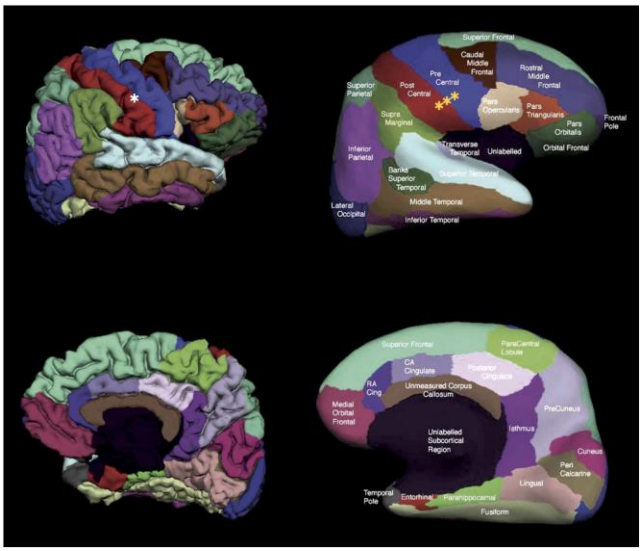


Fig. 8.2. From Desikan et.al (Desikan et al., 2006). Shows pial (left) and inflated (right) cortical representation of regions of interest in one hemisphere. The top row illustrated the lateral view while the bottom row shows the medial view of the hemisphere.

8.2.4.2. Analyses of subcortical areas using FIRST (FSL): pre-processing

FIRST (FMRIB Image Registration and Segmentation Tool) is a fully automated method within the FSL (FMRIB Software Library) suite of tools (Patenaude, Smith, Kennedy, & Jenkinson, 2011). FIRST was used to segment subcortical grey matter structures on the

T1-weighted scans. The structures included were the amygdala, caudate, globus pallidus, nucleus accumbens and thalamus (see Figure 8.3).

FIRST begins by using an affine-registration of all T1-weighted scans to the MNI152 standard-space template in two different steps: first the tool registers the image to the entire template and follows with a separate registration to a subcortical mask of the template in order to optimally align the subcortical structures.

FIRST uses shape and appearance models that have been generated from manually-traced masks combined with signal intensity information that helps guide the segmentation of each subcortical grey matter structure.

The last stage of FIRST is a boundary correction which is done in order to correct for any overlapping voxels. Before extracting the volumes for each subcortical structure, all registrations and segmentations were visually inspected for accuracy. FIRST produces an html link in order to inspect each parcellation. FIRST has been optimized and no discernible issues were found while inspecting the structures.

Volumes for each of the subcortical structures were then obtained using FSL utilities.

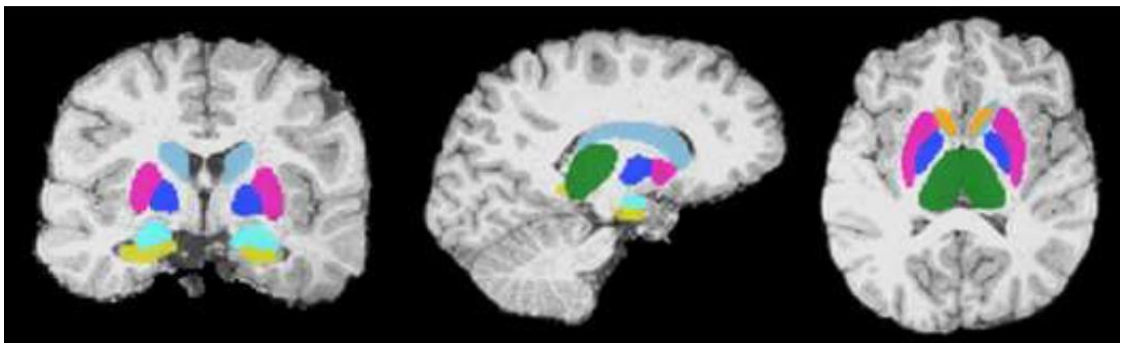


Figure 8.3. shows an example of FIRST output of subcortical structures overlaid on a T1-weighted scan. Coronal, sagittal and axial views are shown (left to right respectively). The following subcortical structures were segmented: caudate (blue-grey), nucleus accumbens (orange), putamen (pink), globus pallidus (dark blue), thalamus (green), amygdala (bright light blue) and hippocampus (yellow).

7.2.4.2. Freesurfer vs. FSL FIRST

Delineation of brain regions in MR images is an important technique that plays an expanding role in neuroscience research. While traditionally, brain regions were segmented manually, this method can be subjective (depending on ability) and very time consuming. Therefore, automated segmentation tools have been proposed with FSL-FIRST and Freesurfer methods being the two most widely used. Despite both being validated, there is evidence that they are not always equally accurate.

Given the importance of striatal structures in the study of ED and because their segmentation can be challenging (Patenaude et al., 2011), we chose to use FIRST as it has been shown to be more accurate (compared to manual tracing) in the automated segmentation of caudate and putamen (Perlaki et al., 2017), and nucleus accumbens (Rane, Plassard, Landman, Claassen, & Donahue, 2017).

Visual inspection showed problems in nucleus accumbens and putamen Freesurfer segmentation in some of our images. Further analyses showed that left nucleus accumbens volumes segmented by FIRST and those segmented by Freesurfer were not correlated (Fig. 8.4).

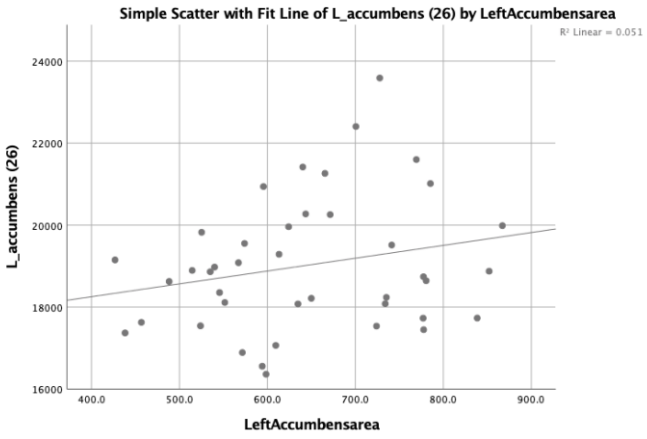


Figure 8.4. Scatterplot showing considerable variability between software's. (Correlation results: r = 0.225, p = 0.156)

8.2.5. Statistical analyses

Independent sample t-tests were used to compare demographic measures between the at-risk and control groups (Chapter 4). All variables were examined to check for inconsistencies and outliers and normality using tabulations, graphs and plots.

Associations between children at risk status and cerebellar and subcortical volumes were investigated using crude and adjusted linear regression analyses. Further hypothesis driven region of interest analyses were performed to investigate associations between specific cortical structures: orbitofrontal cortex (lateral and medial divisions), anterior cingulate (rostral and caudal divisions), and insula. A priori confounders (children age at testing, and total intracranial volume) were included in all adjusted models. Further post-hoc analyses were conducted in order to investigate associations between specific maternal ED diagnoses (lifetime AN) and outcomes under study.

Correlation analyses were run to investigate the correlation between outcomes and neuropsychological variables. All analyses were run using SPSS 23 (IBM Corp, Armonk, NY), and a two-tailed significance level of $p \leq .05$ was used.

8.2.5.1. Freesurfer GM and WM volumes

GM and WM total volumes produced by Freesurfer were initially compared between groups using a one-way between-subjects' analysis of covariance (ANCOVA) to investigate interactive effects between groups (HC and at high-risk) while controlling for age and total intracranial volume (TIV) in SPSS version 24 (SPSS Inc. 2012, Chicago, IL.). Although age did not differ significantly between groups, due to the large age range sampled (8 – 15 years old), age was included as a covariate and could contribute to GM variations.

8.2.5.3. FIRST: subcortical volume analyses

Volume data were extracted from each subcortical area using FSL utilities as described previously and input into SPSS. Data was then checked for normality and outliers and then

the volumes were compared between groups using linear regression with age, gender and whole brain volume as covariates.

8.3. Results

8.3.1. Sample characteristics

Both maternal and child participants' demographic characteristics are summarised in Chapter 4 Table 4.1. Children of HC mothers did not differ from children at high-risk in age or IQ. Groups did not differ in maternal characteristics (maternal age, education, and ethnicity).

8.3.2. Image analyses

8.3.2.1. Whole-brain GM differences between children at high-familial-risk for ED and children of healthy control mothers

See table 8.1. Initial whole brain volume comparisons revealed a significant difference between groups in GM volume after controlling for age and total intracranial volume, with children at high-risk having higher GM absolute volumes than children of HC mothers ($F = 7.53$, $p = 0.01$).

No significant differences were found between children at high-risk and children of HC mothers in WM absolute volume or TIV (Total intracranial volume) (Table 8.1).

Table 8.1. Group comparisons: WM, GM and TIV

	HC (mean mm3, SD)	Children at high-risk (mean mm3, SD)	F[1], p
WM	40,3244.45 (45,853.88)	41,5885.00 (34,319.35)	F = 0.32, p = 0.57
GM	68,3313.60 (54,920.88)	72,9421.98 (48,829.76)	**F = 7.53, p = 0.01****
TIV	1,392,636.85 (119,384.21)	1,400,354.21(104,680.67)	F=0.45, p=0.83

*p≤0.05, **p≤0.01, ***p≤0.001, †p<0.1

8.3.2.3. Hypothesis driven Region of Interest analyses: Cortical parcellation

See table 8.3. Linear regression analyses showed greater volume in the right medial orbitofrontal and left lateral orbitofrontal cortex in children at high-risk relative to children of HC mothers. After adjusting for children's age and total intracranial volume these differences remained significant. A trend towards significance was also found in divisions of the insula and anterior cingulate. No other significant differences in ROI analyses were identified between groups.

Table 8.2. Linear regression analyses coefficients

	Crude (B, 95% CI)	**Adjusted (B, 95% CI)**	
	At risk, N=17	At risk, N=17	HC, N=29
Left insula	0.265 (-50.045, 455.939)	0.256 (-59.776, 452.040)	Ref.
Right insula	0.287 (-25.256, 333.781) †	0.240 (-32.469, 290.752)	Ref.
Left medialorbitofrontal	0.293 (-22.801, 352.236) †	0.244 (-21.530, 296.440) †	Ref.
Right medialorbitofrontal	**0.429 (65.798, 408.673) ****	**0.436 (67.544, 414.970)****	Ref.
Left lateralorbitofrontal	**0.343 (11.360, 482.815)***	**0.296 (48.701, 378.019)****	Ref.
Right lateralorbitofrontal	0.264 (-52.478, 433.178)	0.224 (-36.707, 359.813)	Ref.
Left caudal ant.cingulate	0.000 (-114.786, 114.886)	-0.005 (-123.424, 97.271)	Ref.
Right caudal ant.cingulate	0.087 (-89.295, 148.720)	0.080 (-77.826, 132.739)	Ref.
Left rostral ant.cingulate	0.312 (-6.204, 209.104) †	0.246 (-7.254, 167.351) †	Ref.
Right rostral ant.cingualte	0.134 (-59.600, 134.725)	0.121 (-55.769, 124.032)	Ref.

*p≤0.05, **p≤0.01, ***p≤0.001, †p<0.1; Adjusted: child's age and TIV

8.3.2.4. Subcortical volume differences between children at high-familial-risk for ED and children of healthy control mothers

Initial independent t-test yielded a significant difference in the amygdala and hippocampus between children of HC mothers and children at high-risk and a trend towards significance in the right caudate (Figure 8.5).

Linear regression analyses showed that volume was greater in children at high-risk relative to children of HC mothers in the amygdala (both right and left), left hippocampus and right caudate. After adjusting for children's age and total intracranial volume, children at high-risk were more likely to have higher GM volume in the amygdala (B=0.320 95% CI: 8.231, 228.591, p = 0.041; B=0.320 95% CI: 8.231, 228.59, p = 0.036), both right and left respectively, left hippocampus (B=0.298 95% CI: -0.018,484.011, p= 0.050) and right caudate (B=0.311, 95% CI: 19.093, 647.648, p=0.038). No other associations were found in other subcortical volumes (Table 8.4).

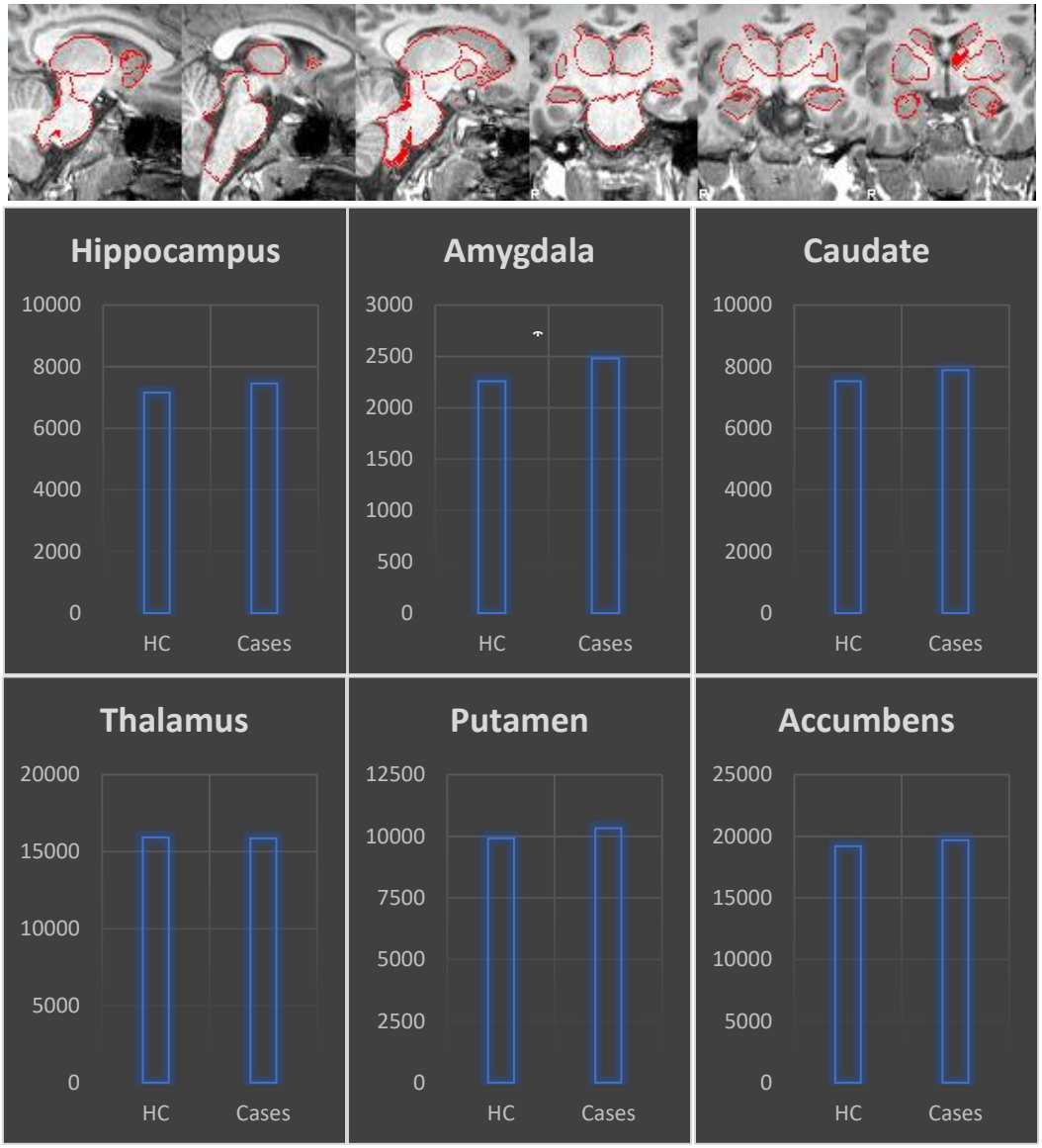


Figure 8.5. Analyses of subcortical volume in children at high-risk compared to healthy controls. Single subject results of FSL FIRST automated subcortical segmentation as exemplified in HC (age = 11) plotted on T1 weighted MRI volume. Grouped bar graphs showing mean volume measurements in mm^3 for each group extracted from each of the subcortical regions of interest. Exact values showed are means per group obtained with an independent sample t-test which revealed a significant difference in volume between children of HC mothers and children at high-risk [t = -2.33 (35.93), p = 0.032].

Table 8.3. Linear regression analyses coefficients

	Crude (B, 95% CI)	Adjusted (B, 95% CI)	
	At risk N = 17	At risk N = 17	HC N=29
Hippocampus	0.220 (-120.302, 728.709)	0.182 (-158.376, 662.007)	Ref.
L Hippocampus	**0.321 (18.039,502.622)* 0.036**	**0.298 (-0.018,484.011)* p 0.050**	Ref.
R Hippocampus	0.060 (-186.181, 273.928)	0.013 (-211.745, 231.382)	Ref.
Amygdala	**0.378 (56.564, 440.884)** 0.012**	**0.349 (37.290, 421.871)* 0.021**	Ref.
L Amygdala	**0.317 (6.273, 220.998)* p 0.039**	**0.310 (4.714, 217.625)* p 0.041**	Ref.
R Amygdala	**0.365 (26.406,243.771)** p 0.016**	**0.320 (8.231, 228.591) * p 0.036**	Ref.
Caudate	0.282 (-37.737, 1045.222) † p=0.067	0.237 (-102.143, 950.828)	Ref.
L Caudate	0.182 (-101.765, 392.701)	0.114 (-145.399, 327.343)	Ref.
R Caudate	**0.334 (39.689, 676.858)* 0.028**	**0.311 (19.093, 647.648)* p 0.038**	Ref.
Thalamus	0.033 (-818.879, 10001.852)	-0.10 (-676.338,618.059)	Ref.
L Thalamus	-0.006 (-485.128, 466.441)	-0.043 (-421.474, 291.942)	Ref.
R Thalamus	0.073 (-344.093, 550.754)	0.025 (-277.446, 348.699)	Ref.

Putamen	0.184 (-275.703, 1080	0.132 (-348.348, 924.184)	Ref.
L Putamen	0.135 (-194.537, 490.823)	0.092 (-233.070, 435.575)	Ref.
R Putamen	0.222 (-98.253, 606.466)	0.163 (-133.187, 506.518)	Ref.
Accumbens	-0.099 (-2692.519, 1406.116)	-0.096 (-2744.203, 1491.161)	Ref.
L Accumbens	-102 (-2700.866, 1374.428)	-0.096 (-2736.025, 1484.918)	Ref.
R Accumbens	0.086 (-53.503, 93.539)	-0.004 (-64.231, 62.296)	ref.

*p≤0.05, **p≤0.01, ***p≤0.001, †p<0.1; Adjusted: child's age and TIV

8.3.2.4. Exploratory analyses

When stratifying analyses by maternal diagnosis, linear regression analyses showed that volume of the right amygdala was greater in children at high-risk (for AN) relative to children of HC mothers. After adjusting for children's age and total intracranial volume, children at high-risk (for AN) were more likely to have higher GM volume in the amygdala (B = 0.33, 95% CI: 21.43, 440.88, p = 0.032), the right amygdala specifically. No other associations were found in other subcortical volumes with previous significant differences in the whole group (Table 8.5).

Table 8.4. Linear regression analyses coefficients divided by maternal ED subtype diagnosis (AN)

	Crude (B, 95% CI)	Adjusted (B, 95% CI)	
	At risk N = 14	At risk N = 14	HC N=28
Hippocampus	0.064 (-372.639, 553.288)	0.097 (-317.388, 590.384)	Ref.
L Hippocampus	0.135 (-154.952, 374.848)	0.165 (-133.515, 402.726)	Ref.
R Hippocampus	-0.025 (-273.979, 234.731)	0.002 (-246.637, 250.422)	Ref.
Amygdala	0.309 (-2.353, 406.462)†	**0.345 (22.225, 429.510)***	Ref.
L Amygdala	0.197 (-46.210, 192.285)	0.254 (-25.198, 213.432)	Ref.
R Amygdala	**0.361 (12.67, 237.814)***	**0.370 (21.991, 241.509)***	Ref.
Caudate	0.139 (-315.023, 786.305)	0.170 (-247.878, 824.878)	Ref.
L Caudate	0.063 (-195.927, 289.602)	0.071 (-177.529, 282.887)	Ref.
R Caudate	0.181 (-148.327, 525.934)	0.226 (-99.416, 571.058)	Ref.

p≤0.05, **p≤0.01, ***p≤0.001, †p<0.1; Adjusted: child's age and TIV

8.3.2.5. Correlation analyses with neuropsychological scores

Partial correlation analyses in the familial high-risk group showed a significant correlation between attention switching measures (congruency cost and switching cost) and hippocampal volume (right and left respectively). No other correlations were found between neuropsychological measures and hippocampus, amygdala and caudate. See table 8.6.

Table 8.5. Partial bivariate correlations in the high-risk group, between subcortical volume and neurocognitive measures (social cognition, cognitive flexibility, spatial working memory, affective response and attention)

	RME	Morphed Emotion Identification	AST congruency cost	AST switching cost	AGN affective response bias	SWM strategy	SWM total errors	RVP probability of hits[a]
Hippocampus								
L Hippocampus	0.140	0.101	-0.375	**-0.550***	0.261	-0.242	-0.365	0.135
R Hippocampus	0.479	0.118	**-0.606****	-0.446	0.137	-0.057	-0.470	0.344
Amygdala								
L Amygdala	0.251	0.140	0.076	-0.233	0.014	0.033	0.088	0.151
R Amygdala	0.279	0.862	-0.144	-0.210	0.003	0.198	-0.341	0.207
Caudate								
L Caudate	0.144	-0.164	-0.244	-0.092	0.018	0.183	0.136	0.143
R Caudate	0.045	-0.022	-0.285	-0.051	0.112	0.187	0.067	0.216

*p≤0.05, **p≤0.01, ***p≤0.001, †p<0.1

[a] Non-parametric correlation due to non-normality of variable

Table 8.6. Significant partial bivariate correlations in the high-risk group, between subcortical volume and neurocognitive measures (reward measures)

Subcortical region	Neurocognitive measures	Rho	Uncorrected P value
Hippocampus - right	BAS Fun seeking	-0.62	0.01
Putamen - right	BAS Drive	-0.62	0.01
Thalamus - right	BAS Drive	-0.56	0.02
Thalamus - left	BAS Drive	-0.54	0.03

8.4. Discussion

We investigated grey and white matter volume alterations in children at high familial risk for ED compared to children of healthy control mothers. Our main findings in this study are of greater GM volume in children at high-familial risk for ED. The second aim of this study was to investigate subcortical volumetric differences. Significantly greater volume was found in the amygdala (both left and right), left hippocampus and right caudate in children at high familial risk for ED compared to children of healthy control mothers. Exploratory analyses showed that children of mothers with lifetime AN diagnosis had significantly greater volume in the amygdala. Hypothesis driven analyses of cortical regions showed greater volume in medial orbitofrontal areas and trends towards significance in the insula in girls at risk compared to girls of healthy control mothers. Lastly, we found significant correlations between measures of cognitive flexibility and hippocampus volume. Reward sensitivity was also correlated with volume in hippocampus, putamen and thalamus.

Interestingly, all significant differences show increased GM, in both cerebellar volumes, sub-cortical regions and specific cortical regions. It is worth noting that although most studies in patients with acute ED have generally found decreased GM in both cerebellar and subcortical volumes this has been hypothesised to be largely due to the effects of starvation, although some studies have shown persistent decreased GM after weight recovery (Katzman, Zipursky, Lambe, & Mikulis, 1997; Swayze et al., 2003). In contrast to studies on adults, that have generally found decreased GM and decreased WM diffusivity, some studies investigating adolescents have found increased GM (G. K. Frank, M. E. Shott, J. O. Hagman, & T. T. Yang, 2013) as well as increased measures of WM diffusivity (see chapter 6). Since the current research studies children and young people it is more likely that results would be similar to those found in adolescents. One possible explanation for these differences is that increased GM volumes may highlight a dysfunction in developmentally appropriate synaptic pruning, which can lead to an increased GM volume and influence connectivity. Inappropriate myelination along the GM/WM border could also be a reason for greater GM volume. In our sample, although not significant, WM volume was also increased in children at risk compared to HC and therefore suggests that myelination is also greater in children at risk. However, speculation on the possible mechanisms involved in larger volumes in children and young people at-risk vs adults in the ill state requires further clarification. The normal trajectory of GM and WM volume during childhood and adolescence is opposite, with GM decreasing (following an inverted

U shaped trajectory) and WM increasing with age (Lenroot et al., 2007). It is possible, that in ED differences between children/young people and adults in these trajectories reflect abnormalities (perhaps delays) in brain maturation, which in turn could underlie risk. Longitudinal studies in ADHD have shown a developmental delay of cortical thickness trajectories compared to healthy controls which could reflect some of the difficulties in this group (Shaw et al., 2007). Longitudinal brain MRI studies in healthy siblings of children with early onset schizophrenia showed initial alterations in cortical GM change that were then normalised in adulthood, unlike their siblings, suggesting that GM longitudinal abnormalities may be, at least in part, a possible endophenotype (Cannon et al., 2003). Clinical studies have shown that diagnosis-specific brain differences are beginning to elucidate the timing and nature of deviations from typical development. Therefore, using trajectories of change in morphometric measures may act as an endophenotype.

Altered subcortical areas found in this study are areas known to play a role in the mediation of processes such as reward and decision making, affect regulation, and interpersonal processes which have been shown to be altered in patients with ED. The main finding in the study was of bilateral increased amygdala volume. This finding persisted in exploratory analyses after stratifying by maternal ED diagnosis, only including children at risk for AN. The amygdala is a region located in the brain's medial temporal lobe forming part of the limbic system, and it is at the core of the brain's emotion circuitry. The amygdala has been described as the fear centre of the brain and has been found to be altered in different psychiatric disorders including anxiety disorders (Scott L. Rauch, Shin, & Wright, 2003), OCD (Milad & Rauch, 2012), ADHD (Cubillo, Halari, Smith, Taylor, & Rubia, 2012). Furthermore, the role of the amygdala in ASD psychopathology, specifically in social cognition, has been highlighted in numerous neuropathological and neuroimaging studies (Park et al., 2016).

Disturbance in interpersonal deficits in AN has been proposed to be a *trait* rather than *state* marker, with evidence suggesting premorbid impairment (C. Gillberg & Råstam, 1992). Interpersonal deficits have also been shown to interfere on treatment outcome and have a negative impact on illness prognosis (P. K. Keel et al., 2005). Studies have identified premorbid traits of anxiety, patterns of comorbidity with social phobia, ASD and OCD as being of importance for a cognitive interpersonal model of AN. As discussed in chapter 2, Zucker and colleagues (Zucker et al., 2007) identified premorbid traits of anxiety as well as comorbidities with ASD (social withdrawal and rigidity as well as impaired social

information processing), social phobia and OCD (excessive rigidity), as the aspects of central importance in the development of their risk model for AN. Anxiety traits have been shown to emerge early in AN and to be family transmitted (Strober et al., 2007) (Chapter 1). One possible hypothesis is that cortico-limbic dysfunctions implicated in anxiety might predispose to ED. In line with this hypothesis, in study one (Chapter 5) (Barona et al., 2016), results showed an increased level of emotional disorders in children at risk for ED; it was hypothesised the role that anxiety may play in the development of ED and the possible shared risk between ED and other psychopathology (including anxiety disorders).

At the core of ED pathophysiology there is a debilitating fear of gaining weight that overrides the body's need for calorie intake necessary for survival. As previously stated, research on neural processes underlying anxiety and fear behaviour have the amygdala at its centre. The output connections from the medial part of the amygdala go to areas responsible for physiological responses of anxiety (such as production of cortisol and autonomic nervous responses through the hypothalamus). Therefore, alterations in the amygdala could play a role in the development of fear and anxiety responses in ED. Extensive research has been conducted studying the role of the amygdala in fear conditioning/anxiety (Lupien, McEwen, Gunnar, & Heim, 2009). For example, increased GM volume in the amygdala has been repeatedly found in Generalised Anxiety Disorder (GAD) (Etkin, Prater, Schatzberg, Menon, & Greicius, 2009) patients and increased right amygdala volume has specifically been associated with prolonged reaction times in GAD patients, indicating attentional difficulties (Makovac et al., 2016). A study by De Bellis and colleagues (De Bellis et al., 2000) found increased amygdala volumes in paediatric Generalised Anxiety Disorder, suggesting an increased sensitivity to threat cues creating a vulnerability to the disorder. Alternatively, the authors considered a developmental hypothesis where increased amygdala volume might be the result of increased anticipatory anxiety during development (A pilot study of amygdala volumes in pediatric generalized anxiety disorder). A study by Qin and colleagues in 2014 (Qin et al., 2014) found that in children as young as 7 to 9 years old, anxiety can be reliably predicted by the volume and connectivity pattern of the amygdala. Animal models have also shown that early life stress can lead to increased formation of synapses which can lead to an increased rate of growth of the amygdala (Lupien et al., 2009; Vyas, Jadhav, & Chattarji, 2006). Electrical stimulation of the amygdaloid region has also been associated with fearful behaviours and physiological responses of anxiety such as increase heart rate, blood pressure, freezing and activation of fear-related movements (review Davis 1992) (Davis, 1992). A study on autistic children also found a correlation between anxious symptoms

and increased amygdala volume (Juranek 2006; Association Between Amygdala Volume and Anxiety Level: Magnetic Resonance Imaging (MRI) Study in Autistic Children). It is possible that enlargement of the amygdala is not unique to specific disorders but that cuts across diagnostic boundaries when there is an underlying element of anxiety.

Most of the work conducted to date exploring fear responses in AN has been focused on symptoms of AN related to eating and body image. In these studies, greater amygdala activation has been demonstrated in response to cues relating to food or weight relative to healthy control participants (G. K. Frank, Bailer, Henry, Wagner, & Kaye, 2004), which is in line with increased activation in anxiety disorders. Interestingly, in our study we did not find significant differences in internalising disorders measures between the at-risk group and HC (as measured by the SDQ). Given the measures used, it is possible that we were not able to identify specific high anxiety traits, measures such as the STAI, which was one of the first anxiety measures to measure both trait and state anxiety, may be worth exploring in the future. Interestingly, when stratifying by maternal diagnosis, results showed that girls of mothers with AN had higher sensitivity to punishment as measured by the BIS/BAS. The BIS system mediates reactivity to threat and punishment and can predict response to anxiety cues. In the 2004 review of the model, McNaughton (McNaughton & Corr, 2004) described the amygdala as having a central role in anxiety due to its participation in both the processing of warnings of punishment and promoting arousal reaction of the BIS. In this study, we did not find any correlations between amygdala and BIS/BAS measures.

More recently emerging evidence suggests emotion regulation and social cognition as possible endophenotypes for ED (Ambwani et al., 2016; Lang, Larsson, et al., 2016) (see chapter 2). The amygdala has also been identified as an area of importance in the study of social cognition. Ocshner (Ochsner, 2008) in his development of constructs for social cognition identified the amygdala as the area of importance in the acquisition, recognition and response to social-affective stimuli. In 1990 Brothers (Brothers, 1990) proposed that the amygdala is a key component of a circuit that forms the basis of social perception and studies of lesions in the amygdala have been shown to result in impaired social functioning in animal research (Emery et al., 2001). These last studies have also shown that the amygdala is involved in the recognition of emotional facial expressions (in particular fear) as well as in the development of “theory of mind” (Adolphs, Tranel, Damasio, & Damasio, 1994; Shaw et al., 2004).

The association between the amygdala and social cognition suggests that alterations in this limbic structure could play a pivotal role in the at-risk status for the development of ED. Extensive research has been conducted in social cognition in patients with ASD, with amygdala alterations being consistently found. However the evidence is ambiguous: studies report both amygdala enlargement (Abell et al., 1999; Howard et al., 2000) and reduction (Rojas et al., 2004) in ASD and fMRI studies have found both hypoactivation and hyperactivation in response to social cognition tasks. A study by Gibbard (Gibbard, Ren, Skuse, Clayden, & Clark, 2018) found larger right amygdala in young adults with ASD. Gibbard also investigated amygdala connectivity and found reduced microstructural integrity of amygdala-parietal and amygdala-temporal connections all related to ASD difficulties. Enlarged amygdala has also been found to be associated with anxiety in ASD and therefore it is possible that amygdala enlargement is associated with anxiety levels (known to be high in ASD) rather than social cognition. Correlations between subcortical regions (and the amygdala specifically) and emotion recognition and theory of mind tasks in our sample were not statistically significant. This could be due to many reasons, the main one being that our sample once divided into high risk and controls is small and it is possible that we did not have the power to find significant differences in the at-risk group (N = 17). Interestingly, Kothari and colleagues (R. Kothari et al., 2015) found that children at high familial risk for ED performed worse than children of healthy control mothers in tasks of facial emotion recognition. In this large longitudinal sample children at high familial risk for ED were more likely to misattribute faces as fearful than children of healthy control mothers. Another possibility is that differences in the findings could be due to heterogeneity of the measures used to assess social cognition. While this study used a validated online version of Reading the mind in the Eyes and facial emotional recognition tasks, a limitation was the lack of control in the way children completed the task (as this could be done without the supervision of a researcher). Therefore, the results might not be an adequate representation of their social cognition abilities. Theory of mind and emotion recognition both require involvement of different areas and connections, therefore looking at the connectivity and alterations between the amygdala and other areas involved in social cognition processing might further explain differences in this group. It is possible that correlations lie within a specific amygdala-cortical connectivity, which will be explored in the next chapter. Of course, we cannot disregard the possibility that there is no relationship between measures of social cognition and alterations in the amygdala and that differences in amygdala volume in this group are unrelated to social cognition difficulties and more related to underlying trait anxiety.

Main regression analyses showed significantly increased volume in the right caudate, an area that has been investigated in relation to reward sensitivity. The caudate and the putamen together comprise the striatum, two areas which form the main input nuclei to the basal ganglia, receiving axons from nearly every area in the cortex. The caudate and the putamen are then reciprocally interconnected with the substantia nigra, the main output area for the basal ganglia. This cortico-striatal loop has been widely studied (Alexander, DeLong, & Strick, 1986). These relationships show that the putamen plays a critical role within the so called 'motor circuit' whilst the caudate forms part of the oculomotor, dorsolateral and ventral/orbital circuits which is of importance in goal-directed behaviour and is sensitive to reward and punishment. The ventral striatum (which also incorporates the nucleus accumbens) has been proposed as integral for coding the pleasure of a reward and its motivational salience (K. C. Berridge & Robinson, 1998). It has been suggested that eating disorders are associated with alterations in cortico-striatal circuits given the role these circuits play in reward-guided learning, decision-making, affect regulation and reappraisal. Findings from this chapter have shown alterations in striatal areas, which could suggest alterations in cortico-striatal circuits including the OFC. The OFC occupies the entire ventral surface of the frontal lobes and plays a role in many of the complex functions that are central in cognition, such as processing of reward expectation and value as well as controlling aspects of behaviour (such as how much we eat and food avoidance (Plassmann, O'Doherty, & Rangel, 2010; Rolls, Rolls, Rowe, & Sweeney, 1981)). The OFC has been suggested to play a role in many psychiatric disorders (Drevets, 2007; Melloni et al., 2012; Shott et al., 2015) including ED (J. Cha et al., 2016a; Schafer et al., 2010). Findings from supplementary region of interest (ROI) analyses showed a significant difference between children at high and low risk in the right medial OFC and left lateral OFC. Surface area data was obtained from the automatic surface parcellation in Fresurfer using the Desikan-Killiany atlas (Desikan et al., 2006). Larger OFC has been suggested by Frank *et al* (G. K. Frank, 2016) in his bio-psycho-social model of risk as a possible risk marker for ED and could play a role in reward and compulsive behaviours, both of which have been suggested as possible endophenotypes for ED. Interestingly, results from surface area analyses showed that the differences were in the medial OFC, which has been previously found to be altered in both adults and adolescents with ED (G. K. Frank, M. E. Shott, J. O. Hagman, & V. A. Mittal, 2013; G. K. Frank, M. E. Shott, J. O. Hagman, & T. T. Yang, 2013).

Alterations in the cortical-striatal system connecting the caudate and prefrontal cortex have also been found in other disorders such as OCD and been proposed to underlie compulsive behaviours. OCD is characterised by obsessions (recurrent intrusive thoughts) and compulsions (repetitive actions or mental acts) aimed at reducing the anxiety created by the obsessions. It has been suggested that some characteristics may be shared with other psychiatric disorders, and specifically the overlap between OCD and AN has been widely studied. It has been suggested that some of the stereotyped and often ritualistic behaviours seen in AN have the same neurobiology as those in OCD (J. Steinglass & Walsh, 2006). In fact, comorbidity between AN and OCD is often found (Halmi et al., 1991) and it has been found that the presence of obsessive-compulsive symptoms is a risk factor for developing AN (Anderluh et al., 2009). A study by Godier and Park (Godier & Park, 2014) suggested the compulsive nature of weight loss behaviour which are central in AN, as a transdiagnostic concept. Models of neurocircuitry involved in compulsive behaviour suggest involvement of a cortico-striatal circuit, with the caudate (striatal component) suggested to be responsible for the compulsive behaviour (while the OFC – prefrontal component is supposed to inhibit behaviour). Several studies have found alterations in the caudate in OCD, although there are some conflicting results (Radua & Mataix-Cols, 2009; Rotge et al., 2009). The caudate has also been associated with cognitive processes that are altered in OCD patients (Britton JC, Rauch SL, Rosso IM, Killgore WD, Price LM, Ragan J et al (2010) Cognitive inflexibility and frontal-cortical activation in pediatric obsessive–compulsive disorder. J Am Acad Child Adolesc Psychiatry 49(9):944–953) as well as in AN. While to our knowledge, there are no studies investigating children at risk for OCD, recently, a study by Dong et al. (Dong et al., 2019) suggested impairment in the cortico-striatal learning system as a biomarker for OCD after alterations in both patients and their unaffected first-degree relatives. It is therefore possible, that alterations in the caudate and the OFC could underlie difficulties in cognitive inflexibility and leading to compulsive behaviour that could be considered a potential endophenotype in AN.

Sensitivity to reward has been hypothesised as a risk and/or maintenance factor in ED. In AN, the persistence of dieting to the point of starvation has led to the proposal that dieting is rewarding for these patients (Charlotte Keating, 2010; C. Keating, Tilbrook, Rossell, Enticott, & Fitzgerald, 2012; Zink & Weinberger, 2010). In these hypotheses, restriction of calories may become rewarding due to several factors, including an initial social reinforcement due to weight loss, development of a sense of achievement or possibly as an alleviation of negative effect. In BN, it has been hypothesised that food becomes more rewarding than in healthy controls and drives the need to eat even when not hungry. A

meta-analysis in 2010 concluded that patients with ED had higher sensitivity for punishment compared to controls, while those with bingeing type disorders (BED, BN and AN-B/P) were more sensitive to reward and those with restricting type disorders (AN-R) were less sensitive to reward (A. Harrison, O'Brien, et al., 2010). This meta-analysis included studies using behavioural measures of reward and highlighted the heterogeneity of measures used as well as the need for more studies using similar measures. More recent findings have shown higher sensitivity to reward across all ED subtypes (rather than differentiating between restrictive and binge/purge phenotypes) (Glashouwer et al., 2014; Jappe et al., 2011). A more recent meta-analysis focused on neuropsychological measures on reward-related decisions found that “altered general reward-related decision making” is altered across all eating disorders (in adults) (Wu et al., 2016). Interestingly, in our study we found results in line with newer findings, of higher sensitivity to reward in children at risk as shown by higher scores in BAS drive when compared to children of HC mothers. Interestingly we also found an association between BAS Drive and putamen volume.

Imaging studies have also investigated reward sensitivity and its neural correlates in patients with ED. In AN, studies have demonstrated functional and structural abnormalities in areas of the brain known to be involved in reward processing (W. H. Kaye, Fudge, & Paulus, 2009), including the ventral striatum. Most functional MRI research has focused on reward to food cues (by delivering visual or actual food stimuli) although other studies have also studied monetary reward. Both types of paradigms have generally found alterations in reward processing across all ED subtypes (Guido KW Frank, 2013). These findings further point to altered reward processing as a potential biomarker in ED, with women recovered from AN and BN also showing altered response in reward tasks (Wagner et al., 2010; Wagner et al., 2007). Findings also correspond to the hypothesis that altered eating is a consequence of dysregulated reward, and/or awareness of homeostatic needs, which could also be related to the executive ability to inhibit some motivational drives (Walter H Kaye et al., 2013).

Interim conclusion

In summary, findings from this study show alterations in a more ventral (limbic) neurocircuit that includes the amygdala, hippocampus, and caudate which are associated with identification of the importance of emotional stimuli and generation of affective response

to these (M. L. Phillips, Drevets, Rauch, & Lane, 2003a, 2003b), as well as playing a role in reward processes (Walter H Kaye et al., 2013) and behaviour regulation.

Chapter 9. Microstructural and connectivity differences in children at familial high-risk for ED: whole brain WM differences and amygdala connectivity findings from BREDS

9.1. Introduction

Given our lack of understanding of the pathophysiology of ED, gaining a better understanding of the underlying neurobiology will be key in aiding more effective treatments. As seen in Chapters 2, 6 and 8, research has been conducted to further develop our understanding of brain differences in ED. Although this has focused mostly on patients with active illness (with the large majority of studies conducted in patients with AN), more recently studies have also included recovered patients in order to help elucidate what could be 'trait' vs. 'state' of each disorder. Specific alterations in both GM and WM structure and volume have been identified although findings are inconsistent (Seitz, Buhren, Von Polier, et al., 2014; Seitz et al., 2016; Titova et al., 2013; F Van den Eynde et al., 2012). Similarly, findings for white matter integrity and connectivity are not conclusive (Barona et al., 2019; S. Gaudio, Carducci, Piervincenzi, Olivo, & Schioth, 2019; Martin Monzon et al., 2016). This can be due to a number of reasons, including the heterogeneity of ED presentations, not only in their different types (AN, BN, BED as well as subtypes) but also due to the permanent effect that malnutrition and altered eating behaviours may have in neurocognitive and neural markers.

Given the complexity of ED it is expected that alterations will not only appear at the structural level but that connectivity between brain areas might also be affected. Understanding neural computations that underlie cognitive abilities in humans requires a framework that studies neural events in the context of overall brain connectivity. Diffusion tractography studies aim to identify anatomical connections in the brain ("Diffusion tractography," ; Jones et al., 2013). This method shows how strongly anatomical areas connect by measuring diffusion in WM tracts expressed by streamlines as an indicator of fiber count (Jones et al., 2013). These connections show the pathways of information transfer between remote brain regions and are therefore of great importance to our understanding of brain structural organisation and tractography is a non-invasive and in-vivo method for identifying and measuring these pathways. Multimodal neuroimaging analyses (DWI data in combination with data from segmentation of grey matter tissue

(derived from T1-weighted images)) allow for the quantification of WM pathways connecting grey matter ROI's (Hagmann et al., 2008). Therefore, the structural brain connectome (an individualized whole-brain map of WM connectivity) (Hagmann et al., 2008) can be obtained by looking at the DWI connectivity between all possible combinations of grey matter ROI's. The use of DWI provides us with the ability to identify altered tracts and their integrity, which will in turn help interpret the alterations underpinning the illness. As described in chapter 6 (meta-analysis of WM differences in AN), little research has been conducted studying WM microstructural differences in ED.

Following up from findings in Chapter 8 of increased gray matter in the amygdala in girls at high-risk compared to girls of healthy control mothers, we wanted to further explore connectivity from this region. The amygdala plays a central role in anxiety which has been found to pre-date ED (Schaumberg et al., 2019) and may play a role in an increase in vulnerability to the disorder. Amygdala functions are served through several connections with cortical and subcortical areas including sensory and perceptual association cortices, limbic-paralimbic affective systems, fronto-parietal network and medio prefrontal network (Dolan & Vuilleumier, 2003; J. E. LeDoux, 2000; O'Doherty et al., 2004; Pessoa & Adolphs, 2010). The amygdala can be divided into distinctive areas based on its connectivity with each area playing a distinctive role and therefore investigations of these connectivity patterns can help elucidate altered circuits that have the potential to be considered for aetiological models of the disorder.

As established earlier, the current study aims to be exploratory in nature and therefore the main aim was to conduct whole brain WM voxel-based analysis in children at high-risk for ED compared to those at low risk. Since previous chapters have resulted in findings that can lead to hypotheses of altered connectivity we wanted to explore this using a Region Of Interest (ROI) approach. Given results of altered GM in the amygdala this study aims to explore connectivity from the amygdala to five cortical areas (frontal, parietal, temporal, occipital and insula)(the insula was included given its hypothesised role in ED).

9.2. Methods

9.2.1. Participants

Data from both cases and controls was collected as part of the Brain in high-Risk for Eating Disorders Study. A total of 20 healthy controls and 16 at risk cases were recruited as part of the study. Data on a further nine healthy controls were included as part of a sharing data initiative in the department. For inclusion in this study, data on maternal lifetime ED and children's data on IQ, development stages and DTI data were necessary. The nine extra healthy controls were contacted after taking part in another study and underwent screening for inclusion and exclusion criteria for the current study.

Full details on the study's protocol, and inclusion and exclusion criteria can be found in chapter 4 (Methodology).

8.2.2. Clinical measures

9.2.2.1. Demographics

Information on maternal demographic details (age, marital status, ethnicity and education) was collected at the time of testing.

Information on children's height and weight was also collected.

9.2.2.2. Exposure

Data on maternal eating disorders was gathered via an in depth interview using the Structured Clinical Interview for DSM-IV-TR (Research version)(First et al., 2002) which was adapted for the new DSM-V diagnoses. A full explanation of both the measure and the interview protocol can be found in chapter 4 (Aims and methodology). Mothers received a diagnosis of AN or BN, current or past with a detailed description of lifetime exposure to ED.

9.2.2.3. General Intelligence

General Intelligence was assessed at the time of testing using the Wechsler Abbreviated Scale of Intelligence, 2nd edition (WASI)(Wechsler, 2011). For this study, full scale IQ (FSIQ) was used to control for differences in brain volume and regional cortical thickness.

Full details can be found in Chapter 4 (Methodology, section 4.2.4.1).

9.2.2.4. Neurocognitive measures

Explanation on neurocognitive measures used in this study can be found in Chapter 4 (section 4.2.4.) with explorations of differences between groups in Chapter 7.

As a summary, this study explored measures of general intelligence (WASI), social cognition (Reading the Mind in the Eyes and Morphed Emotion Recognition) (Baron-Cohen et al., 2001), CANTAB neurocognitive measures: motor screening, inhibitory control, set-shifting of cognitive flexibility, risk-taking behaviour, sustained attention and visual working memory (J. Fray et al., 1996) and reward measures (BIS/BAS) (A. Cooper et al., 2007). Significant differences between groups were found in set-shifting (girls at risk took a significantly longer time to switch when compared to controls) and BAS drive (girls at risk had higher drive towards pursuing goals when compared to controls) (see Chapter 7 for detailed information).

9.2.3. MRI Data Acquisition and preprocessing

Whole-brain MRI was carried out on a 3T Siemens Prisma at Great Ormond Street Hospital (GOSH) using a 64-channel head coil by experienced research radiographers. For inclusion in this chapter, T_1 – weighted and diffusion weighted scans were used. More details on acquisition parameters and pre-processing are described in Chapter 4.

All scans were visually inspected for abnormalities, motion and other artefacts. One scan was discarded due to image artefacts. T_1 – weighted images were processed as per Chapter 4. The DTI data was preprocessed using TractoR (Tractography with R) (Clayden et al., 2011), a project which includes R packages for reading, writing and visualising MRI data in DICOM format (as well as others). Further details on procedures were described previously in Chapter 4.

9.2.4. Tract Based Spatial Statistics (TBSS) Whole-Brain Analysis

TBSS was carried out using FSL version 5.0. The software has a comprehensive manual, which was used to follow the required steps to carry out TBSS analyses.

1. Participants' FA images are first slightly eroded and the end slices are zeroed (to remove outliers). An overview containing a static view of each inputted image is created so that they can be visually inspected for errors.

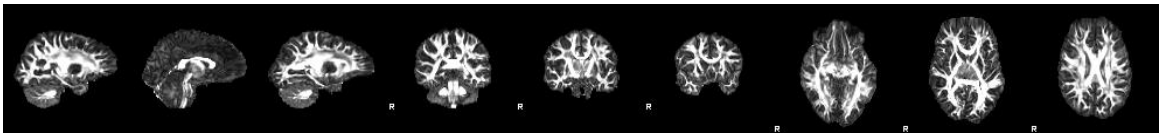


Figure 9.1. Example of static view of participant's images (Patient B46)

2. All FA images are aligned to a 1x1x1mm standard space using nonlinear registration. The target image used was the recommended FMRIB58_FA standard-space image. This step involves carrying out just one registration per subject and generally gives good alignment results.

3. The nonlinear transform found in the previous stage is applied to all subjects to bring them into standard space. All images are then merged into a single 4D image. The mean of all FA images is created, which is then used to derive the FA skeleton (figure 8.2). Finally, the FA data for each individual scan is projected onto the skeleton to ensure that only the centre of the white-matter tracts are included in the analysis (the maximal FA orthogonal to the skeleton is projected onto the skeleton. This is done for every voxel on the skeleton and for every subject).

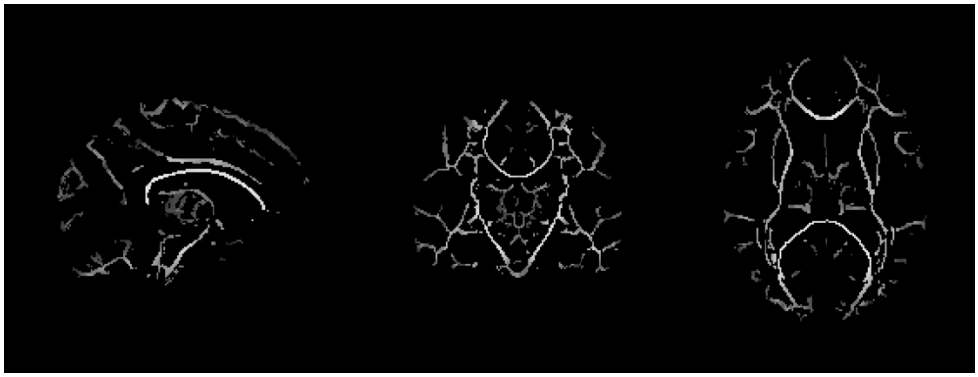


Figure 9.2. Mean FA Skeleton

4. In the last step, the mean FA skeleton image is thresholded at a specified value (recommended is 0.2) in order to remove any voxels that have a low FA and are therefore unlikely to represent white matter or be highly variable between subjects.

MD was also projected onto the skeleton using the transforms calculated for the FA

maps, and these data were used for voxel-wise permutation-based analysis.

Each participant's aligned FA and MD data were projected onto the skeleton and voxel-wise cross-participant statistics were applied. Age and whole brain volume were entered as covariates in the TBSS analyses. Results were corrected for multiple comparisons using family wise error (FWE) correction, in which each p-value is adjusted in light of the number of statistical tests performed, thus reducing the likelihood of false positives. Threshold-free cluster enhancement (TFCE) was applied, as per the TBSS protocol. TFCE compares neighbouring voxels in order to identify clusters of similar voxels; this increases confidence that each voxel's results are genuine and not an isolated chance occurrence. Only clusters surviving FWE $p<0.05$ are reported.

9.2.5. Region of interest volume estimation and parcellation

Whole brain volume was calculated as described in the previous chapter from the T_1 – weighted images using Freesurfer (version 6.0. for Linux, documented at the website: https://surfer.nmr.mgh.harvard.edu/) (Fischl, Sereno, Tootell, & Dale, 1999). Motion correction was carried out prior to removal of non-brain tissue and transformation to Talairach space. Intensity normalization was then applied prior to estimation of great matter/WM and grey matter/cerebrospinal fluid boundaries. Finally, cortical and subcortical regions of interest were generated by segmenting the T_1-weighted image using Freesurfer (Desikan et al., 2006) . Cortical grey matter regions of interest were grouped into frontal, parietal, occipital, temporal and insula lobes. All registrations and segmentations were visually inspected for accuracy. Subcortical segmentations had been previously calculated using Freesurfer, and amygdala volume had been found to be significantly increased in children at-risk (both left and right side) (Chapter 8).

Regions of interest were registered from T_1 space into diffusion space using a combination of NiftyReg (http://cmictig.cs.ucl.ac.uk/wiki/index.php/NiftyReg). Specifically, the T1W image was aligned to an unweighted b0 image using a rigid transform. The same transform was then applied to each of the segmentations, using nearest-neighbour interpolation, to transfer them to diffusion space. All registrations were visually inspected.

TractoR was used to seed probabilistic tractography from each amygdala voxel using the five ipsilateral cortical regions of interest as targets. A hemisphere mask was used in order

to prevent streamlines from crossing the midline. A maximum of 5000 streamlines were seeded from each amygdala seed voxel. Only portions of the streamlines that proceeded from the seed in the direction of the target and terminated at the target were retained. The number of streamlines connecting the seed to each cortical target was recorded in a connectivity matrix.

The connectivity matrix was used to parcellate the amygdala based on their connectivity to the cortical targets. One common technique, known as 'winner takes all' (Behrens et al., 2003), achieves this by assigning to the seed voxel the label of the cortical region that receives the greatest number of streamlines. However, the approach does not allow for overlap in the parcellations and may therefore underrepresent some connections. In order to allow for overlap in connected regions, we calculated the proportion of streamlines connecting a seed voxel to a given cortical target, where 1 is fully connected (i.e. all streamlines seeded in the voxel connect to one target) and 0 is not connected. The volume of a parcellated region that connects to the target was then calculated by summing the weighted values and multiplying it by the voxel dimensions. Only voxels with at least 0.25% of streamlines seeded reaching the cortex were included to reduce the effect of spurious streamlines. The weighted means of FA and MD within each of the parcellated regions were also calculated. Statistical analysis was performed using linear regression to investigate group differences in the volumes, MD and FA metrics of the parcellated volumes.

8.2.7. Estimation of DTI parameters in WM tracts

FA and MD values in each voxel of WM connecting amygdala clusters to their cortical target were weighted by the number of streamlines in that voxel. The weighted FA and MD values were averaged across the whole tract (in order to prevent rarely-visited voxels from affecting the average and ensuring that the tract microstructure was accurately represented).

The DTI metrics (FA and MD) were then compared between groups using linear regression. Ipsilateral amygdala cluster volume was additionally included as a covariate when comparing DTI metrics.

8.2.7. Association between amygdala structural connectivity measures and neurocognitive scores

In order to investigate the specificity of associations between amygdala structural connectivity and specific neurocognitive measures, amygdala-cortical WM tract microstructure was correlated with: Reading the mind in the Eyes, Morphed Emotion Recognition, Attention switching task and reward measures.

8.3. Results

8.3.1. Whole-Brain Tract Brain Analyses

Whole brain TBSS analyses did not yield any significant differences between both groups in either Fractional Anisotropy or Mean Diffusivity measures. This did not change when controlling for age. Given the exploratory nature of these analyses and relatively small sample, the threshold was changed to explore possible findings that did not survive correction. No significant findings were highlighted.

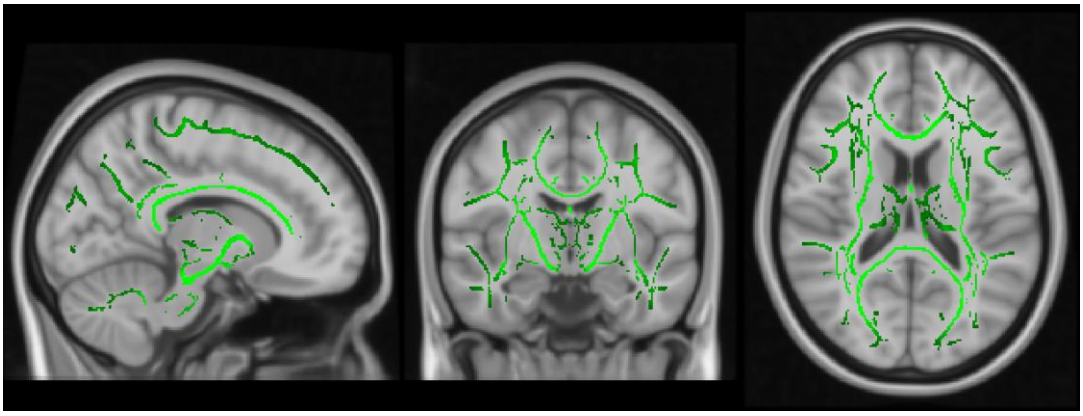


Figure 9.3. TBSS results maps showing no significant differences. Green represents major white matter tracts with a minimum FA value of 0.2 across the sample. Significant clusters would be shown in red.

9.3.2. Amygdala parcellation results

Amygdala parcellation identified clusters of voxels demonstrating maximal structural connectivity to the same cortical target, thus segmenting the amygdala *in vivo* based on its structural connectivity (see figure 9.4). Bilaterally this resulted in five possible clusters connecting to the frontal lobe, parietal lobe, temporal lobe, occipital lobe and insular cortices. Linear regression analyses showed significantly larger volume in right amygdala temporal cluster in girls at high-risk compared to controls (β = 0.312 95% CI (9.158, 277.143), p = 0.037). Results became a trend towards significance in analyses adjusted for amygdala volume for the right side (β = 0.282 95% CI (-13.864, 272.060), p = 0.076). No other significant findings were found in other amygdala clusters.

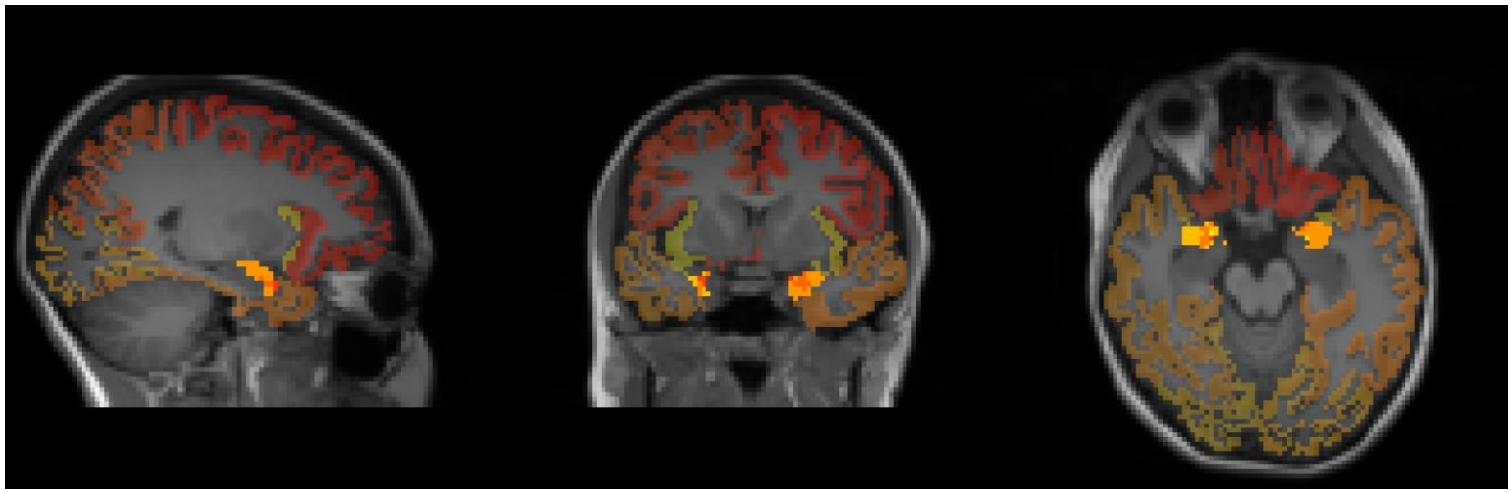


Figure 9.4. A representative subject showing the results of VCP 'winner takes all' parcellation of the amygdala *in vivo* overlaid on the participants' T_1-weighted scan. Parcellation was based on the amygdala's structural connectivity profile: probabilistic tractography was seeded from each amygdala voxel to ipsilateral frontal, parietal, temporal, occipital and insula (as per colours above) cortices. Each voxel was assigned a target representing its maximum connectivity.

9.3.3. Amygdala WM tract microstructure group comparison

There were no significant differences between girls at high-risk and girls of healthy control mothers in amygdala clusters WM tract microstructure as measured by FA and MD.

9.3.4. Association between amygdala structural connectivity measures and neurocognitive scores

Correlations between measures of social cognition/emotion identification and reward and measures of FA and MD in WM tracts connecting the amygdala clusters to the cortex were investigated in the at-risk group. There were no significant correlations in the at-risk group between measures of social cognition and emotion identification. There were significant correlations in the at-risk group between measures of reward and WM microstructure in tracts connecting amygdala clusters to the cortex (see table 9.1).

Table 9.1. Results of correlations between amygdala cluster white matter tract microstructure and neurocognitive measures in girls at risk.

White Matter tract	DTI metric	Neurocognitive measure	Rho	Uncorrected P value
Right amygdala insula cluster - right insula cortex	FA	BAS reward responsiveness	0.541	0.046
Right amygdala frontal cluster - right frontal cortex	FA	BAS reward responsiveness	0.628	0.009
Left amygdala temporal cluster - left temporal cortex	FA	BAS reward responsiveness	0.502	0.048
Left amygdala insula cluster - left insula cortex	FA	BIS	0.581	0.018

9.4. Discussion

The aim of the study was to investigate whole brain WM microstructural integrity and structural connectivity from a specific region of interest (amygdala). The study did not yield significant differences in whole brain WM microstructural integrity analyses as analysed

using Tract-Based Spatial Statistic (TBSS) analyses. TBSS uses a non-linear image transformation that carries out localised statistical testing of FA and MD data (aimed to alleviate alignment problems), and aims to combine the strength of voxelwise and tractography based analyses (S. M. Smith et al., 2006). The targeted hypothesis driven analyses of the structural connectivity of the amygdala yielded no differences between groups in white matter integrity in tracts studied. There was a significant difference in volume in one of the amygdala parcellations (cluster connecting to temporal cortex), although it became a trend towards significance after adjusting for total amygdala volume.

As discussed in Chapter 6, research investigating WM microstructure in ED lags behind that of other psychiatric disorders, and to date, less than 20 studies have been published and overall, only few studies have compiled samples of more than 20 participants. Given the variability in the methodology and samples studied it is not surprising the results are mixed: with different sample sizes (few studies had more than 20 participants in their case group), scanner type, software used for analysis as well as the differences in the group of patients: mostly AN (only two BN studies have been published to date (G. K. Frank, Mettler, Shott, & Yang, 2013; He et al., 2016; Mettler, Shott, Rollin, & Frank, 2012)), combined binge/purge and restrictive, recovered and acute, long and short term recovered. However, it is important to note that most studies have shown widespread alterations in projection, association and commissural WM fibres during the acute stage in adults with AN (Frieling et al., 2012b; Hayes et al., 2015; Kazlouski et al., 2011b; Nagahara et al., 2014). Results for adolescents have been less consistent with findings of both increased (Travis et al., 2015; Vogel et al., 2016) and reduced FA (G. K. W. Frank, M. E. Shott, J. O. Hagman, & T. T. Yang, 2013; Travis et al., 2015). On the other hand, some studies have not found any differences between patients and healthy controls (Olivo et al., 2019; Pfuhl et al., 2016), although most have been those studying recovered patients (Frieling et al., 2012b; Olivo et al., 2017; Pfuhl et al., 2016; von Schwanenflug et al., 2018). It has therefore been hypothesised that alterations in WM microstructure in patients with acute illness could be due to a reduction of myelin content secondary to malnutrition. Myelin is composed of various types of lipids and may therefore be particularly vulnerable to injury from malnutrition during adolescence (Giedd, 2008). To help elucidate differences between ‘state’ (secondary to undernutrition) and ‘trait’, three studies have longitudinally investigated WM microstructure alterations in AN (J. Cha et al., 2016b; Vogel et al., 2016; von Schwanenflug et al., 2019). Vogel and colleagues found that differences in bilateral frontal, parietal and temporal areas in acute adolescent AN patients were partially normalized after nutritional treatment, however, this study only had a small sample of 9

patients. Cha and colleagues (J. Cha et al., 2016b) did not find any differences after short-term weight restoration (although they did find increased WM FA in the fronto-accumbal pathway in a hypothesis driven ROI analysis). Lastly, von Schwanenflug and colleagues (von Schwanenflug et al., 2019) also found normalizing of alterations in FA after weight-gain. Findings from this study could therefore be pointing to alterations in WM microstructure in patients with ED being secondary to malnutrition rather than being a risk factor for its development. It is of course important to consider the methodology and sample size of this study as further research with larger samples and targeted hypothesis are needed to help elucidate these findings.

Overall findings in patients after weight restoration and in recovered patients point to alterations in WM microstructure being highly dynamic and critically dependent on weight status, however, as discussed, most studies have small sample sizes and have used exploratory methodological approaches (mostly whole brain comparison analyses). Whole brain analyses allow the investigation of differences without *a priori* hypotheses regarding possible specific alterations, mainly based on a good understanding of the pathophysiology of the disorder and provide better comparability across studies. From a methodological perspective, most studies undergo whole brain comparisons which are exploratory in nature, this however means that generally they do not have the statistical power to find significant differences in small brain regions. This was recently highlighted by Phillipou and colleagues (Phillipou, Castle, Abel, Gurvich, & Rossell, 2018; Phillipou et al., 2014) who after adopting a behaviourally driven ROI approach identified structural and functional dysfunction in a hypothesised area (superior colliculus) that had not yielded significant findings in their initial whole-brain analysis. Given the small sample size of most studies to date, it is possible that a lack of power is responsible for non-significant findings. However, in order to be able to conduct more hypothesis-driven studies, specific hypotheses with regards to ‘state’ vs ‘trait’ WM microstructural differences need to be developed.

Given some of the above limitations to whole brain analyses, targeted, hypothesis-driven analyses were used to investigate structural connectivity of the amygdala after findings of increased GM in the amygdala in girls at-risk compared to girls of healthy control mothers in the previous chapter. Volume differences of the amygdala based on its structural connectivity yielded a trend towards significance in the right amygdala temporal cluster (cluster based on the connectivity from the right amygdala to the right temporal cortex).

While these results are only showing a trend towards significance, it is possible that this is due to our sample size and a larger study may be able to confirm this finding. If so, this suggests that heterogeneous abnormalities in specific amygdala subdivisions may underlie the development of vulnerability to eating disorders.

A larger volume of the amygdala cluster with winning connections to the temporal cortex may indicate altered structural connectivity in that region. It is therefore reasonable to hypothesise that alterations in the subdivision of amygdala with main connectivity to a cortical area may underlie alterations in this cortical area as well. The temporal cortex automated parcellation in Freesurfer includes regions that lie within the temporal cortex as well as the fusiform gyrus, which extends to the occipital lobe as well. This is of importance given the functions of this region. The extrastriate body area (EBA) (Downing, Jiang, Shuman, & Kanwisher, 2001) is located in the lateral occipito-temporal cortex and responds to visual images of human bodies and body parts. This region has been of interest in the study of ED given its role in body perception and the significance for the ED behavioural phenotype. Concerns about weight and shape are central to ED and related to these, “a disturbance in the way in which one’s body weight or shape is experienced” (APA, 2013) is central for the development of the disorder. This is manifested by an overestimation of one’s own body size, with the bias being much stronger in patients with AN than in BN (T. F. Cash & Deagle, 1997). It has been suggested that this disturbance is likely to involve both perceptual and affective components and therefore engage a neural network of different regions involved in these processes (Thompson, Heinberg, Altabe, & Tantleff-Dunn, 1999). Functional MRI studies in patients with ED have previously supported the concept of dysfunction in areas of the brain (such as occipital-temporal) that are of importance for analysing perceptual aspects of body image. In a study by Uher and colleagues (Uher et al., 2005), patients with ED had lower activation in these regions when presented with drawings of body images. The authors suggested that low functionality in this ‘body perception’ network might facilitate the development of body image disturbance in ED. Selective display of images of bodies that express emotions (anger, disgust, happiness, fear) have also been shown to activate the EBA, and this activation seems to be supported by a close correlation with the amygdala, suggesting a modulation of emotional information (Myers & Sowden, 2008). Research suggests that increased brain activity in the amygdala might be involved in fearful emotional processing concerning body images (Y. Miyake et al., 2010; Pruis, Keel, & Janowsky, 2012). On the other hand a number of fMRI studies of perception of self-body image and visual self-other body discrimination in patients with AN have also shown contradictory results with regards to

the role of the EBA and therefore further investigation is needed (Esposito et al., 2018). Whilst further investigation in larger studies is required, considering the evidence, it is possible to hypothesise and altered structural connectivity between amygdala and temporal cortex may underlie alterations in self-perception given the role that the EBA (found partly in the temporal cortex) plays in processing of body images and previous findings of this region being altered in patients with ED.

Interestingly, there were no differences in diffusion measures in WM tracts connecting the amygdala to their cortical targets. Therefore, results point to alterations in amygdala volume but no differences in the microstructure of WM tracts connecting amygdala clusters to their winning targets. This suggests that while the amygdala may be functioning differently in children at-risk, the microstructure of its connections to the investigated cortical targets are intact at this stage. It is interesting that we found a trend towards significance in amygdala volume cluster based on its winning connectivity as this may suggest differences in the way the amygdala is structurally connected in girls at-risk but not in the microstructure of the WM tracts connecting to the target. However, since this was only a trend it requires further investigation. Given that this study is an exploratory pilot study, it is important to still consider that findings are due to small sample size and therefore it is important to investigate this further in a large sample.

It is also possible that non-significant findings are due to methodology used and connectivity is indeed altered in a more global manner. One possible way of investigating structural connectivity from the amygdala is based on its anatomical subregions (rather than based on structural connectivity), which have distinct roles in the modulation of cognitive and affective functions (Dolan & Vuilleumier, 2003). The basolateral (BLA) and centromedial amygdala (CMA), are the two most widely characterized subdivisions of the amygdala and service distinct functions as well as having distinct connectivity profiles to both cortical and subcortical regions (J. E. LeDoux, 2000). The BLA plays an important role in perception and regulation of emotionally significant events and does this via interaction with multiple brain regions (Dolan & Vuilleumier, 2003; J. LeDoux, 2007; J. E. LeDoux, 2000), while the CMA is essential for controlling the automatic expression of emotion (such as the fight-flight response) through projections to the brainstem, cerebellum and sensorimotor system (J. E. LeDoux, 2000; Roy et al., 2009). Enlarged left BLA has been shown to be present in children with subclinical anxiety as young as 7 years old (Qin et al., 2014), as well as being a consistent finding in adults with generalised

anxiety disorder (Hettema et al., 2012; Schienle, Ebner, & Schafer, 2011). Given the BLA's correlation with anxiety and previous findings of premorbid anxiety in patients with ED (Schaumberg et al., 2019) and the role this may play in ED development, it is possible that by dividing the amygdala in these regions and studying its connectivity we may be able to see different results to those found in this study. From previous findings of enlarged amygdala in the at-risk group, it is possible to hypothesise that increased GM in amygdala is related to increased/heightened anxiety in our group. This heightened anxiety can lead to an exacerbation of vulnerability to ED by biasing the reward system towards withdrawal or avoidance behaviour to alleviate anxious states. It is possible that increased GM in the amygdala acts as a risk factor which is exacerbated by other biopsychosocial risk factors as explained by the diathesis-stress model of mental health (Monroe & Simons, 1991). Findings from this study suggest that a possible diathesis of ED may be alterations in amygdala structure leading to the emergence of trait-like susceptibility to anxiety. It is possible that interactions between such diathesis as well as other risk factors can lead to negative consequences such as ED.

Another possible hypothesis worth exploring is that connectivity from the amygdala to other subcortical regions is altered as this was not investigated in this study. The amygdala has connections with the ventral striatum, which is important for reward and therefore may be of interest as a hypothesis driven analysis. A ventral (limbic) neurocircuit that includes the amygdala, insula, ventral striatum and ventral regions of the anterior cingulate cortex (ACC) and the orbitofrontal cortex (OFC) is of importance for identifying stimuli that are emotionally significant and generating an affective response (M. L. Phillips et al., 2003a, 2003b) and may underlie some of the common behaviours found in both ill an recovered ED patients such as anxiety and obsessionality. Prior knowledge about WM tract anatomy and those that have been found to be altered in patients with ED may also be of guidance in future hypothesis driven analyses. Chapter 6 highlighted the role that the corpus callosum may play in the neurobiology of ED and a recent systematic review by Gaudio and colleagues (S. Gaudio et al., 2019) suggested altered thalamo-cortical WM connections (including corona radiate, thalamic radiations and internal capsule) as well as occipital-parietal-temporal-frontal WM connections (such as the inferior fronto-occipital fasciculus, superior longitudinal fasciculus and cingulum).

Lastly, given the stage of development at which this study was performed and the development of myelination during childhood and adolescence, it is possible to

hypothesise that changes in WM connectivity in children at high-risk may happen at different stages of development. Given our sample size it was not possible to stratify by age as this made groups too small (with the majority of participants being over the age of 11). As this is a pilot study that will be informing a larger study, it will be important to have equal numbers of two age groups (8 to 11 and 12 to 15) to help elucidate some of the differences that may be due to different stages of brain development.

Correlation analyses between specific neurocognitive measure and WM connectivity from amygdala parcellation yielded significant results for reward measures. Specifically, BAS reward responsiveness was correlated with FA in WM tracts connecting the right frontal and insular amygdala and the left temporal amygdala clusters with their corresponding cortical target. BIS scores were also correlated with FA in WM tracts connecting the left insular amygdala cluster with its corresponding cortex target. Although we did not find any differences in diffusion metrics between the groups, it is interesting that we found correlations with reward measures in the at-risk group which were not present in the low-risk group. Importantly, these differences are relating to reward mechanisms which have been widely explored in connection to ED, both behaviourally and in neuroimaging studies (J. Cha et al., 2016b; McAdams & Smith, 2015; O'Hara, Campbell, & Schmidt, 2015; Olivo et al., 2017; J. E. Steinglass, Berner, & Attia, 2019; Wu et al., 2016). Due the exploratory nature of this study, it is worth further exploring the association between reward responsiveness and amygdala connectivity.

This study did not find any WM microstructure alterations in tracts connecting the amygdala and insula cortex. Given the proposed role that the insula plays in ED one hypothesis in this study is that connectivity between amygdala and insula may be altered. The insular cortex lies deep within the lateral fissure and is located between the anterior and posterior cerebral hemispheres. Importantly, this cortex contains a complex set of connections between anterior and posterior structures, left and right hemispheres and cortical and sub-cortical structures. Among others the insula has been proposed to play a central role in the regulation of appetite and eating, monitoring of body state, perception and integration of disgust and taste and integration of thoughts and feelings (Nunn, Frampton, Gordon, & Lask, 2008). Given the role that the insula plays in these regulatory processes it is not surprising that a central role of the insula in ED has been proposed. Nunn *et al* (Nunn et al., 2008) proposed that individuals at risk for AN have a dysfunctional neural network converging upon the insular cortex leading to an alteration in the capacity

to effectively integrate visual and body perception with emotions and the inhibition of higher cognitive processes. This study did not find altered connectivity between amygdala and insula, however this could be due to a lack of power or limitations in the methods used. Connectivity with insula was based on parcellation of the amygdala based on connectivity and whole amygdala-insula connectivity was not studied. Equally, only measures of FA and MD were investigated in this study. Other WM neuroimaging biomarkers axial and radial diffusivities and fibre count. Axial and radial diffusivities are simply the diffusivity along the principal axis of diffusion (former) and an average of diffusion along its two minor axes (later) and have been associated with axonal damage, axonal diameter and fibre coherence. While fibre count is not strictly a DTI parameter it can also provide information with regards to tracts connecting ROI (Curran, Emsell, & Leemans, 2016). It is also possible that the insula and its connectivity to other regions of the brain may be altered, but not its connectivity with the amygdala.

Limitations

This study has some limitations. Overall while this is only a pilot study aimed at forming hypotheses to inform a larger study, our sample size was not large enough for the number of tests carried out. There was no correction for multiple comparisons given the aim of exploring possible hypotheses, which means that false negatives would be more costly than false positives. Connection probability decreases with distance; to limit the effects of this a high number of streamlines were seeded and DTI values were averaged over the tracts. The low resolution of MRI scans relative to actual white matter tract dimensions may cause partial volume effects, and the directionality of white matter connections cannot be determined using tractography; post-mortem or tracer studies would be necessary. Errors may be introduced during registration and segmentation, although visual inspection aimed to mitigate this. It is not possible to infer causality using correlation analyses, and some of these might be driven by outliers.

Interim conclusion

Results from this chapter highlight the need for hypothesis driven brain imaging analyses. As highlighted by Phillipou and colleagues, ROI driven analyses may result in significant findings that were not previously highlighted by whole brain analyses. In this study, no significant findings were found using a whole brain approach while a trend towards significance was found in ROI analyses based on results from the previous chapter.

Further exploration of amygdala connectivity using different methodology is warranted given previous results of alterations in the amygdala in this at-risk group.

Chapter 10 Considerations and limitations, final discussion, and future work

10.1. Considerations

10.2.1. Recruitment

One main limitation in this study was recruitment which led to delays in the study and a reduced sample size (compared to initial proposal). Several factors played a role, and given the exploratory nature of this study it is important to discuss them:

1. Although not only a limitation in this study, given the relative low prevalence of ED, finding participants with a current ED or a history of one in the community can be difficult. While a lot of studies in the field will recruit currently ill patients from clinics, there is a drive to focus more on recovered patients in order to help elucidate mechanisms involved in the development of the disorder. I believe that the stigma still associated with the disorder makes it difficult for recovered patients to be found in the community and for them to want to take part in a research study. While as a society, over the last decade, we have become more aware and accepting of mental health and its biological underpinnings, there is a sense that we are not there when it comes to ED.

2. Specifically, for this study, most of the mothers recruited had a history of an ED, and surprisingly (or maybe not as much), they felt that this was not something that they could share with their family and therefore they felt they could not take part in the study. Although not formal questions within the initial interview, during recruitment calls, it was common for mothers to say that they did not ‘want their daughters to go through what they went through’. Of course, this is very understandable, however, there was a sense that if they told their daughters that they had an ED, this would affect their daughters. Equally some mothers felt that given their daughters already had increased risk of having an ED, talking about it would only drive attention to an issue that is better kept undiscussed and might increase their daughter’s chances of developing the disorder even further. While this fear might be understandable given the severity of ED

and the consequences for the young person, it poses an interesting question about why is it difficult for parents to discuss EDs? A similar situation was also present in recruitment of healthy control mothers. It is important to note, that in general, healthy controls were recruited mostly at the university (UCL) and attached hospital (Great Ormond Street) with a couple of schools and community and therefore this might not apply to the whole of the community. Healthy control mothers who initially showed an interest in the study felt that they might find it difficult to explain to their daughters what the study was about as they did not feel comfortable (given the girls ages) discussing eating disorders. This was interesting given that these mothers had never had a difficulty with ED, but felt that the topic was not one that they would approach with their children. While we have become more ready to discuss worries and 'feeling low' in schools and at home, worries and thoughts about shape and weight continue to not be discussed. It is possible, that given that dieting and/or ideas about body shape are very present in everyday life we feel that this is not something we can influence change in. Furthermore, there may be a perception that ED are caused only by pressure to have a specific body shape and therefore there may not be such a drive to discuss them.

While recruiting for this study it was difficult not to wonder about what people in the community know and understand about ED; as well as wanting to understand better what patients who have recovered from an ED feel about causes of ED. Mothers (with a history of ED) reluctance to speak about ED (even after being recovered for over 20 years in some cases) with their daughters poses an interesting hypothesis about the way individuals think about their own ED and the way they approach discussions about weight/shape or even food with their own children. Could this possibly play a role in their children's own view of weight/shape and/or food? We know from phobia studies that even when parents do not discuss their phobia with their child, driven by a worry that their child will develop the same phobia, the child might pick up the cues from the parents' behaviour, which in turn can influence their views and beliefs about the phobic situation.

Given the stigma associated with ED, do people with a past history of an ED feel judged when discussing ED? Studies have found negative stereotypes and social rejection of individuals with eating disorders including attributions to personal responsibility (Puhl & Suh, 2015). Unlike people with other psychiatric disorders, individual with ED are frequently regarded as personally responsible for and to blame for their condition. Furthermore, stigmatization of ED has been found to predict alienation and social

withdrawal which in turn predicts symptoms severity (Griffiths, Mitchison, Murray, Mond, & Bastian, 2018). It has also been shown to be a barrier to seeking help (Ali et al., 2017). As a result of these problems, some studies have aimed to reduce ED stigma in the community. One of these techniques (used in general to help people change their perception of mental health disorders) has been to inform of etiological explanations to the disorders. While attributing the development of the disorder to biological factors may help reduce the stigma, it doesn't solely explain the disorder, and therefore it may cause more harm. Other strategies have used education about ED and breaking barriers by encouraging discussions with patients with ED.

In this study, it is clear that there continues to be some stigma related to ED and this can in turn lead to secrecy and reduced understanding of the disorder. Research aimed at elucidating biological mechanisms that play a role in the development of the disorder can not only help our understanding but would also shape our attitudes towards diagnosis and treatment. This will in turn help reduce the stigma by helping people understand the complex nature of these disorders.

10.2.2. Controlling for multiple comparisons

When performing a large number of statistical tests, by chance, some of these will have *p* values that are less than 0.05 (even if all the null hypotheses are true). Therefore, controlling for multiple comparisons is a way of reducing false positives. Adjusting for multiple comparisons has received increased attention given the development in techniques such as neuroimaging, in which results are estimated from 100,000 or more three dimensional bits of the brain (voxels).

A classic approach to multiple comparisons is to control for the familywise error rate, by which, instead of having a critical *p* of 0.05, we use a lower critical value. The most common used method has been to use the Bonferroni correction (Simes, 1986). This correction method uses the number of tests to find the new critical *p* value (by dividing the alpha for an individual test between the number of tests). However, this type of correction comes with its limitations. If you are hoping to find multiple results that might be significant, the Bonferroni correction can lead to a high rate of false negatives. Another issue to take into account is deciding what a "family" of statistical tests is. An alternative approach is to

control for the false discovery rate (Benjamini & Hochberg, 1995) (proportion of discoveries that are actually false positives). This approach, allows the researcher to set a false discovery rate based on what the percentage of acceptable false positives that they might allow is. As an example, if the cost of additional experiments is low and the cost of false negatives is high, then a fairly high false discovery rate might be used (Akey).

Overall, the goal of multiple comparisons is to reduce the number of false positives, however, this may lead to an increase the rate of false negatives (which can be very costly). Therefore, one could argue that given the exploratory nature of this study, the costs of false negatives is far too great, as the aim of this study is to help develop new hypotheses that can lead to later findings in better powered studies. The main aim of the study was to cast a wide net, which means performing a large number of statistical tests in a relative small sample size where false negatives would be very costly. Therefore, while I can't conclude that the findings of this thesis are not false positives, I can hypothesise what the findings might mean and how hypotheses can be developed further in future studies.

10.2. Final discussion

As established in Chapter 1, ED are complex psychiatric disorders that should be considered within a biopsychosocial model, with risk factors that operate within a genetic/epigenetic predisposition, a cultural context that idealizes a thin female body, as well as psychological, neurocognitive and neural endophenotypes or biomarkers. This PhD has initially focused on gaining an understanding of psychopathological, neurocognitive, and neural biomarkers in children at high-familial risk for developing an ED (and who are young enough that they might go on to develop the disorder). In order to do this, the first chapter explores psychopathology in children at high-familial risk compared to healthy controls in a large community sample. The next chapter is a meta-analysis of white matter alterations in patients with AN to help inform hypothesis to be explored in a larger study in children at high-familial risk for ED. Finally, the last three chapters show results from a pilot study (BREDS) undertaken during this PhD, aimed at exploring neurocognitive and neural (volumetric and white matter) alterations in girls at high- familial risk for developing ED. This section will summarise findings from each result chapter and propose final conclusions and hypotheses based on results:

Chapter 5 demonstrates in a large community sample that children at high-risk of ED have higher odds of having emotional difficulties (internalising difficulties such as anxiety) whilst boys showed higher odds of having hyperactivity and social communication difficulties. The first results related to increased odds of internalising difficulties confirm previous studies, that have found heightened anxiety as a risk factor for the development of an ED. Whilst results relating to boys may be shedding some light into shared vulnerabilities between psychiatric disorders (ADHD and ASD and ED) as well as gender-specific vulnerabilities. While it has been suggested that girls with AN may have increased levels of ASD traits (Zhou et al., 2018) it is also true that individuals with ASD (the majority of which are boys) have increased difficulties with eating compared to healthy controls. It is of course important to consider the shared diathesis of psychiatric disorders and therefore results shown could simply show the direct link between psychiatric disorders at a genetic level. While this finding may not be specific to ED, it is important to consider when working on early interventions that will help encourage better mental health in young people and may therefore reduce the risk of developing any disorder.

Chapter 6 highlights how research into WM microstructure in ED lags behind other psychiatric disorders (Barona et al., 2019; S. Gaudio et al., 2019), with less than 20 studies published, and only three focused on patients with BN. It is also important to note that a large number of studies had relatively small samples (with some studies published with samples under 10). Furthermore, within such a small sample of studies, many different methodologies for analysing data were used. Given all these factors, it is not surprising that results are mixed. One of the main findings in the meta-analysis in Chapter 6 is of alterations of WM microstructure (as measured by reduced Fractional Anisotropy) in the corpus callosum which has been reported in several studies of patients with AN (J. Cha et al., 2016a; G. K. W. Frank et al., 2013; Nagahara et al., 2014; Olivo et al., 2019; Travis et al., 2015; von Schwanenflug et al., 2019). The corpus callosum is the main interhemispheric commissure involved in multimodal sensory and motoric signal processing and altered WM microstructure in the peri-splenial region (as highlighted in the meta-analysis – with involvement form the cuneus and precuneus regions) may contribute to distorted body perception in AN (Santino Gaudio & Riva, 2013; S. Gaudio et al., 2016). A second cluster in the corpus callosum extended to anterior thalamic projections. Despite some discrepancies between directionality of DTI specific measures, several studies have found altered thalamo-cortical connections in patients with AN (Frieling et al., 2012a; S. Gaudio et al., 2017; Hayes et al., 2015; Olivo et al., 2017; Vogel et al., 2016), including

one study with recovered patients (Shott et al., 2016). It has been proposed that posterior thalamic-radiations play a role in body-image disturbances in AN as well.

Results from the high-risk for ED study (BREDS)

The remaining three chapters show results from the main study of this PhD: the Brain in high-Risk for Eating Disorders Study (BREDS) which aims to further our understanding of neurocognitive and neural differences in children at high-risk for ED compared to those at low-risk for ED (based on maternal diagnosis of ED – both past and present).

Chapter 7 demonstrates alterations in set-shifting (cognitive flexibility) and reward processing (measured by BAS drive) in girls at high risk for ED. Difficulties in cognitive flexibility refer to the ability to transition from thinking in one dimension/concept to another, and can therefore affect how quickly we are able to adapt to our environment. Alterations in this process have been found in individuals with different psychiatric disorders, including ASD and OCD and they have been suggested to underlie AN phenotypic presentation (rigidity in restricting calories and inability to change behaviour in the presence of extreme low weight). Findings from this study build upon findings in healthy sisters of patients with AN (Holliday et al., 2005; Tenconi et al., 2010) which suggest that cognitive inflexibility may be considered an endophenotype of the disorder and therefore may play a role in its development.

My study found higher sensitivity to reward in girls at high-risk compared to controls as reflected by higher scores in the BAS drive sub-scale. Given that the BAS drive subscale reflects persistence in pursuing goals even in the event of delayed reward or potential for reward, it is possible to hypothesise that this measure is an index of perfectionistic traits, know to be maintaining and risk factors for AN (Bardone-Cone et al., 2007). Interestingly, it can also be argued that drive persistence matches with the phenotypic expressions of AN (and binge-purge type disorders to some extent), characterised by the pursuit of a goal such as thinness even in the presence of little reward.

Interestingly, in this sample, we did not replicate findings of increased difficulties in identification of emotions from facial expressions (R. Kothari et al., 2015), however, this could be due to limitations inherent in the measures used as well as administration method, and small sample size.

Chapters 8 and 9

Chapters 8 and 9 explore volumetric and white matter alterations in girls at high-risk for ED compared to controls. The main findings from chapter 8 are of altered GM volume in subcortical regions: amygdala, hippocampus and caudate. Chapter 9 builds upon findings from the previous chapter by further exploring connectivity from the amygdala.

Amygdala, hippocampus and caudate nucleus are regions which are known to play a role in the mediation of processes such as reward, decision making, affect regulation and interpersonal processes, all of which have been found to be altered in patients with ED. The caudate and putamen together form the striatum and play an important role in reward circuitry and together with cortical areas play a crucial role in goal directed behaviour (which is sensitive to reward). Whilst not in the main analyses, a larger OFC was also found in this sample, suggesting an alteration in fronto-striatal structures. Alterations in these regions have been implicated in hypotheses regarding an imbalance between the influence of the goal directed and the habit system (with the dorsal striatum at its core).

Findings regarding connectivity between amygdala and cortical areas (frontal, parietal, temporal, occipital and insula), which were based on amygdala parcellation (depending on best connectivity with each area) did not yield any differences. It is possible that connectivity from the amygdala to cortical areas is not altered in children at risk, however, it is also possible that these findings are due to our relative small sample size or the methodology used. Further exploration of connectivity from the amygdala is warranted.

Final conclusions and theoretical implications

This thesis highlights our lack of understanding of the neurobiological biomarkers or mechanisms responsible for ED development and/or maintenance; and the importance of

developing research methods and hypotheses that can clarify these. Given the complexity of ED and the effect that undernutrition has on neurocognitive and neural mechanisms, elucidating what are 'state' (due to undernutrition) and what are 'trait' mechanisms has proven complex. To better understand the aetiology of psychiatric illnesses, the Research Domain Criteria (RDoC), resulting from the National Institute of Mental Health (NIMH) 2008 strategic plan (Cuthbert & Insel, 2013; Thomas R. & Jeffrey A., 2013) encourages a transdiagnostic approach in the research aimed at advancing our understanding of psychiatric disorders. Central to this approach is the search for endophenotypes (which reflects the function of a discrete biological system and is heritable) that can be common to several disorders. Results from this PhD highlight the role that negative affect, cognitive flexibility and reward play in the aetiology of ED. These alterations map into negative and positive valance, as well as cognitive control and social processing systems mapped in the RDoC cognitive systems (Morris & Cuthbert, 2012). These proposed domains also identify specific neural correlates that have been shown to be involved in these processes. The primary neural mechanisms of reward sensitivity involve the ventral striatum and the orbitofrontal cortex (OFC), and involves other neural regions including the amygdala (Baxter & Murray, 2002; Zalla et al., 2000), dorsal anterior cingulate cortex (ACC) (Bush et al., 2002), and the hippocampus (Berns, McClure, Pagnoni, & Montague, 2001). The negative valence system includes responses to acute threat (fear) and potential harm (anxiety). Fear conditioning has been most consistently associated with amygdala (R. G. Phillips & LeDoux, 1992) and insular cortex (Etkin & Wager, 2007), while ventral and dorsal striatum are associated with anticipation of negative outcome (loss or punishment) (Delgado, Locke, Stenger, & Fiez, 2003).

From the literature review in this thesis, it is clear that the complex interaction between psychosocial, neurocognitive, and neural abnormalities in ED have made the study and development of neurocognitive and neuroscientific treatments more complex than those in order disorders. In the past decades, research techniques have facilitated better understanding of the bi-directional 'vectors of influence' that link the genes, brain and social behaviour and it is clear the search for biomarkers in psychiatric disorders is of great importance and an important path towards better understanding the development of ED. Biomarkers or endophenotypes are of critical importance for diagnosis as well as predicting treatment response. Furthermore, the study of biomarkers can help us study disease mechanisms by detecting pathology early and following its development.

One well-known difficulty in ED is the instability of diagnoses (AN, BN and OSFED) (Milos et al., 2005). Since the introduction of DSM-5, there has been a reduction in number of OSFED (previously EDNOS) diagnoses, however, instability in cross-over in diagnoses continues to be problematic. Results from my PhD show how most of the research available has been conducted with patients with AN, therefore hypotheses with regards to cognitive and neural biomarkers are mostly based on patients with these ED subtype. While the aim of the search for endophenotypes is to improve our understanding of the pathophysiology of ED, the majority of research investigates endophenotypes for DSM or ICD disorders. However we still do not know if ED diagnoses reflect different pathophysiological and biological phenotypes. In a recent study by Kothari and colleagues (R. Kothari et al., 2015), one way of addressing the instability and heterogeneity in ED diagnosis was to use observable phenotypic features of ED (i.e. ED behaviours), which may be more directly associated with differences in cognitive functioning than DSM or ICD diagnoses. Another possibility is to focus on processes common across ED diagnoses to investigate neural and neurocognitive differences associated with them. Fairburn and colleagues proposed a model suggesting that common processes operate across all ED diagnostic categories (Z. Cooper & Fairburn, 2011; Fairburn et al., 2003): clinical perfectionism, low self-esteem and interpersonal difficulties. While this pilot study was not able to distinguish between diagnoses or ED behaviours because of its sample size, this will be crucial when trying to understand neural and neurocognitive commonalities between ED diagnostic categories.

My first chapter, with data from a large community sample, allowed for gender differences in the exploration of psychopathology in children at high-familial risk for ED. Results show interesting gender differences, specifically when exploring differences of internalising vs. externalising difficulties. While overall children at familial-high risk showed an increased risk for emotional difficulties (internalising), when stratified by gender, boys showed a specific increase in odds for hyperactivity and social communication difficulties (considered more externalising difficulties). While the inclusion of both girls and boys was possible in this large community based study, when designing BREDS, only girls were included. While it is a limitation, and may restrict investigation of specific hypothesis with regards to gender differences, it would be difficult to recruit a sample big enough to allow for these investigations. ED are more common in females with sex ratio estimates ranging from 3:1 to 18:1 (Raevuori, Keski-Rahkonen, & Hoek, 2014) which would affect the sample size needed.

The RDoC encourages a transdiagnostic approach to help explore shared diathesis of psychiatric disorders that can in turn help develop a hierarchal structure of definition of psychiatric disorders with broad genetic vulnerability factors which may branch into more narrowly defined clinical phenotypes. The co-occurrence of disorders and symptoms (within different disorders) has been of great interest in research for some time and can help point to different ways of understanding the neural and genetic underpinnings of these psychiatric illnesses that commonly co-occur. More importantly, leading to the study of underlying processes and neural circuits that can be shared biomarkers for a larger number of disorders. It is possible to use our developing understanding of brain alterations in patients with ED to help guide our focus outside of diagnostic boundaries, understanding the underlying neurobiology of ED 'type' and how this can help create more individualized treatments. The study of overlapping features between ED and psychiatric disorders such as ASD, OCD, and social anxiety is important and can help guide research and development of testable hypotheses. Fronto-striatal alterations have been found in several psychiatric disorders, including OCD, schizophrenia and depression and neurodevelopmental disorders such as ASD and ADHD. Findings of the overlap in brain alterations between several psychiatric disorders may be viewed as a supporting argument for reformulation of the nosological status of ED in DMS-5 to a broader taxonomy of psychiatric disorders that are hierarchically structured, defined at the highest level by broad, genetic vulnerability factors which may branch into more narrowly defined clinical phenotypes. This may help combine research efforts in the search for candidate genes and neural endophenotypes causally related to fundamental characteristics of ED.

My PhD also highlights the role that altered developmental neural processes may play. It is possible that abnormalities in GM and WM highlighted in this pilot study reflect not just pure structural alterations, but also altered developmental processes (e.g., pruning, myelination) that are then further disrupted by the onset of ED and its correlates (such as malnutrition). A major phase of synaptogenesis, pruning and myelination occurs around the time of puberty and adolescence (predominantly in frontal and limbic areas) (Benes, 1998) and alterations in these processes may play a role in the integration of emotional processing with cognition. It has been demonstrated that until pruning improves the efficiency in frontal circuity, reaction time for emotional recognition can be altered as well as speed in cognitive flexibility (Casey et al., 1997; McGivern, Andersen, Byrd, Mutter, &

Reilly, 2002). Therefore, it is possible that alterations in the form of later pruning are a biomarker for the onset of ED.

Theoretical implications

Overall, the differences in cognitive, structural and neural alterations observed in this sample of children at high risk may work in combination to increase the risk for development of an ED. Heightened anxiety could result in a hypervigilant behaviour with a specific bias towards body related stimuli (which is already present during adolescence given the bodily changes during puberty and the pressure for an ideal body type). Cognitive inflexibility may further enhance difficulties by leading individuals to be unable to shift from their negative thoughts. Higher drive may also work against them by enhancing their pursue of unhealthy goals. While this study did not find differences between neurocognitive measures of social cognition, alterations found in amygdala and its connectivity overlap with those found in patients with ASD and therefore are suggestive of difficulties in social cognition in this group. If this is true, this may play a role by making it difficult for individuals to navigate a social life and increase heightened anxiety. These neurocognitive processes and heightened anxiety can lead to specific behaviours that are repeated after time and are more likely to become habitual, and altered fronto-striatal pathways may play a role in these mechanisms of habit formation.

One of the main findings in this thesis is in relation to higher odds of internalising difficulties (such as anxiety) in children at risk for ED and grey matter alterations in regions of the brain which are central in anxiety disorders. Anxiety, fear and reward play an important role in ED and have been proposed as central in the development of AN (and other ED to some extent). Anatomic structures of importance to the anxiety circuit include the amygdala, hippocampus, ventral striatum and OFC, all of which have been shown to be altered in this PhD. In both human and animals studies, a wide range of anxiety phenotypes have been linked to altered volume in areas that regulate anxiety and fear-related behaviours (M. L. Phillips et al., 2003a, 2003b), overactivity of the amygdala and of the hippocampus (which plays a critical role in emotional memory) (J. Gray & McNaughton, 2000) (Scott L. Rauch et al., 2003) and dysfunction in frontal systems that regulate amygdala activation by threatening stimuli (reduced top-down control over threat-related distractors) (Bishop, Duncan, Brett, & Lawrence, 2004; S. L. Rauch et al., 2005). This finding not only confirms retrospective research about the role that anxiety may play in the development of an ED (J. M. Swinbourne & Touyz, 2007) but builds upon research

in children at risk for ED which has demonstrated higher odds of psychopathology in this group (Nadia Micali et al., 2014; N. Micali, Stahl, Treasure, & Simonoff, 2014). Importantly, understanding the role that anxiety plays in the course of the development of an ED may help elucidate the mechanisms by which ED develops and lead to earlier interventions.

As said in an earlier paragraph, it is possible that heightened anxiety could result in hypervigilant behaviour (with a specific bias towards body related stimuli given the age of onset and the gender bias) leading to dieting behaviours which can be in itself rewarding and is thereby positively reinforced. Patients with AN also report that dieting helps them cope with negative effect which makes the behaviours even more reinforcing, becoming more rewarding. The response of the ventral striatum (central for the assessment of the potential reward value of stimuli) has been shown to be greater in adolescents than in children and adults (suggesting that the same reward is viewed as more rewarding at this stage of development) (Somerville, Hare, & Casey, 2011). The attraction of rewarding stimuli paired with an underdeveloped inhibitory neural system, may contribute to the development of an ED. In Frank's paper in 2016 (G. K. Frank, 2016), he proposes a model of risk which includes predisposing traits including a disposition to heightened anxiety, increased sensitivity to salient stimuli and a larger OFC which could alter the individuals ability to stop eating.

AN is one of the deadliest psychiatric disorders and one possible reason for its chronicity is how remarkably persistent and resistant it is to current interventions. A shorter duration of illness is amongst the factors associated with a more favourable outcome (Steinhausen, 2002). Given the possible protective factor of a shorter duration of illness, combined with the persistence of behaviours (despite the risk to health associated with them), it has been suggested that reward processing and habit formation plays an important role in the maintenance of AN. The ventral striatum and OFC have been hypothesised to play a role in habit formation which has been suggested to explain extreme weight restriction in AN (with anxiolytic effects reinforcing effects of starvation which then become a habit reducing the function of the goal-directed system) (Lloyd et al., 2017). Individuals with AN achieve and maintain a significantly low body weight mainly through food restriction, which over time becomes highly entrenched and resistant to change, and has therefore been suggested to fulfil the definition of a habit "behaviours that are not innate, that are engaged in repeatedly and become fixed, that appear to occur without conscious effort, and that are elicited by a variety of stimuli" (Graybiel, 2008). While initially a behaviour may be goal-

directed and associated with the receipt of a reward, as it gets repeated overtime, it becomes automatic and far less depended in reward. As this change happens, so do the neural systems that support this behaviour. Animal and human research show that once a behaviour becomes habitual it is under the control of neural systems that include the dorsal striatum (including basal ganglia, caudate and putamen) and associated dorsolateral frontal cortex (Balleine & O'Doherty, 2010). Interestingly, these neural systems have been identified as being of importance in persistent behaviours across psychiatric illnesses (Robbins et al., 2012). It is therefore possible, that alterations in these systems may play a role the process by which behaviours become habitual.

Future work

Given the age of our sample it is important to continue to consider the role that changes in development may play in the onset of an ED. To better understand the role of cognitive control and flexibility in ED it is important to think about it within a developmental context, since the onset of the disorder is generally during adolescence. Brain development undergoes significant changes during adolescence, and the development of executive functions (driven by frontal lobes development) is especially dynamic during this time (Nelson, Leibenluft, McClure, & Pine, 2005). Of special importance are connections with the PFC, which help modulate interconnected subcortical structures. My results suggest that developmental factors related to executive functioning and cognitive processing might be important as biomarkers of ED. Being able to study different age groups at risk may shed more light into biomarkers/endophenotypes within a developmental framework. Being able to study different age groups at risk may shed more light into biomarkers/endophenotypes within a developmental framework. In ED, it is worth considering studying both younger patients and those who have short duration of illness to help our understanding of biomarkers. This is an important factor to consider not only in ED but generally in mental health disorders with an onset in adolescence. The study of major mental health disorders and age of onset would also help shed some light into the overlap between these disorders (such as OCD, anxiety, depression) and create a roadmap for the study of neural correlates for these disorders. Future studies in young people with ED could consider stratifying patients by age in order to see neural developmental changes and influence of the disorder.

This work established alterations of the fronto-striatal circuit, which have been suggested as possible biomarkers for ED (as well as other psychiatric disorders). Given some of the

behavioural differences between different ED types, it is possible that a dysregulation of the frontostriatal (or fronto-amygdala) circuits can lead to under- or overcontrol depending on how reward is experienced. Therefore, the study of connectivity of cortical and sub-cortical regions may be of special importance in understanding what regions may drive altered behaviour.

Results from this thesis show greater GM volume of the OFC which has been suggested to be a trait marker for AN after findings of altered GM volume in healthy sisters of patients (G. K. W. Frank, Shott, & DeGuzman, 2019). While it is unclear what the implications for illness are, further investigation of volume and connectivity of this region is warranted. The OFC plays a central role in reversal learning, a cognitive function whereby behaviour is flexibly altered after negative feedback and has been suggested to play a central role in OCD and be found to be altered in healthy first degree relatives (Chamberlain et al., 2008). As highlighted in early chapters, OCD and AN can present together and patients with AN have been shown to present with numerous OCD traits which has led to research on the overlap between the disorders (Dong et al., 2019; C. A. Levinson et al., 2019; N. Micali, Hilton, et al., 2011; Murphy et al., 2010; Serpell et al., 2002). Results from neurocognitive testing highlight set-shifting difficulties as being present in children at risk, making it a good candidate for an endophenotype. Lesion studies in animals have shown a link between specific regions of the PFC and ability to perform in Cantab AST task. Specifically the OFC has been shown to be linked to performance in AST (McAlonan & Brown, 2003). Given similar results in OCD and the link between OFC and cognitive inflexibility, further investigation is warranted on the influence that increased or decreased OFC volume and it's connectivity may have in increased vulnerability towards ED and the link with inflexibly thinking.

While this study was not able to stratify by maternal diagnosis or ED phenotype, future studies should do so in order to help clarify whether differences in fronto-striatal connectivity might be an important biomarker and therefore future studies should explore this further using both structural and functional imaging techniques Given results from this thesis future studies should consider an approach differentiating patients by phenotypic presentation to help elucidate neural differences in patients with ED.

It is important to note how difficult it is to integrate findings from this study, especially given the complexity of ED and the number of different processes that play a role in their development. The main aim of this study, could be seen as 'casting a wide net', where results may be related to different processes but not necessarily paint a coherent picture, but that can help guide a number of later investigations. I believe that it is possible to tentatively assume that high trait anxiety can lead to hypervigilance which can lead to behaviour changes: possibly more goal directed behaviour (partly influenced by how reward is processed given results from reward measures and alterations in the caudate, or by a hypothesised perfectionistic behaviour) as well as a difficulty to shift from anxious thoughts (explained by cognitive inflexibility). This study did not find any differences in FA between the at risk group and healthy control girls, however, it is possible that hypothesise, that in time, these behaviour changes (led by anxiety and reward sensitivity), can lead to increased coherence of WM through a process of specialisation (increased FA found in adolescent studies) in areas responsible for reward and behaviour control, such as results on increased FA in orbitofrontal areas found by Frank in 2013.

Finally, this thesis shows the importance of at-risk studies to further our understanding of the complex bio-psycho-social model of ED. Given the role that malnutrition plays in neural mechanisms, it's important that we investigate healthy children and first degree relatives in order to understand what neural differences can be considered traits.

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Appendix A: Publications attributed to this Thesis

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ACTA PSYCHIATRICA SCANDINAVICA

Childhood psychopathology in children of women with eating disorders

Barona M, Nybo Andersen AM, Micali N. Childhood psychopathology in children of women with eating disorders.

Objective: We aimed to investigate the effect of maternal eating disorders (ED) on childhood psychopathology, early delays in cognitive, motor and language development, mother and child relationship, and child temperament in a community-based cohort: the Danish National Birth Cohort (DNBC).
Method: Data were obtained prospectively on 48 403 children at 18 months and 46 156 children at 7 years. Data on cognitive, motor and language development, temperament and attachment were obtained at 18 months; data on child psychopathology were obtained at 7 years of age, using the Strengths and Difficulties Questionnaire (SDQ). Children of mothers with lifetime diagnosis of anorexia nervosa (AN, *n* = 931), lifetime diagnosis of bulimia nervosa (BN, *n* = 906) and both (AN & BN = 360) were compared to children of mothers without an ED (*n* = 46 206).
Results: Girls of women with lifetime AN had higher odds of having emotional problems, and girls of women with lifetime BN of having conduct problems compared with children of healthy women. Boys of women with lifetime AN had higher odds of total, emotional and conduct problems; boys of women with lifetime BN had higher odds of total, conduct, hyperactivity and peer difficulties compared to children of women without an ED. Boys of women with lifetime AN and BN had higher odds of total, emotional and peer problems compared to children of healthy women.
Conclusion: Maternal ED is associated with childhood psychopathology in both boys and girls. Boys seemed at higher risk for psychopathology in this sample. Associations between emotional disorders across genders in children of mothers with lifetime AN, and hyperactivity and peer difficulties in boys of mothers with lifetime BN confirm and extend previous findings and point to possible shared risk between ED and other psychopathology.

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Key words: Danish National Birth Cohort; eating disorders; psychopathology; Strengths and Difficulties Questionnaire; temperament; children

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Significant outcomes

- Children of women with ED are at increased risk of psychopathology across several domains.
- The links between maternal BN and childhood hyperactivity and conduct problems, and maternal AN and childhood emotional problems might underlie shared genetic risk; however, this requires further research.
- Studying children at risk for eating disorders helps clarify possible intermediate phenotypes for the disorder as well as shared vulnerabilities with other disorders.

Limitations

- Maternal eating disorder status was obtained using self-report during interviews.
- The data on the children were obtained from mothers (shared method variance).
- We found evidence of selective attrition amongst women with ED; therefore, generalizability of this study might be affected.

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Review article

White matter alterations in anorexia nervosa: Evidence from a voxel-based meta-analysis

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DTI

ABSTRACT

Anorexia nervosa (AN) is a severe psychiatric disorder with a complex and poorly understood etiology. Recent studies have sought to investigate differences in white matter microstructure in AN, with significant results in several brain regions. A systematic literature search of Embase, PubMed and Psychinfo databases was conducted in order to identify Diffusion Tensor Imaging (DTI) studies of patients with AN and controls. We performed a meta-analysis of studies that met our inclusion criteria (N = 13) using effect size-signed differential mapping (AES-SDM) to detect differences in Fractional Anisotropy (FA) in patients with AN (N = 227) compared to healthy controls (N = 243). The quantitative meta-analysis of DTI studies identified decreased FA in the posterior areas of the corpus callosum, the left superior longitudinal fasciculus II, and the left precentral gyrus, as well as increased FA in the right cortico-spinal projections, and lingual gyrus in AN vs. controls. Studies of WM architecture are still limited in AN; further studies with longitudinal design are needed to better understand the complexity of abnormalities, and their persistence.

1. Introduction

Anorexia Nervosa (AN) is a severe psychiatric disorder with a complex and poorly understood etiology and the highest mortality rate (12%) of any other psychiatric disorder (Arcelus et al., 2011). AN commonly begins during adolescence and affects mostly females (APA, 2013); it is characterized by food restriction leading to low body weight, intense fear of gaining weight or persistent behavior that interferes with weight gain; and weight or shape concern, or persistent lack of recognition of the seriousness of the current low body weight (APA, 2013). AN is not only a heterogeneous disorder with a complex multifactorial etiology, involving an interaction of neurobiological, psychological, and environmental mechanisms (Bulik, 2005), but is further complicated by significant medical complications, including severe metabolic, electrolyte, and endocrine disturbances, as well as psychiatric comorbidity. Furthermore, it remains one of the most challenging psychiatric disorders to treat; especially in adults, with low rates of full recovery, about 25% of individuals develop a chronic course of illness (Berkman et al., 2007).

In the last two decades, neuroimaging studies investigating brain structure and function in AN are beginning to provide valuable information on the neural correlates of the disorder. Recent systematic reviews have concluded that structural brain changes are frequently observed in patients with AN (Seitz et al., 2014; Phillipou et al., 2014; Van den Eynde et al., 2012; Titova et al., 2013). AN has been associated with increased Cerebral Spinal Fluid (CSF) volume in the inter-hemispheric fissure, cortical sulci, and ventricles. In 2014 a meta-analysis by Seitz et al. (2014) found that global grey matter (GM) was reduced by 5.6% on average in individuals with acute AN compared to healthy controls (HC) and global white matter (WM) was reduced by 3.7%, both in adults and adolescents with AN. Differences between groups after recovery (2–8 years) were no longer significant.

The heterogeneity of findings and large number of brain areas involved in the pathophysiology of eating disorders (ED) suggests a critical role of interconnections of cortical and subcortical regions, warranting therefore further exploration of connectivity of WM structures in ED. Diffusion MRI allows the estimation of brain fiber structures using water diffusion properties as a proxy. Water molecules diffuse freely in a random manner (isotropic), however, in brain tissue water molecules diffuse more freely along the axon but are constrained from

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0149-7634/

Appendix B: Presentations attributed to this Thesis

Poster presentation: Eating Disorders Research Society – 2015

Childhood psychopathology in children of mothers with eating disorders: findings from the DNBC birth cohort

Manuela Barona[1], Anne Marie Nybo-Andersen[2] & Nadia Micali[1,3]

[1]Institute of Child Health UCL, [2]University of Copenhagen, Dept of psychiatry, [3]Icahn Medical School at Mount Sinai, New York, NY

1. Introduction

There is good evidence that parental psychiatric disorders are associated with psychopathology in the offspring, however, the effect of maternal eating disorders (ED) on offspring's development and psychopathology has only been recently researched.

We know that maternal disordered eating is associated with adolescent disordered eating[1,2] and maternal ED with eating problems in childhood.

Small studies in early childhood[3-5] and a couple of large longitudinal studies[6,7] have confirmed higher risk for psychopathology in children of women with ED compared to those with no ED.

Studying children of mother with an ED might not only clarify risk mechanisms but also shed some light into shared vulnerabilities and endophenotypes for ED.

2. Our Aims

1. Investigate the effect of maternal eating disorders on early child development: motor, cognitive and language; as well as child attachment difficulties and child difficult temperament at 18 months.
2. Investigate the effect of maternal eating disorders on children's psychopathology.
3. Based on previous findings we were interested in gender differences in the associations

3. Method

Participants

The Danish National Birth Cohort (DNBC) established to explore foetal growth, early life and its determinants.

	N
Whole sample	47,933
BN	937
AN	910
BN+AN	360
Unexposed	45,726

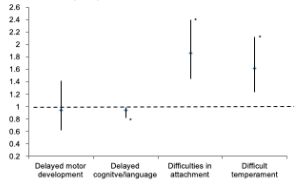
Measures

- Maternal eating disorder
  - Obtained by interview (around 12 weeks gestation)
  - Three groups: AN, BN and AN+BN
- **18 month interview**
  - *Developmental milestones:* 11 questions 5 questions on motor development 6 cognitive and language development questions
    Infants on the top 5% of the age distribution were categorized as delayed (>9 months for sitting and >16 months walking)
  - *Child temperament*
- **7 years assessment**
  - *Strengths and difficulties questionnaire :* Validated tool for child psychopathology 25 item questionnaire
  - Coded in a 3-point Likert scale
  - Measures levels of conduct, emotional, hyperactivity, peer problems, pro-social difficulties and overall psychopathology

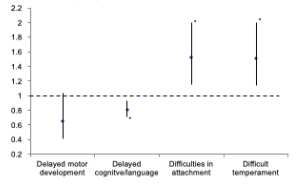
4. Results

4.1. Development at 18 months

OR (95%) Associated with maternal BN

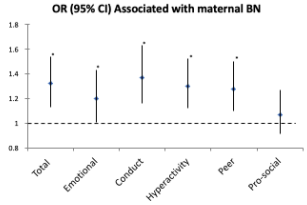


OR (95%) Associated with maternal AN

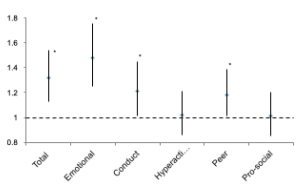


4.2. Psychopathology at age 7

OR (95% CI) Associated with maternal BN



OR (95%) Associated with maternal AN



4.3. Gender differences

Significant findings in psychopathology at 7 when stratified by gender

	BN	AN	AN+BN
Female			
Emotional difficulties		1.28 (1.0-1.6)	
Conduct difficulties	1.41 (1.1-1.8)		
Male			
Total difficulties	1.51 (1.2-1.8)	1.37 (.1-1.7)	1.91 (1.4-2.6)
Emotional difficulties		1.56 (1.2-1.7)	1.92 (1.4-2.7)
Conduct	1.32 (1.1-1.7)	1.32 (1.0-1.7)	
Hyperactivity	1.33 (1.1-1.6)		
Peer difficulties	1.37 (1.1-1.8)		1.71 (1.3-2.3)

Significant findings in attachment and temperament at 18 months when stratified by gender

	BN	AN	AN+BN
Female			
Attachment	2.07 (1.4-3.0)	1.69 (1.1-2.6)	1.96 (1.0-3.74)
Temperament	1.85 (1.3-2.7)		
Male			
Attachment	1.71 (1.2-2.4)		
Temperament		1.56 (1.1-2.3)	2.3 (1.3-4.1)

5. Conclusions

- Children of women with ED are at increased risk of psychopathology across most domains, with boys at higher risk than girls.
- The links between maternal BN and childhood risk for hyperactivity, and maternal AN and childhood risk for emotional problems could be highlighting the possible genetic links in the transmission of the disorder.
- Studying children at risk for eating disorders helps clarify possible phenotypes for the disorder as well as shared vulnerabilities with other disorders.

Bibliography

1 Field et al., 2008; 2 Pike & Rodin, 1991; 3 Franzen & Gerlinghoff, 1997; 4 Stein et al., 2006; 5 Timimi, 1996; 6 Reba-Harrelson et al., 2010; 7 Micali et al., 2014

Oral presentation: London International Eating Disorders Conference – 2015

UCL

Childhood psychopathology in children of mothers with eating disorders

Manuela Barona, Institute of Child Health, UCL
Nadia Micali, Institute of Child Health, UCL
Anne Marie Nybo Andersen, University of Copenhagen

Correspondance: m.martinez-barona@ucl.ac.uk

ED & AMH
Eating Disorders & Adolescent Mental Health

Workshop: Eating Disorders International Conference – 2016

ED & AMH

UCL

Eating disorders in pregnancy:
The potential for adverse outcomes for mother and infant, and the barriers to identifying in antenatal care

Manuela Barona Soyer
PhD Student and Research Coordinator at UCL

Amanda Bye
PhD Student at UCL
Research Worker at KCL

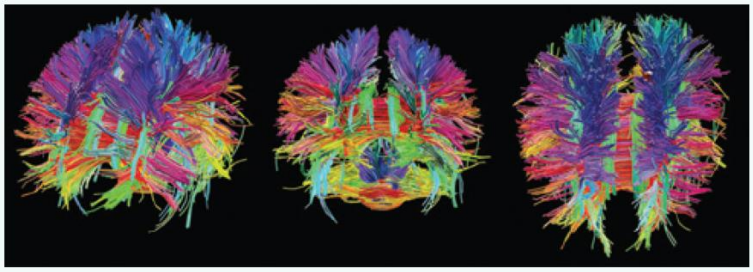
Supervised by: **Dr Nadia Micali**
Senior Lecturer and Honorary Consultant Psychiatrist

Oral presentation: International Conference on Eating Disorders – 2017

UCL

White matter alterations in anorexia nervosa: evidence from voxel-based meta-analysis

Barona, Manuela; Brown, Melanie; Clark, Christopher; Frangou, Sophia; White, Tonya & Micali, Nadia



Manuela Barona
m.martinez-barona@ucl.ac.uk

Oral presentation: European Council of Eating Disorders – 2019

Subcortical and cerebellar volumetric differences in children at high-risk for Eating Disorders

Great Ormond Street, Institute of Child health, UCL

UCL
GREAT ORMOND STREET
INSTITUTE OF CHILD HEALTH

Manuela Barona
m.martinez-barona@ucl.ac.uk

"The author declares that she has no conflict of interest"

Appendix C: Ethics approvals

UCL INSTITUTE OF CHILD HEALTH

Great Ormond Street Hospital for Children NHS Foundation Trust

Joint Research and Development Office
Division of Research and Innovation

Direct Line: 020 7905 2698
Email: R&DGovernance@gosh.nhs.uk

11/09/2014

Dear Dr Radha Kothari,

Project Title	Brain Structure and Function of Children at High Risk of Developing an Eating Disorder: Risk, Identification, and the Potential for Prevention
R&D Number	13BS06
Protocol version	UCL REC application
Protocol date	UCL REC application
Funder	Swiss Anorexia Nervosa Foundation
Sponsor	UCL Institute of Child Health (ICH)

This project has been granted Management Approval by the Joint Research & Development Office.

Approval Conditions:

- You must submit an annual report which will be sent to you by the Joint R&D Office when it is due.
- The PI must inform the Joint R&D Office of any changes to the start and end dates of the project, or if there are any changes to the protocol or personnel. At the end of the study the PI will be sent a final report form to complete and return to the Joint R&D Office.

Please be aware that although you have been granted R&D approval you will not be authorised to spend against your award unless there is a signed contract with the research funder / lead site.

Please contact the Joint R&D Office if you require any further guidance or information on any matter mentioned above. We wish you every success in your research.

Yours sincerely,

[redacted]

Dr Thomas Lewis
Research Management and Governance Officer
Joint Research and Development Office

cc: GOSH Finance and/or ICH Finance

Joint Research and Development Office
Division of Research and Innovation
UCL Institute of Child Health, 30 Guilford Street, London WC1N 1EH
Tel: 020 7905 2700 Fax: 020 7905 2201
www.gosh.nhs.uk

The child first and always

NHS
Health Research Authority
London - London Bridge Research Ethics Committee
Skipton House
80 London Road
London
SE1 6LH
Telephone: 020 7972 2580

06 April 2016

Dr Nadia Micali
UCL Institute of Child Health
Institute of Child Health, BBSU, 4th Floor
30 Guilford Street, London, WC1N 1EH

Dear Dr Micali

Study title:	**Neurocognitive function, brain structure and function of children at familial high-risk of developing eating disorders**
REC reference:	**16/LO/0256**
IRAS project ID:	**194268**

Thank you for responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Vice-Chair.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to make a request to postpone publication, please contact the REC Manager, Miss Kirstie Shearman, nrescommittee.london-londonbridge@nhs.net.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission must be obtained from each host organisation prior to the start of the study at the site concerned.

Appendix C: Webpage developed

HELP US UNDERSTAND EATING DISORDERS

Some feedback from mum's and daughters who have already visited us!

"If research proves that the disease is genetic or that it can be prevented developing in children or finds anyway of controlling eating disorders it would be amazing so that others didn't have to live the life I did for so long. Research and support is vital and I am so glad I took part."

"...she was super impressed with the fact that she was going to have pictures of her brain."

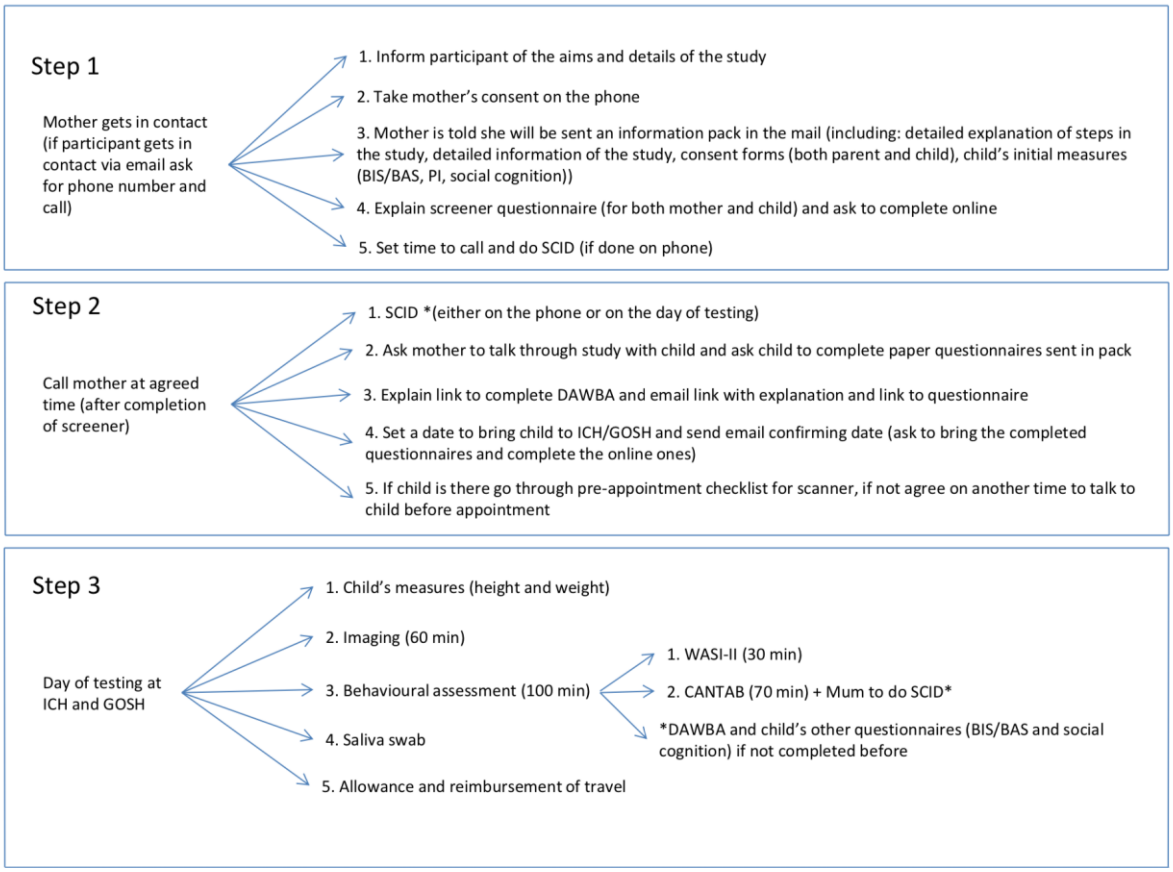
"Everything was very relaxed and there was nothing uncomfortable and no intrusive questions"

"My daughter enjoyed the various tests and questionnaires except for the MRI scan; this was however handled with patience and understanding."

"I felt it was important to take part in the study. The more research and understanding of eating disorders and their causes the better"

"My daughter enjoyed taking part and found the experience a lot of fun!"

Appendix D: Visual protocol



Appendix E: Puberty questionnaire

UCL INSTITUTE OF CHILD HEALTH

Project ID: .....

Maternal Eating and Adolescent Brain Development Study

1. Has your daughter starter her menstrual periods yet?

   ☐ Yes ☐ No

2. How old was your daughter when she had her first period?

   ☐

3. When was her first period?

   Month ☐ Year ☐

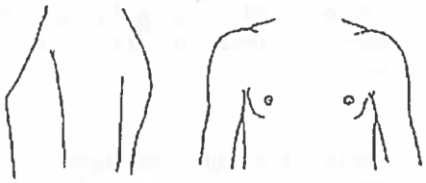
We would like to assess the stage of your daughter's physical development using some a set of drawings on the next pages. These indicate various stages of puberty commonly used by doctors to assess the growth and development of girls. Not all children follow the same pattern of development.

We need to know which drawings most closely match your daughter's stage of development at the moment. **Just pick the stage that is closest, based on both the picture and the description.**

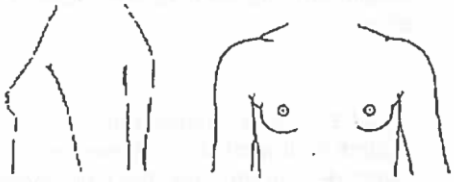
The drawings below show stages of the way the **breasts** develop. A girl can go through each of the five stages shown, although some girls skip some stages. Please look at each of the drawings. It is also important to read the descriptions.

Put a tick in the box to the right of the drawing that is **closest** to your daughter's current breast stage.

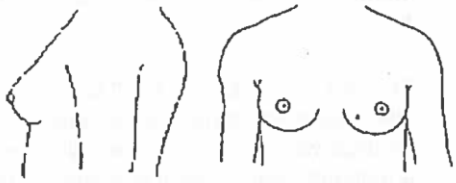
☐ 1 The nipple is raised a little in this stage.The rest of the breast is still flat



☐ 2 This is the breast bud stage. In this stage the nipple is raised more than in stage 1. The breast is a small mound. The dark area around the nipple (areola) is larger than in stage 1



☐ 3 The areola and the breast are both larger than in stage 2. The areola does not stick out away from the breast



☐ 4 The areola and the nipple make up a mound that sticks up above the shape of the breast (Note: This stage may not happen at all for some girls. Some girls develop from stage 3 to stage 5 with no stage 4)

☐ 5 This is the mature adult stage. The breasts are fully developed. Only the nipple sticks out in this stage. The areola has moved back in the general shape of the breast

☐ 6 Not sure

The drawings below show different amounts of **female pubic hair**. A girl can go through each of the five stages shown. Please look at each of the drawings. It is also important to read the descriptions.

Put a tick in the box to the right of the drawing that is the **closest** to the amount of pubic hair your daughter has.

☐ 1 There is no pubic hair

☐ 2 There is a little long, lightly coloured hair. This may be straight or a little curly

☐ 3 The hair is darker in this stage. It is coarser and more curled. It has spread out and thinly covers a bigger area

☐ 4 The hair is now as dark, curly and coarse as that of an adult woman. However, the area that the hair covers is not as large as that of an adult woman. The hair has not spread out to the legs

☐ 5 The hair is now like an adult woman. It also covers the same area as that of an adult woman. The hair usually forms a triangular pattern as it spreads out to the legs

☐ 6 Not sure

NOTE: Your daughter's pubic hair stage may or may not be the same as her stage of breast development

Appendix F: MRI safety screening

Maternal Eating and Child Brain Development

Please read through this safety screening sheet and answer the following questions carefully. Ask if anything is not clear. If you answer 'yes' to any questions or have any questions about the study please contact Manuela Barona (m.martinez-barona@ucl.ac.uk, 020 7905 2166) before taking part. All information is held in the strictest confidence.

MRI scanning uses strong magnetic fields. For your own safety and the safety of others it is **very important** that you do not go into the magnet halls with any metal in or on your body or clothing, as well as any eye-makeup.

1. Do you have any implants in your body? e.g. replacement joints, drug pumps	Y/N
2. Do you have aneurysm clips (clips put around blood vessels during surgery)?	Y/N
3. Do you have a pacemaker or artificial heart valve? *(These stop working near MR Scanners)*	Y/N
4. Have you ever had any surgery?	Y/N
5. Are you currently on any medication?	Y/N
6. Do you have any foreign bodies in your body (e.g. shrapnel)?	Y/N
7. Have you ever worked in a machine tool shop without eye protection?	Y/N
8. Do you wear a hearing aid or cochlear implant?	Y/N
9. Have you ever suffered from tinnitus?	Y/N
10. Do you wear dentures, a dental plate or a brace, or have you had any recent metallic dental work?	Y/N
11. Are you susceptible to claustrophobia?	Y/N
12. Do you have any problems with lying still on your back for extended periods of time?	Y/N
13. Do you suffer from blackouts, epilepsy or fits?	Y/N
14. Do you have any tattoos?	Y/N
15. Are you short sighted?	Y/N
If so do you wear glasses or contact lenses?	
16. Is there anything else you think we should know?	Y/N